Adaptive plant plasticity to light and disease: Perspective from natural variation in *Camelina sativa*

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THESIS DECLARATION

I, Maria Indah Purnamasari, certify that:

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ABSTRACT

Environmental factors determine plant performance in the field. An individual plant genotype can produce multiple phenotypes in response to changes in environmental conditions, known as phenotypic plasticity. *Camelina sativa* (L.) Crantz (Brassicaceae) is an oilseed crop with value in the production of functional foods, industrial oils and biofuel. However, current knowledge is incomplete regarding this plant's plasticity to two economically important agricultural challenges: shade and Sclerotinia rot (SR; disease caused by a broad-host pathogen *Sclerotinia sclerotiorum* (Lib.) de Bary). This thesis aimed to a) determine the population structure within a panel of Camelina lines and b) use a Camelina-shade–*S. sclerotiorum* model system to study plant plasticity in different environments to identify the key genetic causes underlying these responses.

The first experimental chapter characterises intraspecific diversity within a Camelina germplasm panel of 31 lines using DArTseqLD genotyping-by-sequencing, flow cytometry and phenotypic evaluation. While the experiments revealed relatively low genetic diversity among lines, the lines were separated into two subpopulations using STRUCTURE. The nuclear genome size of lines indicated low but significant intraspecific variation. Significant variation was also identified for eight morphological and biochemical traits. Despite these intraspecific variations, no clear association was detected between phylogenetic and phenotypic traits from lines of a particular genome size. Nevertheless, the intraspecific diversity analysis revealed variation among the studied Camelina lines that could be used for Camelina improvement and pangenome studies.

The second chapter explores the plasticity pattern of Camelina under varying light qualities [measured as red to far-red ratio (R:FR)], from shade-mimicking light (R:FR=0.2) to full light (R:FR=10). Twelve plastic traits were measured for this assessment. We performed a comparative analysis between Camelina and two well-established related species, *Arabidopsis thaliana* (L.) Heynh. (model) and *Brassica napus L.* (agricultural crop). Camelina exhibited a plastic response typical of shade-avoidance syndrome (SAS) due to low R:FR. The three Brassicaceae species have different patterns of plasticity to R:FR changes, perhaps due to their crop's domestication history. Leaf area was identified as an important trait for maintaining biomass under simulated shade, providing a breeding goal trait for shade-resilient Camelina.

In the third experimental chapter, we evaluate the reactions of Camelina lines to two important Brassicaceae pathogens with different pathogenic lifestyles: broad-host *S. sclerotiorum* (a causal agent of SR) and narrow-host *Leptosphaeria maculans* (Desm.) Ces. & de Not (a causal agent of blackleg). Camelina exhibited a variable response to two pathotypes of *S. sclerotiorum* during the seedling and adult stages and high-level resistance to six isolates of *L. maculans* representing five

different avirulent loci. The study confirmed partial resistance of genotype C370 and susceptible genotype C2305, as identified in my previous study. The F_5-recombinant inbred line (RIL) population from a cross between C370 and C2305 at the cotyledon stage varied in disease response to *S. sclerotiorum*, suggesting quantitative disease reaction phenotypes.

The last experimental chapter aims to understand the plasticity of Camelina defence against *S. sclerotiorum* under different light qualities and identify the complex genetic regulation underlying this response. QTL mapping was conducted in the 106 F5 RIL population from crosses between C370 and C2305 using 515 markers obtained by DArTseqLD. The genomic locations of Camelina responses to SR, both in full light and shade-mimicking conditions, were mapped for the first time in this work. This study also revealed the mechanism of defence attenuation under shade in Camelina, by compromising RSR (the locus for SR resistance under full light) and inducing the susceptibility locus SIS under simulated shade. These results support the application of natural variation to reveal the genetic architecture of complex traits.

Overall, the research presented in this thesis contributes to Camelina genetic improvement efforts and provides valuable information on the genetics of plant defence mechanisms under shade. The results and their interpretation address previous gaps in knowledge of Camelina plasticity in response to shade and SR. The findings also highlight the major contribution of the analysis of natural variations to expand the relevance of model systems for understanding complex traits.

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"The company." said Tiny Dragon.

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AUTHORSHIP DECLARATION: CO-AUTHORED PUBLICATIONS

This thesis contains one paper published in a peer-reviewed scientific journal, plus three additional manuscripts prepared for publication.

Details of the work:

A research paper published in peer-reviewed scientific journal *Plant Disease*.

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Location in thesis:

Chapter 5.

Student contribution to work:

- Conceived the design of this study with WE, JC and MJB.
- Grew plant samples and inoculated plants in the glasshouse.
- Performed all statistical analysis.
- Wrote the manuscript, with comments and suggestion from co-authors.

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Student contribution to work:

- Performed genome size measurement for half the samples.
- Phenotyped plants for flowering time, seed size and seedling vigour index.
- Prepared samples for genotyping.
- Genetic analysis with the assistance of JL.
- Performed all statistical analysis.
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- Performed all statistical analysis.
- Completed QTL mapping using genotype data provided by DArT Pty Ltd with the assistance of JL.
- Wrote the manuscript, with comments and suggestion from co-authors.

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I, Professor William Erskine certify that the student statements regarding their contribution to each of the works listed above are correct.
As all co-authors' signatures could not be obtained, I hereby authorise inclusion of the co-authored work in the thesis.

Coordinating supervisor signature:


Date: 23/2/2021

CHAPTER 1

General introduction

Environmental changes in both natural and agricultural ecosystems highly influence the growth and fitness of plants. Environmental changes can be short-term (*e.g.,* diurnal or seasonal variation) or long-term (*e.g.,* climate change). Furthermore, plants are often exposed to the effects of other organisms' responses to environmental changes. For example, rising temperatures due to climate change increase the severity of plant–pathogen interactions (Walker *et al.,* 2019). Based on these cues, plants respond with a molecular process that determines their status in their current environments. Both phenotypic plasticity and heritable genetic changes underlie and facilitate plant responses to the environment. A lack of plasticity to adapt to environmental changes often harms crop productivity in the field and can put species survival at risk in some ecosystems.

This study tracks a crop's responses to multiple environmental cues that can affect sustainable food production. I selected an understudied crop *Camelina sativa* (L.) Crantz (Brassicaceae), environmental cues by simulating shade and a recalcitrant broad-host pathogen *Sclerotinia sclerotiorum* (Lib.) de Bary [causal agent of Sclerotinia rot (SR)] as the subjects of my study into environmental plant responses and to identify key genetic links between them. Camelina is an oilseed crop that produces high-quality oil for many applications (Nguyen *et al.,* 2013; Berti *et al.,* 2016). This oilseed crop has considerable agronomic potential, as it can be grown in marginal cropping environments under comparatively lower rainfall than similar oilseed crops, such as canola (*Brassica napus* L.), and requires relatively low inputs, particularly in terms of weed control (Gugel & Falk, 2006; Matteo *et al.,* 2020). Due to its ability to produce the plant phytoalexin camalexin, Camelina has a low level of disease response to pathogens that cause significant yield losses in other Brassicaceae species, such as canola (reviewed in Séguin-Swartz *et al.,* 2009). Despite its agronomic potential, the adoption of Camelina has been hampered by low genetic diversity in available germplasm and insufficient agronomic knowledge (Brock *et al.,* 2018; Hotton *et al.,* 2020).

At the time of this study, little is known about the response of Camelina to shade and the Brassicaceae pathogen *S. sclerotiorum*. In high-density planting or mixed cropping systems, Camelina is often subject to self-shading and shading from neighbouring plants, both of which reduce photosynthate accumulation and total oil yield (Waraich *et al.,* 2020). Moreover, after blackleg, SR disease is the second-most prevalent disease in Australian *Brassica* oilseed cultivation (Khangura *et al.,* 2011). This generalist pathogen threatens the adoption of this crop in Australia and worldwide. Therefore, expanding our understanding of Camelina responses to varying light qualities and broad-host pathogen attacks, as independent or co-occurring cues, could improve future Camelina breeding efforts and add to the collective understanding of these factors on other species. Furthermore, this research has practical implications for food security in light of the economic importance of the crop, its diseases and the role of shade stress.

To better understand the genetic and phenotypic potential of such an understudied plant as Camelina, I implemented a comparative analysis involving two well-established species: *Arabidopsis thaliana* (L.) Heynh and canola. Camelina is closely related to Arabidopsis (both located in lineage 1 of Brassicaceae) and canola (lineage 3 of Brassicaceae). I expected to gain an insight into Camelina responses given the similarities in the genomes and transcriptome sequences, the extensive collections of phenotypic data for the model plant and the information related to the life history (*e.g.*, geographical distribution) of these two Brassicaceae species.

The thesis comprises seven chapters and is presented as a series of scientific reports, including one peer-reviewed article. Chapter 1 contains the background of the study and research aims. Chapter 2 presents a review of the literature relevant to this thesis to identify knowledge gaps and define the study's research objectives.

Chapter 3 is the first experimental chapter. This chapter revisits the genetic diversity among Camelina accessions held at The University of Western Australia by evaluating their sequencing, genome size and phenotypic data.

Chapter 4 characterises Camelina lines in response to different R:FRs (from R:FR=0.2 to mimic shade to R:FR=10 to represent full light). A comparative analysis with Arabidopsis and canola is used to contextualise the response of Camelina. The hypotheses tested were: (i) Camelina exhibits plasticity when exposed to different R:FRs, including the shade-mimicking condition (*i.e.*, a single genotype responds in accordance to the R:FRs); (ii) the three Brassicaceae species display different patterns of plasticity in response to light quality changes; and (iii) there is a direct link between shade-avoidance traits and plant biomass at high and low R:FRs.

Chapter 5 presents an experiment designed to confirm earlier observations on the response of 30 Camelina lines to SR at the seedling stage (Purnamasari *et al.*, 2015) using additional inoculation methods and isolates. The responses of Camelina to different *Leptosphaeria maculans* (Desm.) Ces. & de Not. isolates are also presented. This chapter has been published in a peer-reviewed journal (*Plant Disease*) and reformatted for consistency within the current thesis.

Chapter 6 presents the first comprehensive genetic exploration of plant defence under shade. Information on Camelina response to *S. sclerotiorum* under full light (R:FR=10) is used for comparison, which independently is of value as little is known about the SR-resistance mechanism in Camelina. The hypotheses tested were: (i) Camelina defence against *S. sclerotiorum* is compromised under shade and (ii) quantitative trait loci affect defence under shade and/or full light.

Chapter 7 presents a general summary, discussion and future work related to these findings as they concern plant responses to simultaneous environmental cues and Camelina development.

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CHAPTER 2

Literature review

Camelina sativa is a promising crop that produces high-quality oil and has positive agronomic traits associated with sustainable agriculture. Despite the potential of Camelina, its responses to some significant challenges that face agriculture (*e.g.*, broad-host pathogen attack and shade-avoidance response) have not been addressed adequately. This chapter introduces Camelina and discusses the potential of using a comparative analysis of related Brassicaceae species to understand the response of Camelina to various environmental challenges. Subsequent sections provide a brief description of shade and plant plasticity in response to shade before discussing relevant molecular components in the signalling network. The review also describes relevant knowledge on the fungal pathogen, *Sclerotinia sclerotiorum*, and recent advances in plant responses to this pathogen. Recent progress in understanding the regulatory networks that underlie plant defence responses under shade is also reviewed. The chapter concludes by describing genetic tools that can be used to evaluate complex mechanisms underlying plant responses to various environmental factors and summarises current knowledge and gaps where further research is needed.

2.1. *Camelina sativa* – A component in sustainable intensification of agriculture

2.1.1. Multiple potentials of Camelina

Camelina sativa, also known as false flax, has received recent attention as an environmentally friendly oilseed crop due to its high-value oilseed and positive agronomic traits (Song *et al.*, 2020). Camelina has three different subspecies, spp. *sativa* (spring form), spp. *foetida* (spring form) and spp. *pilosa* (winter form), based on vernalization requirement. Worldwide, the spring forms are most widespread and commonly cultivated (Kurasiak-Popowska *et al.*, 2018). Camelina is native to Eastern Europe and Western Asia (Vollmann & Eynck, 2015). It has been cultivated since the Bronze Age in Europe (Bouby, 1998). According to archaeological studies, the oldest remains of Camelina were found in Armenia and date back to 6000 BC (Hovsepyan & Willcox, 2008). It was extensively grown in Europe in the 19th century but almost disappeared in the mid-20th century due to competition with other high-yielding crops, such as wheat (*Triticum aestivum* L.), soybean (*Glycine max* L. Merrill) and canola (Zubr, 1997). Camelina, as a 'secondary crop', is a well-documented example of the mode of evolution called 'Vavilovian mimicry', in which a weedy race evolves to become a crop in its own right by mimicking real crops; in the case of Camelina, the mimicked crop was flax (Zohary *et al.*, 2012). In line with this theory, Brock *et al*. (2018) proposed that Camelina is a domesticated form of the Camelina weedy species, *Camelina microcarpa Andrz. ex DC*. Their hypothesis is supported by similarity in morphology, ploidy number and phylogenetic relationship of the two species.

Renewed interest in Camelina in the 21st century was sparked by the valuable unique fatty acid profile of its seed oil, which is important for food, feed and industrial applications. Camelina seeds contain

high levels of oil (36–47%), proteins (30%) and a variety of natural active compounds, including polyphenols and carotenoids (Moser, 2012; Pagnotta *et al.,* 2019). Moreover, Camelina oil is considered a 'superfood' as it is rich in unsaturated fatty acids (90%), particularly *n-3* (omega-3) fatty acids (40%), which have high nutritional value and a significant function in improving human and animal immunity (Ibrahim & El Habbasha, 2015; Gutiérrez *et al.,* 2019). Due to Camelina oil's unusually well-balanced fatty acid profile, it has several potential non-food-related applications (*e.g.,* as an ingredient in oleochemicals, polymers and cosmetics) (Iskandarov *et al.,* 2014; Kim *et al.,* 2015; Berti *et al.,* 2016; Popa *et al.,* 2017). Perhaps the most important Camelina oil application is as aviation biofuel due to its high long-chain hydrocarbon content. Besides having advantages in biofuel production (Camelina-derived fuel can be used directly without processing), it can reduce CO_2 emissions by up to 80% compared to traditional petroleum jet fuel (Shonnard *et al.,* 2010). Although some improvements need to be made to Camelina oil (*e.g.,* reducing erucic acid and glucosinolate content) (Amyot *et al.,* 2018), the high quality of Camelina oil has earned the nickname of 'gold-of-pleasure.'

Another significant reason for the recent adoption of Camelina is its agronomic features, making it an ideal crop for sustainable agriculture. Such favourable agronomic qualities include its ability to adapt to a wide range of climatic conditions and its good response to low-input farming practices (Gugel & Falk, 2006; Zanetti *et al.,* 2017; Matteo *et al.,* 2020). Camelina is a remarkably robust crop that can adapt to semi-arid regions, infertile or saline soils, marginal land and limited rainfall. This oilseed crop has the capacity for summer and winter production, with short life cycles of 70 to 100 days (Schillinger *et al.,* 2012). Camelina is also suited to intercropping and/or double cropping with cereals such as wheat. As such, it could be a viable, more stress-resistant replacement for other oilseed crops, such as canola (Chandra *et al.,* 2014; Obour *et al.,* 2018). Of special interest among the essential traits of Camelina is its resistance to many common parasitic insects and pathogens that usually cause significant yield losses in other Brassicaceae oilseed crops, such as canola (Séguin-Swartz *et al.,* 2009). Camelina also displays allelopathic traits that suppress weed growth (Lovett *et al.,* 1989). Despite its potential, the agronomic benefits of Camelina remain largely underexploited (Obour *et al.,* 2015; Hotton *et al.,* 2020).

2.1.2. Genetic analysis of Camelina

It has been suggested that the genetic improvement of Camelina is hampered by low genetic diversity in the available germplasm and limited effective population sizes within the species (Brock *et al.,* 2018; Luo *et al.,* 2019). Most Camelina varieties were lost when the focus of oilseed production shifted from Camelina to canola in the latter half of the twentieth century (Brock *et al.,* 2018).

Currently, major germplasm collections of Camelina are maintained at the N.I. Vavilov Research Institute of Plant Industry, European Catalogue of Plant Germplasm Collection, Plant Gene Resources of Canada, USDA National Plant Germplasm System, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) and National Genetic Resources Program (NGRP) (Vollmann & Eynck, 2015; Sainger *et al.,* 2017; Kurasiak-Popowska *et al.,* 2018, 2020; Vera *et al.,* 2020). These centres contain germplasm of mostly spring cultivars from various countries, such as the former Soviet Union, Germany, Poland, Sweden, Denmark, United States, Georgia, Slovenia and Austria. A considerable amount of research has been published on the genetic and phenotypic diversity in this Camelina germplasm (Vollmann *et al.,* 2005; Gehringer *et al.,* 2006; Ghamkhar *et al.,* 2010; Manca *et al.,* 2013; Galasso *et al.,* 2015; Singh *et al.,* 2015; George *et al.,* 2017; Luo *et al.,* 2019; Kurasiak-Popowska *et al.,* 2020; Hotton *et al.,* 2020). All these studies underline the paucity of diversity in Camelina germplasm. Recently, two subpopulations were identified within the Camelina spring panel (Luo *et al.,* 2019). Nevertheless, the collection, conservation and genetic characterisation of Camelina germplasm remain essential to future agronomic improvement of the species.

The use of Camelina winter types could increase genetic heterogeneity of the species and, in turn, improve the cultigen, as winter Camelina has low erucic acid content and produces abundant seed (Walia *et al.,* 2018; Kurasiak-Popowska & Stuper-Szablewska, 2020). Similarly, crossing with the wild relative, *C. microcarpa* could increase the germplasm pool of Camelina, as it is possible to obtain F_2 plants with different lipid profiles than the parental species (Tepfer *et al.,* 2020). However, the success of interspecific crossing depends on which parent of *C. microcarpa* is used. Therefore, further studies on the genetic characterisation of Camelina-related species are required (Séguin-Swartz *et al.,* 2013; Martin *et al.,* 2019; Tepfer *et al.,* 2020).

Genetic studies of Camelina have established its allohexaploid genome structure (Hutcheon *et al.,* 2010; Galasso *et al.,* 2011; Kagale *et al.,* 2014a). The ancient subgenomes of Camelina remain almost undifferentiated, forming the current diploid 2n=40 chromosomes [CsG1x=6, CsG2x=7, CsG3x=7]. The CsG3 subgenome appears to be preferentially expressed over the other two (Kagale *et al.,* 2014a, 2016). Recently, Mandáková *et al*. (2019) suggested that the three subgenomes of Camelina originated from two hybridization steps. The first step involved intraspecific hybridization between the diploid *Camelina neglecta* J.Brock, Mandakova, Lysak & Al-Shehbaz sp. nov., resulting in a tetraploid. The second step involved further hybridization between the tetraploid and a diploid *Camelina hispida* Boiss.

The Camelina genome size has been calculated using flow cytometry (~750 Mb) and genome sequencing (~785 Mb) (Hutcheon *et al.,* 2010; Kagale *et al.,* 2014a). However, only 77.5% of the

estimated genome size can be anchored to the 20 chromosomes of Camelina (608.54 Mb) in the genome assembly. Transcriptomic studies of Camelina at different developmental stages could facilitate genome annotation (Liang *et al.,* 2013; Mudalkar *et al.,* 2014; Abdullah *et al.,* 2016; Kagale *et al.,* 2016). The widespread availability of Camelina molecular kits (*e.g.,* genome sequencing, transcriptomic data) have facilitated attempts to increase seed yield and oil quality through genetic engineering (*e.g.,* Nguyen *et al.,* 2013; Jiang *et al.,* 2017; Morineau *et al.,* 2017; Chhikara *et al.,* 2018; Na *et al.,* 2019; Yuan & Li, 2020). The genetic basis of Camelina tolerance to various environmental factors, such as pathogen attacks and shade, remains unknown.

2.1.3. Comparative analysis of Brassicaceae species: Approach to impart information in understudied species

Camelina belongs to the mustard family (Brassicaceae), which includes ~4000 species currently delineated into 52 tribes, 372 genera and six major clades (lineage 1–6) (Nikolov *et al.,* 2019). The Brassicaceae comprises many economically and scientifically important plants distributed across a wide range of habitats worldwide (Franzke *et al.,* 2011; Kiefer *et al.,* 2014; Koenig & Weigel, 2015; Nikolov & Tsiantis, 2017). Some Brassicaceae species experienced a series of polyploidy events across roughly 100 million years, providing a model family for comparative and evolutionary studies (Franzke *et al.,* 2011; Kagale *et al.,* 2014b; Edger *et al.,* 2015). Indeed, comparative studies in Brassicaceae suggest a highly conserved gene sequence, content and order in this family and rapid rates of gene duplication, chromosome rearrangement and alterations in ploidy (Hall *et al.,* 2002; Cheung *et al.,* 2009; Sharma *et al.,* 2014; Chica *et al.,* 2017).

Comparative analysis, or the study of similarities and differences in closely related species, is a powerful approach for understanding conserved phenotypic traits/genetic architecture, traits/genome evolution and information transfer (Frankel *et al.,* 2011). Comparative studies among Camelina and its related species could be used to assess Camelina response under specific conditions and better understand the biological mechanisms controlling this response. Fortunately, a well-established plant model, *Arabidopsis thaliana,* is in the immediate phylogenetic vicinity of Camelina, which co-locates in lineage 1 of Brassicaceae (tribes: Camelineae) with shared common ancestors that diverged ~22 million years ago (Mya) (Figure 2.1) (Kagale *et al.,* 2014b). Accordingly, the genome sequence and transcriptome of Camelina are related to Arabidopsis, with 70% of Camelina genes being in synteny with Arabidopsis genes (Nguyen *et al.,* 2013; Kagale *et al.,* 2014a). This similarity allows Arabidopsis to be used as a reference for information in phenotypic and molecular studies. Genetic protocols developed for Arabidopsis can be used on this crop with ease, from the transformation technique using *Agrobacterium tumefaciens* (Lu & Kang, 2008) to sophisticated methods such as

RNAi (Na *et al.*, 2018) and CRISPR/Cas9 (Jiang *et al.*, 2017; Morineau *et al.*, 2017). Together, the information from Arabidopsis facilitates discoveries in a closely related crop, Camelina.

Despite its similarities with Arabidopsis, the polyploidy of Camelina has increased the complexity of the genome. Furthermore, because Arabidopsis is not a crop, a comparative study of Camelina and Arabidopsis would provide little information on the evolutionary differences between domesticated plants grown in agricultural settings. For these reasons, expanding the comparative analysis with canola provides opportunities to examine genetic alterations that contribute to crop domestication and how such changes are displayed phenotypically. Supporting our hypothesis, Liang *et al*. (2013) reported that Camelina has a high level of similarity of disease resistance genes to *Brassica rapa* L. (lineage 2). A possible explanation for this remarkable similarity between defence genes is that Camelina and *B. rapa* might have undergone selective pressure associated with the high prevalence of pathogens in their natural environments. Canola is located in lineage 2 (tribes: Brassiceae) and separated from lineage 1 by ~27 Mya (Figure 2.1) (Kagale *et al.*, 2014b). Taken together, a study comparing Camelina and its related species (*i.e.*, Arabidopsis and canola) would significantly improve the current understanding of functional traits and genomics in Camelina.

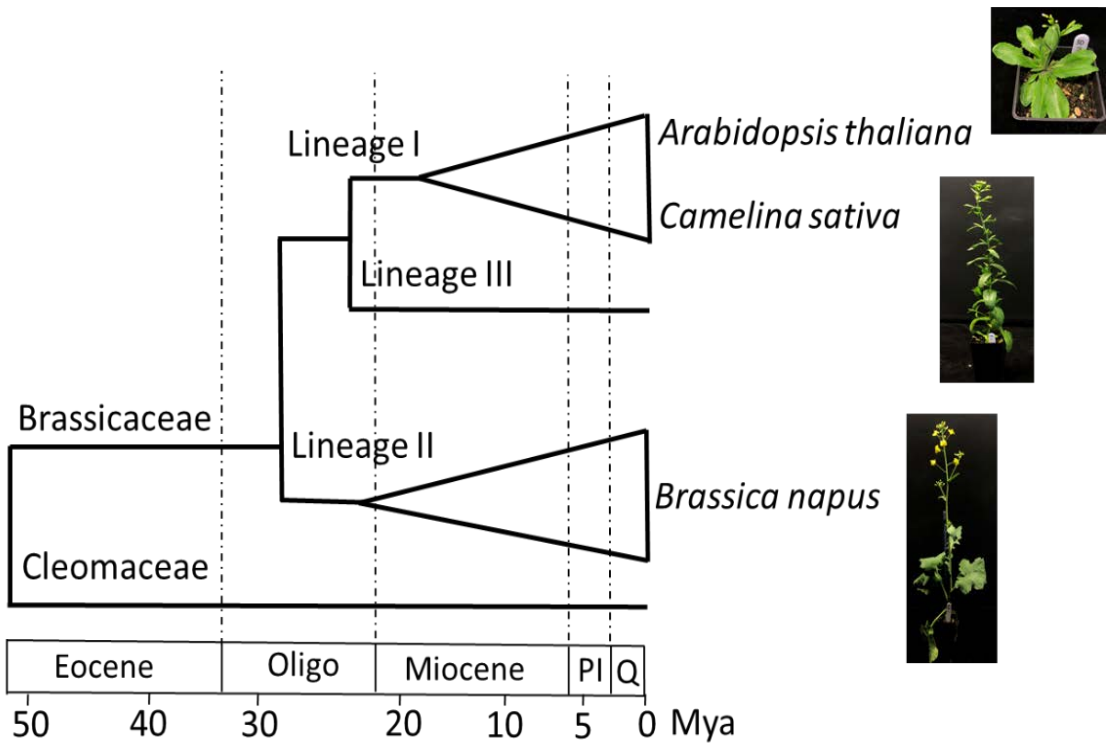

Figure 2.1: Lineage separation events in the three species of Brassicaceae presented in a phylogenetic context. Species included in this study and their morphologies at flowering. Oligo, Oligocene; PI, Pliocene; Q, Quarternary. Figure source: Adapted from Kagale *et al*. (2014b).

2.2. Shade – An inevitable factor in agricultural intensification

2.2.1 Shade-avoidance syndrome

As light is the source of energy for photosynthesis, reduced light quality under shade affects plant growth and reduces yield in the field (Chen *et al.,* 2019). Unfortunately, being shaded is a common problem that plants encounter in the field, especially under close planting conditions and/or intercropping systems (Yang *et al.,* 2015). The light spectral composition is altered under shade due to wavelength-selective attenuation by neighbouring plants. The green tissues of neighbour plants absorb blue (B; 400–500 nm) and red (R; 600–700 nm) light wavelengths, which are included in photosynthetically active radiation (PAR) (400–700 nm) (Casal, 2013). However, because they do not use far-red light (FR; 700–800 nm) or transmit it to the environment, this enriched FR condition leads to a lower photon flux distribution ratio in the R than in FR (R:FR). Many studies have used R:FR as the primary parameter for understanding plant responses to shade. This is because R:FR is unaffected by other environmental factors and could be used to separate light-quality effects (Casal, 2013). In nature, shade is characterised by changes in spectral composition, lower irradiance and changes in relative humidity and temperature (Casal, 2013; Lee *et al.,* 2016). Furthermore, R:FR is related to the activity of photoreceptors and initiation of various phenotypically plastic traits (Smith, 1995; Casal, 2013). In addition to R:FR, the amount of depleted B-light signals the presence of neighbouring plants (Keuskamp *et al.,* 2011).

The development and deployment of light-emitting diodes (LEDs) coincided with the advance in plant photoreceptor studies (Ptushenko *et al.,* 2020). LED technology makes it easy to select and customise specific light wavelengths and control the spectral intensity when studying plants. This, in turn, enables reliable experimental settings to investigate and evaluate the effects of shade on wild types or mutants with optical-signal defects (Massa *et al.,* 2008; Sellaro *et al.,* 2011). LEDs also offer many advantages over other artificial lighting sources, including energy efficiency, low heat emission, long lifespan and low-cost (Darko *et al.,* 2014; Miler *et al.,* 2019). Several approaches have been developed to mimic shade and study plant responses to shade, including using a photoselective filter (Studzinska *et al.,* 2012), applying FR at the end of the day (Dubois *et al.,* 2010), or providing continuous FR light (Kalaitzoglou *et al.,* 2019).

Plants are extremely sensitive to a drop in R:FR below 1 (R:FR for sunlight) (Smith, 1982). Detecting this cue early is crucial to plant survival, as low R:FR signalling produces molecular, metabolic and developmental responses that allow plants to adapt their photosynthetic machinery to a limited light environment (Morelli *et al.,* 2020). After detecting a low R:FR signal, plants exhibit two alternative growth strategies: shade tolerance or shade-avoidance (Martinez-Garcia *et al.,* 2010, 2014). Shade-

tolerant species develop conservative resource-use strategies, such as slowing down their growth rate, reducing apical dominance and increasing branching to cope with the shady conditions (for a detailed review, see Valladares & Niinemets, 2008; Gommers *et al.,* 2013). In contrast, shade-avoiding species, such as the model plant Arabidopsis, adapt their development to compete for light resources by promoting elongation growth in petioles and stems (Casal, 2012). Shade signals also lead to fewer branches on this plant as branch production increases the proportion of shaded tissue (Wang *et al.,* 2020). In response to the shaded environment, Arabidopsis leaves undergo morphological changes, including upward bending of the cotyledon and leaves or hyponasty, inhibition of leaf blade expansion and enhanced leaf flattening (Roig-Villanova & Martínez-García, 2016). Hyponasty heightens the position of the leaf lamina, removing it from the shade of neighbours. This combination of altered leaf morphologies is thought to increase photosynthetic activity overall. Exposure to shade also significantly accelerates flowering in Arabidopsis to quickly complete their life cycle and enhance reproductive success (Dorn *et al.,* 2000). Together, the phenotypic changes in response to shade are called shade-avoidance syndrome (SAS).

Characterisation of SAS traits in different plants is a prerequisite, as not all SAS traits are observed in every crop that displays shade-avoidance. SAS traits are known to be species-specific and sometimes even ecotype-specific (Botto & Smith, 2002; Schmitt *et al.,* 2003; Botto, 2015; Demotes Mainard *et al.,* 2016). For example, in *Medicago sativa* L., typical elongation is observed and surprisingly accompanied by delayed flowering (Lorenzo *et al.,* 2019). Moreover, many studies indicate associations between different SAS traits (Weinig, 2000; Heuvelink *et al.,* 2004; Ta *et al.,* 2020). For example, Weinig (2000) showed that leaf elongation under shade indirectly affects plant biomass and fitness. Therefore, it is essential to examine more than one trait when attempting to understand SAS mechanisms in plants.

2.2.2. Important regulatory pathway of SAS

Over the last 20 years, researchers have made considerable efforts to understand the molecular network underlying SAS using Arabidopsis as a model system. The response to a low R:FR is initially perceived and transduced through sophisticated sensory photoreceptors. Phytochrome B (phyB) is the primary photoreceptor for perceiving R:FR (Quail, 2002), whereas phyA, cryptochromes (crys) and UVR8 are thought to enhance or repress SAS (Martınez-Garcıa *et al.,* 2014; Franklin, 2016; Yang *et al.,* 2018). A recent summary of each photoreceptor's role regulating SAS is in Küpers *et al*. (2020). The interactions between photoreceptors and phytochrome-interacting factors (PIFs) are the main switch for activating a downstream SAS signalling cascade. PIFs act as signal integration hubs and control plant developmental responses to shade by directly or negatively regulating shade-

transcription regulators (Li *et al.,* 2012; Leivar & Monte, 2014; de Wit *et al.,* 2016). These shade-regulator genes are mostly related to auxin biosynthesis and signalling genes, which are important for elongation growth under low R:FR (Hornitschek *et al.,* 2012; Reed *et al.,* 2018; Tavridou *et al.,* 2020). In addition to auxin, other phytohormones, including gibberellins (GAs), brassinosteroids (BRs) and ethylene (ET), are essential for a plant's response to shade. Detailed overviews of the connection between these phytohormones and SAS are in Stamm & Kumar (2010), Yang & Li (2017) and Wang *et al*. (2020). Further work is required to determine whether similar mechanisms occur in other plants.

Characterising natural variation in crops, like shade-tolerant responses, is essential for a more comprehensive understanding of such processes, as plants increase their plasticity of some traits to adapt to shade. However, this topic has not received much attention, and the regulatory network of shade tolerance is not well-understood (Valadares & Niinemets, 2008; Gommers *et al.,* 2013). A recent transcriptomic study demonstrated the important roles of three regulators [FERONA (FER), THESEUS1 (THE1) and KIDARI] that promote adjustments in petiole elongation between two Geranium species with contrasting responses to shade (Gommers *et al.,* 2017). Using Arabidopsis and its shade-tolerance relative, *Cardamine hirsuta* L., several studies have explored contrasting mechanistic and regulatory adjustments under different light conditions (Hay *et al.,* 2014; Molina-Contreras *et al.,* 2019; Paulišić *et al.,* 2020; Strauss *et al.,* 2020). The findings indicate that the stronger phyA activity and more stable HFR1 protein in *C. hirsuta* suppress elongation growth when the plant is exposed to low R:FR (Molina-Contreras *et al.,* 2019; Paulišić *et al.,* 2020). *C. hirsuta* also produces 20% more photosynthetic pigment content than Arabidopsis when R:FR is low. This finding indicates that shade-tolerant species can better maintain photosynthesis activity under such conditions (Molina-Contreras *et al.,* 2019).

It is important to note that Arabidopsis, as a shade-avoidance plant model, displays variation in hypocotyl elongation, flowering time responses and reproductive traits under different R:FRs (Botto & Smith, 2002; Botto, 2015). Many studies have identified the genetic basis for various SAS traits using the natural variation in Arabidopsis ecotypes via various molecular techniques, including quantitative trait locus (QTL) mapping and genome-wide association studies (GWAS) (*e.g.,*Yu *et al.,* 2008; Jiménez-Gómez *et al.,* 2010; Coluccio *et al.,* 2011; Filiault & Maloof, 2012; Schwartz *et al.,* 2017). Recently, Ta *et al*. (2020) found 17 QTL for four important SAS traits—bolting time, rosette size, inflorescence growth rate and inflorescence size—using seven nested association mapping populations of Arabidopsis. The researchers also used path analysis to characterise colocalising QTL to separate the direct effects of allelic series from the indirect effects. Their findings highlight the importance of studying the relationships among traits to understand complex developmental responses. Knowledge on the natural variation in different shade response strategies in the plant

model is valuable for expanding our understanding of the genetic architecture of plant responses to dense vegetation.

2.2.3. Considering phenotypic plasticity to understand plant responses to shade

Any evaluation of a species evolutionary and ecological dynamics or its resilience to unpredictable environments requires an understanding of how the species adapts to changing light environments. Such flexibility for an individual genotype displaying contrasting phenotypes in response to different environments is known as phenotypic plasticity (Bradshaw, 1965; Sommer, 2020). Phenotypic plasticity is an adaptation strategy that allows organisms to maximise their fitness under contrasting ecological conditions (Kusmec *et al.*, 2018). Phenotypic plasticity is believed to instigate adaptive divergence that leads to genetic variation in a species, known as the 'plasticity first' evolution theory (Levis & Pfennig, 2016). However, not all plastic traits are adaptive; some are maladaptive traits (Fox *et al.*, 2019). For example, SAS traits hinder plant productivity as stems and harvestable organs engage in resource allocation competition (Roig-Villanova & Martinez-Garcia, 2016). Information on evolutionary dynamics could be gained by identifying the extent to which and circumstances under which SAS traits facilitate species persistence or limit its ability to adapt. Some reports indicate that reduced plasticity is selected in breeding for stable yields when crops encounter high planting density in the field; thus, it is a common crop-breeding target in modern agriculture (Carriedo *et al.*, 2016; Wille *et al.*, 2017).

The degree of plasticity can range from zero (indicating a stable phenotype) to one (indicating a plastic phenotype). Phenotypic plasticity is shaped by genetics, the environment and their interaction, genotype by environment (G×E). To elucidate each of these plasticity components, the study of plasticity benefits from high-throughput phenotyping, a suitable mapping population, a wide range of environments and robust statistical frameworks (Arnold *et al.*, 2019). A reaction norm is commonly used as a framework to express phenotypic plasticity (Via *et al.*, 1995). As Figure 2.2 illustrates, the direction and magnitude of phenotypic response to environments can be examined (Figure 2.2A), as can genetic variation and G×E interactions (Figure 2.2B). Reaction norms can be used to develop frameworks for comparing plasticity across genotypes, populations, or species, as shown by Bakhtiari *et al.* (2019).

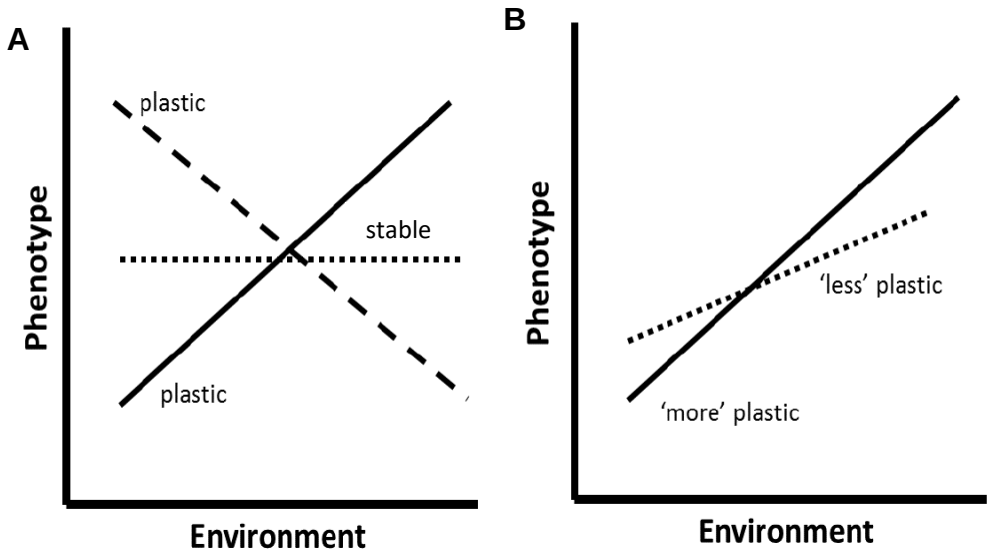

Figure 2.2: Illustration of a reaction norm representing the degree of phenotypic plasticity: **A**, Plastic phenotype vs. stable phenotype; **B**, 'less' plastic phenotype *vs* 'more' plastic phenotype as identified from slope values. Figure source: adapted from Bongers (2017).

2.3. Sclerotinia rot – A serious yield constraint in many crops

2.3.1. *Sclerotinia sclerotiorum*

Sclerotinia rot (SR), caused by *Sclerotinia sclerotiorum*, is among the most impactful non-specific diseases worldwide. SR attacks more than 600 different host plants, including important oilseed crops such as canola, soybean and sunflower (*Helianthus annuus* L.) (Del Río *et al.,* 2007; Peltier *et al.,* 2012), causing substantial economic damage, with up to 80% yield losses reported for canola (Mei *et al.,* 2011). In addition to its direct impact on yield, this disease affects crop oil content and quality by changing its fatty acid profile (McCartney *et al.,* 1999). The pathogen can survive in soil for many years in fungal resting structures known as sclerotia. Sclerotia germinate to produce apothecia under favourable conditions, *e.g.,* when soil water potentials of 100 kPa are reached at temperatures of 10–25°C (Clarkson *et al.,* 2004). Furthermore, apothecia are usually formed in crops when the canopy closes because shading keeps high soil moisture (Bolton *et al.,* 2006). The primary inoculum for SR is windborne ascospores released from apothecia. These spores typically infect plants during and after flowering, as the prolonged moist and humid conditions in lower canopies at that stage support the growth of *S. sclerotiorum* (Moellers *et al.,* 2017). Stem rot begins as a soft, water-soaked lesion, often first appearing at the base of the main stem (Nelson & Lamey, 2000). Eventually, the cankers girdle the stems. Thereafter, the affected stems are bleached, ripen early and tend to break and lodge.

Sclerotinia sclerotiorum is a hemibiotrophic pathogen that reacts as a biotrophic pathogen for a brief period during early infection before advancing to a necrotrophic phase (Kabbage *et al.,* 2015; Liang & Rollins, 2018). During the biotrophic phase, the fungus suppresses and subverts host defence

resistance, partly facilitated by the secretion of oxalic acid and various effectors (Zhu *et al.,* 2013; Nováková *et al.,* 2014). Further, the pathogen uses large numbers of chemical arrays, such as cellulases, peptidases and toxins, to rapidly trigger plant cell death and support the necrotrophic phase (Derbyshire *et al.,* 2017). Remarkably, instead of producing different quantities of secretomes, *S. sclerotiorum* has an effective splicing machinery system that can modify a single secretome to a differently expressed isoform with altered functions (Ibrahim *et al.,* 2020).

2.3.2. Plant response to SR

The primary methods for managing SR in the field are based on agronomic practices, such as rotation with non-host crops and application of foliar fungicides. Due to sclerotia's persistence in the soil and its broad host-range, crop rotations with a non-host crop alone is often ineffective in managing SR (Beulé *et al.,* 2019). Fungicides, such as demethylation inhibitors and dicarboxamides, effectively control *S. sclerotiorum* by inhibiting protein biosynthesis (Derbyshire & Denton-Giles, 2016). However, aside from its negative impact on the environment, the application of fungicide is expensive for relatively low-input farming systems, such as those in Australia. Therefore, developing resistant cultivars is the most effective strategy for managing the disease.

To date, no plant varieties have exhibited high levels of resistance to SR (Wang *et al.,* 2019a). Plants exhibit a continuum in their response to *S. sclerotiorum* rather than a clear separation between resistant and susceptible responses, known as quantitative disease resistance (QDR) (Perchepied *et al.,* 2010; Roux *et al.,* 2014). QDR is a complex trait involving numerous genes from diverse families with small to moderate effects and is often controlled by environmental factors (Roux *et al.,* 2014; Corwin & Kliebenstein, 2017). As such, QDR makes it difficult to precisely and robustly quantify phenotypic variation (Peltier *et al.,* 2012). So far, a series of QDR loci has been identified (Table 2.1). These candidate genes are involved in cell wall development, hormone signalling (jasmonic acid (JA) and ET) and sugar allocation (Guo & Stotz, 2007; Perchepied *et al.,* 2010; Williams *et al.,* 2011; Kabbage *et al.,* 2013; Badet *et al.,* 2019; Wang *et al.,* 2019a,b). A recent transcriptomic analysis revealed that up to 48,910 candidate QDR genes were differentially expressed upon *S. sclerotiorum* inoculation in six plant species (Sucher *et al.,* 2020; Table 2.1). This indicates that the genetic architecture of QDR to *S. sclerotiorum* remains largely unexplored.

The discovery of distinct pathotypes with host resistance being pathotype dependent has spurred the search for sources of host resistance that are pathotype-independent (Ge *et al.,* 2012). In Brassicaceae, pathotype-independent resistance has been identified within breeding populations of canola and *Brassica juncea* (L.) from India and China (Barbetti *et al.,* 2014), making them ideal sources of resistance to target and exploit in developing new commercial cultivars with more effective resistance

to SR across multiple pathotypes of this pathogen. Most studies attempting to identify a complex interaction between this pathogen and plants have been done in controlled environments in full light (R:FR>1). I anticipate that the investigation of the plant–*S. sclerotiorum* pathosystem under shade will substantially enhance our understanding of this complex interaction.

2.3.3. The Camelina–*S. sclerotiorum* pathosystem

As a Brassicaceae family member, Camelina is host to various fungal diseases that threaten other cruciferous crops, such as blackleg, SR, clubroot, Alternaria blight and downy mildew (reviewed in Séguin-Swartz *et al.,* 2009). Interestingly, Alternaria blight caused by *Alternaria brassicae* (Berk.) Sacc. and blackleg caused by *Leptosphaeria maculans*—two fungal diseases of great importance to *Brassica* crop production worldwide—are missing from Camelina disease reports (Jejelowo *et al.,* 1991; Séguin-Swartz *et al.,* 2009). Regarding *S. sclerotiorum,* Camelina lines exhibit varying degrees of resistance that are typical of the QDR phenotype. In one field trial, six Camelina cultivars and seven breeding lines from Denmark and Germany did not significantly differ in their resistance to SR (Föller & Paul, 2002). However, Eynck *et al.* (2012) found variable resistance to this disease when 26 Camelina lines were tested. This result raises the possibility of developing a cultivar resistant to SR. Similarly, Purnamasari *et al.* (2015) showed variable resistance among 30 Camelina lines at the cotyledon stage. The frontline of Camelina defence against *S. sclerotinia* consists of physical barriers such as cell walls and the rapid deposition of lignin-like material around the inoculation site in resistant cultivars (Séguin-Swartz *et al.,* 2009). Enyck *et al.* (2012) showed that SR-resistant Camelina plants strengthen their cell walls by increasing enzyme production to synthesise more lignin monomers. Furthermore, Purnamasari *et al.* (2015) exhibited that the resistance of Camelina to *S. sclerotiorum* does not depend on its phytoalexin production, camalexin. So far, there are no reports on genetic factors controlling the Camelina response to SR.

Table 2.1: List of recent studies on the plant defence mechanism to *Sclerotinia sclerotiorum*.

Host	Plant subjects	Fungal strain	Genetic approach	Molecular pathway/QTL/ genes discovered	Reference
Arabidopsis	100 Arabidopsis inbred lines	Strain 1980	GWAS	Actin-Related Protein Complex isoform 4 (ARPC4)	Badet *et al.*, 2019
	NA	Strain 1980	RNA-seq	Pathogen and abiotic stress response, cadmium tolerance, disordered region-containing (PADRE) genes	Didelon *et al.*, 2020
Canola	347 lines	NA	GWAS & RNA-seq	17 loci, 24 candidate genes, including a tau class glutathione S-transferase (GSTU) gene	Wei *et al.*, 2016
	448 lines	Strain SS-1	GWAS	Three loci: DSRC4, DSRC6 and DSRC8, including 39 candidate genes	Wu *et al.*, 2016
	244 F10 RILs from 888-5 x M083	NA	QTL	30 QTL, 33 candidate genes	Zhang *et al.*, 2019
	150 ZD-DH double haploid lines	Strain SS-1	QTL	4 QTL	Wu *et al.*, 2019
	181 DH lines from J964 x J902	NA	QTL & RNA-seq	17 QTL, 36 candidate genes	Qasim *et al.*, 2020
Brassica juncea - *Brassica fruticulosa* (L.) introgression lines (ILs)	206 ILs	Strain PAU-4	Association mapping	10 marker traits associations	Rana *et al.*, 2017
	88 ILs	Strain PAU-4	GWAS	13 loci, 20 candidate genes	Atri *et al.*, 2019
B. juncea – *Erucastrum cardaminoide* (L.) ILs	96 ILs	Strain PAU-4	GWAS	6 loci	Rana *et al.*, 2019
Common bean (*Phaseolus vulgaris* L.)	14 RIL populations	Strain T001.01	QTL	9 QTL, 5 candidate genes, including cell wall receptor kinase, coronatine-insensitive	Vasconcellos *et al.*, 2017

Host	Plant subjects	Fungal strain	Genetic approach	Molecular pathway/QTL/ genes discovered	Reference
				protein 1 (COI1), ethylene responsive transcription factor, peroxidase and MYB transcription factor	
Pea (*Pisum sativum* L.)	282 pea lines	Strain Scl02-05	GWAS & RNA-seq	Glutathione S-transferase (GST)	Chang *et al.*, 2018
Soybean	474 lines from Groups I, II and III maturity	NA	GWAS	58 loci, 57 candidate genes	Moellers *et al.*, 2017
	405 lines	Strain 105HT	GWAS, RNA-seq and Genomic selection	27 loci	Wen *et al.*, 2018
	185 lines	NA	GWAS	Six SNPs from three major effects genes and eight SNPs from four minor effects genes, including Glyma.18G012200	Sun *et al.*, 2020
	127 soybean lines	Strain NB-5	Genome-wide association mapping (GWAM)	Glyma.01 g048000	Boudhriou a *et al.*, 2020
Sunflower	106 F7 RILs population from HA 441 ×RHA 439	Strain NEB-274	QTL	6 QTL	Talukder *et al.*, 2016
	114 RILs from PAC2 ×RHA266	NA	QTL	36 QTL	Zubrzycki *et al.*, 2017
	Three sunflower ILs: HA89, HA853 and RK416	NA	QTL & RNA-seq	5 genes located near QTL	Fass *et al.*, 2020
Pentapetalae (common bean, castor bean, Arabidopsis, s unflower, tom ato and sugar beet)	Arabidopsis Col-0 , tomato Ailsa Craig, sunflower XRQ, *Ricinus communis* cv Hale PI 642000, beet subsp vulgaris PI 355961 and common bean G19833	Strain 1980	RNA-seq	159 orthogroups featuring genes upregulated by *S. sclerotiorum* in all six plant lineages, including ABCG40 gene	Sucher *et al.*, 2020

2.4. Shade-avoiding plants are susceptible to fungal infections

2.4.1. Profound yet understudied impact of shade in plant–pathogen interactions

The microclimate of the infection site affects the plant host, the pathogen and their interaction. Shady microclimates conducive to disease development not only lower irradiance but also lower temperature and increase humidity, asserting distinct selection pressure on the pathogen. While a plethora of studies provide evidence for the impact of temperature and humidity on disease outcome (Rees *et al.,* 2007; Kazan & Manners, 2011; Lee *et al.,* 2016), the effect of shade *per se* is not as well understood beyond mutant studies on model plants. R:FR could be used to better understand this effect as plants can elicit different defence responses when growing under varying R:FR (Shibuya *et al.,* 2011; Cargnel *et al.,* 2014; Ballaré & Pierik, 2017).

Shade-avoiding plants appear to be predisposed to pathogen infection and/or pathogens have adapted to such conditions (Ballaré & Pierik, 2017). For example, the cell walls of these plants are extended and become thinner under shade. Such changes weaken the plant's first layer defences (Shibuya *et al.,* 2011). PhyB can directly regulate several cellulose synthase (CESA) genes, which synthesise cellulose components in the cell wall (Bischoff *et al.,* 2011). This change in morphology is accompanied by decreased production of phenolic compounds, glucosinolates, latex and volatile organic compound emissions (Agrawal *et al.,* 2012; Kegge *et al.,* 2013; Cargnel *et al.,* 2014; Ballaré & Pierik, 2017). Furthermore, shade affects each line of the plant defence pathway (see Section 2.4.2).

In general, alterations to plant environmental, physiological and molecular networks under shade appear beneficial for pathogens. For example, a low R:FR increases carbohydrate levels in plants to generate defence mechanisms; for example, to activate defence genes and produce secondary metabolites (Bolouri Moghaddam & Van Den Ende, 2012; de Wit *et al.,* 2018; Courbier *et al.,* 2020). However, pathogens can use this sugar as their primary nutrient source (Lapin & Van den Ackerveken, 2013). A recent study by Courbier *et al*. (2020) showed that tomato phyB1phyB2 mutants enhanced glucose and fructose levels in their leaflets, increasing the carbohydrate content to accelerate the growth of *Botrytis cinerea* Pers., *Pseudomonas syringae* pv. *syringae* and *Phytophthora infestans* (Mont.) de Bary in an FR-enriched environment. Therefore, it is not surprising that pathogens have developed mechanisms that enable them to use the effect of SAS to hijack plant cell defence systems. For example, *Hyaloperonospora arabidopsidis* (Gaeum). Weiss & Oberw, the causal agent of downy mildew disease on Arabidopsis, produces the HaRxL106 effector, causing plants to develop shade-avoidance elongation under full light (Wirthmueller *et al.,* 2018). Notably, this effector targets RADICAL-INDUCED CELL DEATH1 (RCD1) that, together with Mut9-like kinases (MLKs), forms a nuclear hub integrating light signalling and pathogen attacks to suppress

salicylic acid signal transduction. Furthermore, fungal phytochromes might help pathogens recognise low R:FR conditions, enabling them to locate stressed and 'susceptible' host targets (Schumacher & Gorbushina, 2020). Overall, these studies highlight the importance of studying the impact of shade on plant defence and breeding for resistance in the relevant microclimate.

2.4.2. Molecular network that regulates plant defence under shade

Understanding the cause and functionality of reductions in plant defence mechanisms under shady conditions is vital for enhancing plant adaptation in the field, especially in intercropping systems. The relationship between growth and defence under shade relies on highly tuned complex signalling networks that involve crosstalk between light and defence signalling pathways (Ballaré & Austin, 2019). The light signalling network is described in Section 2.2.2 of this chapter. The defence molecular network under full light involves a series of defence events. When pathogens breach the plant's physical barrier, they trigger the plant's second level of defence, called pattern-triggered immunity (PTI), which comprises the recognition of and response to common pathogen-associated molecular patterns (Zipfel, 2014). This response encompasses a broad-spectrum resistance strategy that includes mitogen-activated protein kinases cascade activation, cell wall fortification, stomatal closure and reactive oxygen species (Chisholm *et al.,* 2006; Bigeard *et al.,* 2015; Ye *et al.,* 2020). When this immune response is defeated, resistant plants initiate the next level of defence: effector-triggered immunity. This line of defence is activated once the plant detects pathogen effectors using nucleotide-binding leucine-rich repeat receptors (NB-LRRs) (Dangl *et al.,* 2013; Stotz *et al.,* 2014). Effector-triggered immunity responses include the induction of tightly organised hormonal signalling molecules (*e.g.,* salicylic acid (SA), jasmonic acid (JA) and ethylene (ET)), accumulation of pathogenesis-related proteins and often rapid local programmed cell death (Tsuda & Katagiri, 2010; Robert-Seilaniantz *et al.,* 2011; Gururani *et al.,* 2012). Some details of the molecular mechanisms by which shade influences each defence mechanism layer remain unknown. This section summarises the current understanding of the crosstalk between growth and molecular defence mechanisms under shade, with the caveat that most of the collective knowledge on the plant defence molecular network came from mutant studies with few genotypes.

Pattern-triggered immunity response under low R:FR

Although the exact impact of shade on PTI-defence activation is unknown, a recent transcriptomic study by Gommers *et al.* (2017) indicates the possibility of a direct link between these two components. Using two Geranium species with different responses to light quality, the researchers showed that a low R:FR specifically upregulates two receptor-like kinases—THE1 and FER—in shade-avoiding species. These two proteins regulate cell elongation through the PIFs–auxin

independent pathway and aiding PTI-mediated defence (Stegmann *et al.,* 2017; Qu *et al.,* 2017; Guo *et al.,* 2018). Other mechanisms, such as hormonal crosstalk under shade, might also connect shade-avoidance growth to PTI-mediated defence (reviewed in Huot *et al.,* 2014). For example, low R:FR increases brassinosteroids (BRs), which negatively regulate PTI through BZR1-regulated transcription factors (Lozano-Durán *et al.,* 2013). Further research is required to establish growth and defence crosstalk in PTI-mediated defence.

Jasmonic acid-mediated defence under low R:FR

Perhaps the most comprehensive understanding of growth-defence crosstalk under shade is related to how growth hormonal signalling affects JA-mediated defence. A low R:FR reduces bioactive JA accumulation in various plants, such as Arabidopsis, lima beans (*Phaseolus lunatus* L.) and *Asclepias syriaca* L. (Moreno *et al.,* 2009; Radhika *et al.,* 2010; Agrawal *et al.,* 2012). Recently, the inactivation of phyB under shade directly transformed active JA compounds into inactive HSO4-JA molecules by activating sulfotransferase 2A (ST2a) by PIFs (Fernández-Milmanda *et al.,* 2020). Shade also weakens JA-mediated defence through crosstalk between JA and GA. A low R:FR induces GA biosynthesis, which promotes elongation growth. This reaction, in turn, triggers the proteasome-mediated degradation of DELLA, which is the JASMONATE ZIM-domain (JAZ) and PIFs repressor proteins (Figure 2.3) (Djakovic-Petrovic *et al.,* 2007; Hou *et al.,* 2010; Yang *et al.,* 2012; Leone *et al.,* 2014). DELLA removal releases JAZ proteins, which inhibit MYCs transcription factor and inactivate a downstream of JA-defence responses (Hou *et al.,* 2010; Pieterse *et al.,* 2014). GA-insensitive gai1 mutants increase resistance to *A. brassicicola* and *B. cinerea* by enhancing JA-responsive gene expression (Navarro *et al.,* 2008). The limited accumulation of MYCs is thought to be the main factor compromising a plant's ability to defend itself and exhibit SAS (Chico *et al.,* 2014; Li *et al.,* 2020). In line with this observation, enhanced stability of JAZ10 has been observed in seedlings exposed to an FR-enriched environment and in the phyB mutant background (Leone *et al.,* 2014). However, several studies have also observed that FR light is a positive regulator for a different subset of JA-inducible defences, including defences linked to the wound response gene VSP1 and JA signalling gene MYC2 (Cipollini, 2005; Robson *et al.,* 2010). These findings indicate that plants still attempt to fight against pathogens in shady environments.

Plants appear to have mechanisms that limit excessive growth and repression of their defence systems when under shade. Recently, low R:FRs induced the stabilisation of two novel repressors of SAS: *FAR-RED ELONGATED HYPOCOTYL3* (*FHY3*) and *FAR-RED-IMPAIRED RESPONSE1* (*FAR1*) (Liu *et al.,* 2019). FHY3/FAR1 modulates the expression of PIF negative regulators PAR1/PAR2 to prevent excessive growth under shade (Figure 2.3). JAZ can also bind directly to the FHY3/FAR1-PAR1 complex to control the repressive function of FHY3 and FAR1 on elongation growth.

Interestingly, FH3/FAR1 can interact with MYC2 to activate JA-defence gene expression. Mutants with decreased FHY3/FAR1 activity weakened the expression of PAR1/PAR2 and JA-responsive defence genes (Liu *et al.*, 2019), resulting in excessive elongation, weakened defence mechanisms and disrupted balance between growth and defence. The participation of FHY3/FAR1 in modulating the balance between a plant's defence and growth mechanisms under shade means that phyA might be part of some unknown mechanism because these two regulators are essential components of phyA transportation and subsequent signalling processes (Lin *et al.*, 2007).

Salicylic acid-mediated defence under low R:FR

Besides attenuating the JA-defence mechanism, low R:FR conditions also weaken SA-mediated defences, although little is known about how this occurs. The phyB mutant reduces the accumulation of pathogenesis-related 1 (PR1) protein in Arabidopsis (Genoud *et al.*, 2002) and rice (Xie *et al.*, 2011), which increased their susceptibility to *Pseudomonas syringae* and *Magnaporthe oryzae* B.C. Couch, respectively. Additional FR light translocates and inhibits the phosphorylation of NONEXPRESSOR OF PATHOGENESIS-RELATED GENES 1 (NPR1) (de Wit *et al.*, 2013). The binding of SA to NPR1 leads to the turnover of this protein by phosphorylation and SUMOylation, which is essential for the expression of downstream SA immunity genes (Saleh *et al.*, 2015). Recently, Nozue *et al*. (2018) showed that SA-regulated genes are essential for promoting SAS elongation upon FR exposure in adult plants. The authors suggested that a low R:FR alters NPR1 functioning due to protein modifications related to the control of the plant growth–defence response. The framework of growth–defence crosstalk in adult plants is provided in Figure 2.3.

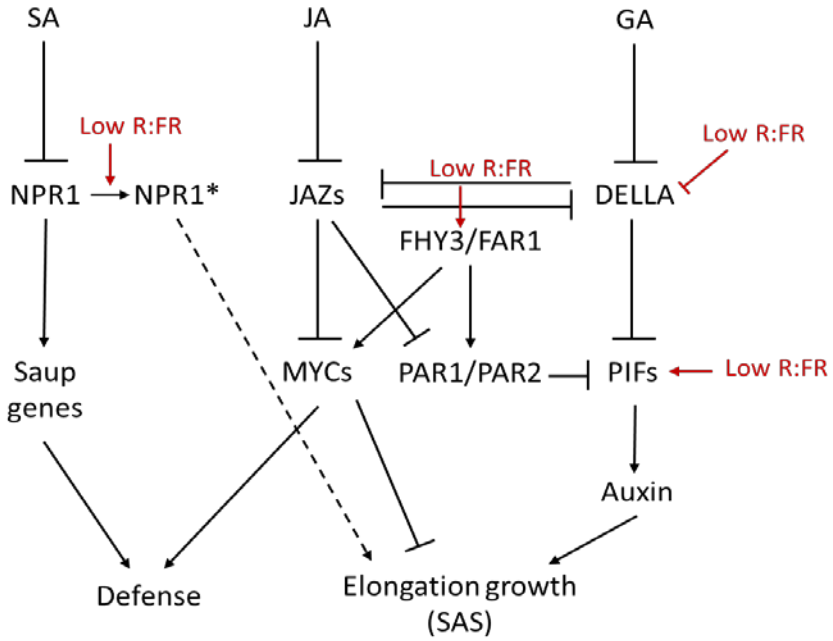

Figure 2.3: The effect of low R:FR on the plant growth–defence signalling network. Figure source: Adapted from Nozue *et al*. (2018).

Other molecular regulation under low R:FR

As shown in the above section, current knowledge has revealed several key components of plant response to diseases, including JAZs, MYCs, FHY3/FAR1 and NPR1, under shade. Many other plant hormones, including auxins, ethylene, cytokinins and BRs, are crucial for mediating growth and defence responses under shade (reviewed in Ballaré, 2014; Yang & Li, 2017). However, the genetic basis of other hormones has not been studied as extensively as those of JA and SA. Therefore, more studies on integrating these hormones in regulating growth–defence crosstalk in plants under shade will provide additional, much-needed insights. Furthermore, elucidating how plants regulate the basal defence response, PTI sensing and signalling under shade will improve the overall understanding of how plants coordinate their signalling pathways to respond to diseases under restrictive conditions.

Exploiting natural variation in Arabidopsis would enhance our knowledge on the existing layered regulation of signalling and could lead to the discovery of a novel molecular pathway of plant plasticity to shade and disease. Several studies have reported that shade-tolerant species can maintain their normal defence levels under low R:FR and some even display enhanced resistance (Kobe & Coates, 1997; Viola *et al.*, 2010; Gommers *et al.*, 2017). However, studies using natural variations are limited. The first such study by Gommers *et al.* (2017) revealed that JAZ3 exhibited higher induction in shade-avoiding *Geranium pyrenaicum* Burm. f. than shade-tolerant *Geranium robertianum* L., which reduced JA-mediated defence expression. This result confirms the findings that emerged from the mutant analysis, suggesting that a similar mechanism of the cellular network operates across species. In a recent study, Ranade & Garcia-Gil (2020) observed greater upregulation of lignin pathway genes in shade-tolerant Norway spruce than shade-avoiding Scott pine. The researchers linked this outcome to the species' different immune responses. Beyond these two studies, little is known about the molecular mechanisms of shade-tolerant plants in response to disease under low R:FR. This is unfortunate because many studies show that plant species that can tolerate shade have evolved transcriptional regulatory networks to respond appropriately to low light and disease (Kobe & Coates, 1997; Valladares & Niinemets, 2008; Viola *et al.*, 2010; Gommers *et al.*, 2017). For example, maintaining photosynthesis activity could be why defences are activated in shade-tolerant plants, as other studies have highlighted this ability as an essential component of defence (Zhang *et al.*, 2013; Mitra & Baldwin, 2014).

2.4.3. Functional explanation for the suppression of defence under shade

Much uncertainty remains regarding why plants repress their defences when in shade. It is well-established that the crosstalk between growth and defence under shade is not due solely to resource constraints (reviewed in Züst & Agrawal, 2017; Ballaré & Austin, 2019). There are two proposed

reasons for the phenomenon. First, phyB mutation leads to the full recovery of jazQ (quintuple *JAZ*) or JAZ10 mutant growth, indicating that growth and defence are genetically uncoupled (Campos *et al.,* 2016; Cerrudo *et al.,* 2017). Second, the Sav3 mutant (fails to induce SAS but has normal phyB function) increases a plant's susceptibility to diseases under shade (Moreno *et al.,* 2009; Cerrudo *et al.,* 2012). In addition, plants that develop SAS due to a cry1 mutation do not exhibit increased susceptibility to *B. cinerea* infections (Cerrudo *et al.*, 2012).

Many prominent researchers have presented theories that explain why plants weaken their defences under shady conditions (Huot *et al.,* 2014; Züst & Agrawal, 2017; Ballaré & Austin, 2019). Huot *et al*. (2014) and Züst & Agrawal (2017) highlight that growth–defence crosstalk initially emerged from plant allocation decisions intended to achieve optimal fitness in restrictive environments. Recently, a comprehensive literature review by Ballaré & Austin (2019) demonstrated that growth–defence crosstalk appears to result from the direct conflict of configurational changes to maximise defence and SAS components. Ongoing efforts into the growth–defence crosstalk mechanism, including insight from shade-tolerant species, will help us to understand this issue. For example, a recent study by Major *et al.* (2020) showed that phyB mutations do not fully protect the growth phenotype of jazD mutants (mutated in 10 of the 13 JAZ genes). The inability of phyB mutants to recover jazD suggests that growth–defence crosstalk results from transcriptional networks and partially depends on strong metabolic constraints. In line with this theory, Izaguirre *et al*. (2013) showed that JA-mediated defence suppression is constrained only in the parts of a plant that are exposed to low R:FR. This observation led to the proposal of the 'self-pruning' strategy, where plants steer nutrient resources towards the most critical tissues.

2.5. Genetic tools to uncover natural variation in the plant response to pathogens under shade

Quantitative trait locus (QTL) analysis is an effective strategy for elucidating the genetic basis of complex traits. QTL mapping is a type of statistical analysis that links phenotypic variation in trait expressions to molecular markers; the genomes causing this relationship can be located using these links. The development of next-generation sequencing provides thousands of molecular markers that substantially increase QTL mapping power (Pascual *et al.,* 2016). Current limitations are that QTL requires a high number of analysed individuals, a considerable level of polymorphism between parents and repeatable phenotyping that is high-throughput and reproducible (Fu *et al.,* 2010; Pascual *et al.,* 2016). Nevertheless, many QTL studies on resistance to SR have been reported in oilseed crops other than Camelina, with the most recent research presented in Table 2.1. QTL studies have identified several SR-resistance genes, including IGMT5 (Wu *et al.,* 2013) and ARPC4 (Badet *et al.,*

2019). Such discoveries indicate the potential of QTL to improve the understanding of the regulatory mechanism of Camelina for complex traits.

While Camelina has a resistance advantage over the major fungal pathogen of *Brassica* species, available genomic studies provide minimal information on the precise mechanisms that play essential roles in conferring resistance. Scholars have suggested that the triplicated gene characteristics of Camelina explain its disease tolerance, as it increases allelic diversity, heterozygosity and fixed heterosis (Kagale *et al.*, 2014a). Studies have identified some genomic locations linked to oil content, seed yield, seed size and flowering time (Gehringer *et al.*, 2006; King *et al.*, 2019; Chaudhary & Parkin, 2020). Nevertheless, no basic genetic map related to Camelina disease resistance has been developed. Most of our knowledge of the Camelina resistance mechanisms come from comparative genomic analysis. Using this approach, Inturrisi (2018) found that Camelina has more plant resistance gene analogues [among which are NBS-LRRs, receptor-like kinase (RLKs) and receptor-like proteins (RLPs)] than the 24 other species within the Brassicaceae. Furthermore, genome-mining analyses for chitinase genes in Camelina and *Brassica juncea* have demonstrated that Camelina has more chitinase genes (47) than *B. juncea* (6) (Mir *et al.*, 2020). This study also showed that the fold-changes of chitinase genes in Camelina were more remarkable than those in *B. juncea* after *A. brassicae* inoculation. This result suggests that such fold-changes contribute to Camelina resistance. Together, these studies highlight the potential of Camelina to act as a reservoir for defence resistance genes. The available information, including that provided by transcriptomic studies and studies on reference genomes and the full genome sequence of related species, provides a powerful platform for identifying the chromosomal locations of genes that influence Camelina disease resistance mechanisms under shade.

2.6. Summary and perspective

Plants encounter different environmental factors, such as plant–pathogen and plant–plant interactions, that threaten their fitness, both in natural and agricultural ecosystems. These threats are worsened by current predictions of the effects of climate change. For example, higher temperatures due to climate change are predicted to enhance Arabidopsis hypocotyl growth under shade (Romero-Montepaone *et al.*, 2020). Traditional approaches to observing plant plasticity to different stresses have been used extensively by studying model plant adaptations to single factors. Although these studies have enhanced our understanding of this topic, there is evidence that plant responses to a combination of stresses differ from those of individual stresses (Rasmussen *et al.*, 2013; Kissoudis *et al.*, 2014; Davila Olivas *et al.*, 2017). Thus, the process of identifying critical regulatory and overlapping stress

signalling pathways involved in adaptation to multiple environmental stressors, such as defence response under shade, needs to be improved. Comparative analyses of related species could provide a framework that offers insights into plant plasticity to various environmental factors, especially in underexploited plants with low genetic diversity such as Camelina. I argue that the current molecular knowledge underlying SAS and defence under shade can be improved by incorporating natural variation. Moving forward, an increased understanding of the dynamics of plant–pathogen–environment interactions through Camelina–Sclerotinia–shade interactions will allow for improved yields on available land and in a sustainable way.

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CHAPTER 3

Genetic, phenotypic, biochemical and genome size variability in a germplasm collection of *Camelina sativa* (L.) Crantz

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3.1. Abstract

Genetic diversity assessment of a species provides important information for crop improvement programs. Here, we characterise diversity in genetic, genome size and phenotype of a germplasm panel of 31 lines of *Camelina sativa* (L.) Crantz. The germplasm was genotyped using 634 DArTseqLD SNP markers. Nei's genetic diversity indicated relatively low genetic diversity among lines (H_S = 0.017). The Bayesian model implemented in STRUCTURE suggests that the germplasm is derived from two subpopulations. The nuclear genome size (2C) (determined by propidium iodide flow cytometry analysis) varied from 1.379 ± 0.009 pg to 1.461 pg ± 0.015, with a 1.06-fold variation. The 2C values indicated low but significant intraspecific variation in genome size in the panel. Significant differences were observed between the lines in eight important agricultural and biochemical traits, including flowering time, seed size, seed weight, seedling vigour index and fatty acid composition. No clear associations were detected regarding the phylogenetic and phenotypic traits of lines of a particular genome size. The genomic and phenotypic characterisation reported in this study provides valuable information for Camelina pangenome analysis and designing experiments to uncover genotype–phenotype interactions.

3.2. Introduction

Camelina sativa L. Crantz (family Brassicaceae, tribe Camelineae), also known as gold-of-pleasure or false flax, is presumed to be native to Eastern Europe and Western Asia (Vollmann & Enyck, 2015). The species has recently received significant attention, as it is a sustainable bioenergy crop with high-value oilseed and positive agronomic traits (Campbell *et al.,* 2013; Matteo *et al.,* 2020). Archaeological, ecological and morphological evidence indicates that Camelina is a secondary crop that evolved from weedy species that infested flax and cereal cultivation (Zohary *et al.,* 2012). Most germplasm available today comprises spring types (Luo *et al.,* 2019), with few reports on winter types, despite being valued for expanding the crop's gene pool (Chaudhary *et al.,* 2020; Kurasiak-Popowska *et al.,* 2020). Germplasm collections of Camelina are maintained at the N.I. Vavilov Research Institute of Plant Industry, European Catalogue of Plant Germplasm Collection, Plant Gene Resources of Canada, USDA National Plant Germplasm System, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) and National Genetic Resources Program (NGRP) (Vollmann & Eynck, 2015; Sainger *et al.,* 2017; Kurasiak-Popowska *et al.,* 2020; Vera *et al.,* 2020). However, incomplete passport data and likely accession duplication remain an issue for researchers using these collections.

Information on intraspecific genome size variation serves to assess potential repetitive elements and complement pangenome sequencing and phylogenetic analyses, in particular for species with recent polyploidisation events (Dodsworth *et al.,* 2017; Píšová & Fér, 2020; Vitales *et al.,* 2020). The first full Camelina genome assembly was released in 2014, with an estimated genome size of 785.5 Mb (Kagale *et al.,* 2014). In other studies, Camelina genome size has been estimated at 743–801 Mb by flow cytometry (Sigareva & Earle, 1999; Hutcheon *et al.,* 2010; Martin *et al.,* 2015, 2017; Brock *et al.,* 2018). Only one study has investigated intraspecific variation in Camelina genome size, which showed insignificant variation (Brock *et al.,* 2018). Camelina genomes resulted from recent allopolyploidisation, ~5.5 million years ago, to form the current diploid 2n=40 chromosomes [CsG1x=6, CsG2x=7, CsG3x=7], with one subgenome (CsG3) showing a distinct expression level advantage over the other subgenomes (Kagale *et al.,* 2014, 2016).

Here, we use genotyping-by-sequencing, flow cytometry and phenotypic evaluation to revisit the characterisation of a set of the Camelina germplasm, including the Camelina genome reference line DH55. The germplasm used in this study comprises 30 Camelina lines, predominantly sourced from the N.I. Vavilov Institute in St Petersburg, Russia. An amplified fragment length polymorphism (AFLP) marker analysis, considerable phenotypic variation in agronomic traits and responses to Sclerotinia rot and blackleg (crucial Brassicaceae diseases) have demonstrated considerable genetic

diversity in this collection (Ghamkhar *et al.,* 2010; Campbell *et al.,* 2013; Purnamasari *et al.,* 2015, 2019). This work addresses the following questions: 1) Do genetic and genome size variations within Camelina lines allow a clear differentiation to infer phylogenetic relations? 2) If variation does exist, does it have phenotypic consequences? An evaluation of this material will provide a starting point for our investigation into genotype–phenotype associations and a basic understanding of this species' adaptive traits. Our findings are relevant to breeding programs seeking to enhance diversity within the spring types of this species.

3.3. Materials and methods

3.3.1. Plant material and growth condition

A total of 31 lines of *Camelina sativa*, representing the various geographical regions of Eastern and Western Europe, were used in this study (Table 3.1). The 30 lines were obtained from the N.I. Vavilov Research Institute of Plant Industry, Russia; prior to this study, these lines were selfed for three generations at The University of Western Australia. Seeds of Camelina DH55 were kindly provided by Dr Isobel Parkin, Agriculture and Agri-Food Canada, Saskatoon, Canada. DNA diversity and genome size were analysed for all 31 lines, while the morphology traits and biochemical analysis were measured for 29 lines (Table 3.1 and 3.2). *Vigna radiata* (L.) Wilczek 'Berken', used as the internal standard for cytometry (Bennett & Smith, 1991), was provided by Dr Colin Douglas from the Department of Agriculture and Fisheries, Qld, Australia. Camelina lines were grown at 22/18 °C under ambient light in School of Agriculture and Environment Glasshouse at The University of Western Australia (Latitude 31° 57' S, Longitude 115° 51' E). All plants were grown in 70 mm square plastic pots with a pasteurised soil mixture composed of finely crushed pine bark/coco peat/sand at 2.5:1.0:1.5 (wt/wt). Plants were watered daily and fertilised fortnightly using Thrive^{TM}, an all-purpose soluble fertiliser, at the recommended dosage of 0.09 g pot^{-1}.

3.3.2. Phenotypic traits and fatty acid composition measurement

For the 29 Camelina lines, eight agricultural and biochemical traits were characterised *viz*. time to flower (day), 100-seed weight (mg), seed size (mm), seedling vigour index and fatty acid composition [% polyunsaturated fatty acids (PUFAs), monounsaturated fatty acids (MUFAs) and saturated fatty acids (SFA)]. Flowering data was collected from June–August 2017 based on glasshouse observations when the first sepals were evident. To measure 100-seed weight, seeds were threshed and aspirated. A Contador (Pfeuffer GmbH, Germany) seed counter with No. 1 seed container was used to count five replicates of 100 seeds, which were weighted on an Ohaus IC-PA413 PioneerTM analytical balance (Ohaus, Parsippany, USA). Furthermore, images of 10 randomly chosen seeds were taken of each genotype using Olympus Z51 stereo microscope (Olympus, Tokyo, Japan) and

Olympus stream image analysis software was used to quantify seed size. The seedlings were harvested and weighed nine days after sowing. Seedling vigour index (SVI) was calculated based on dry matter production, according to a method of Chauhan *et al*. (2019), by multiplying the percentage germination for each genotype by dry shoot weight (mg). For fatty acid analysis, a representative seed sample of 29 lines was harvested and aspirated to clean. A seed sample of between 9 and 11 mg was measured for each genotype, the exact weight of the sample recorded and the sample provided to Metabolomics Australia, Perth, for fatty acid extraction and analysis. The analysis was undertaken using a gas chromatograph mass spectrometry (GC-MS) with an internal standard (C19 fatty acid). The fatty acid profiles were separated based on the retention time dimension (Agilent technologies, 2003).

3.3.3. DNA extraction and genotyping

Young leaves were collected for 31 lines, immediately frozen in liquid nitrogen and stored at –80°C until the tissue was lyophilised. Dried leaf tissue, *c.* 1 g per sample, was sent to Diversity Array Technology (DArT, http://www.diversityarrays.com/) for genomic DNA extraction and genotyping as per the DArTseqLD protocol described by Noyszewski *et al*. (2019). There was no biological replication in genotyping the lines; each line was represented by one plant and one sample per plant. DArT analysed the sequence data using the DArTsoft pipeline (DArT P/L, Canberra, Australia) to generate allele calls for single nucleotide polymorphism (SNP) markers described by Akbari *et al*. (2006). Monomorphic loci among the Camelina lines and those with missing data for more than nine lines were excluded.

3.3.4. Estimation of genome size

Nuclear genome size was estimated using flow cytometry according to Doležel *et al*. (2007) with *Vigna radiata* 'Berken' as a size reference (1C=0.53 pg; Bennett & Smith, 1991). This reference standard was chosen as our preliminary result revealed a genome size close to Camelina, thus avoiding the risk of nonlinearity and offset error (Doležel & Bartoš, 2005). Samples consisted of 10 mg of young leaves from three plants per Camelina genotype and *V. radiata*. The leaf samples were kept on ice until stored at 4°C. Each sample was tested on three different days to account for random technical errors. According to the manufacturer's recommended protocols, nuclei were isolated from leaf samples using CyStain® PI Absolute P Kit (Sysmex-Partec, Germany). Test samples of Camelina and the reference sample, *V. radiata*, were chopped together in nuclei extraction buffer (*CyStain® PI Absolute P Kit*; *Sysmex*-Partec, Germany). The nuclei suspensions were filtered and stained with propidium iodide solution (*CyStain® PI Absolute P Kit*; *Sysmex*-Partec, Germany). After at least 30 minutes of dark incubation, the DNA content in the nuclei was determined using a BD FACSCanto II (BD Biosciences, USA) flow cytometer with a 488 nm laser. The DNA content of at least 3000

stained nuclei was determined for each sample and coefficients of variation averaged below 5% for both peaks. The data were analysed using FlowJo software (Tree Star Inc., version 10). The experimental genome size was calculated based on the value of G1 phase of the cell cycle of Camelina samples and internal standard, *V. radiata*, as described in Doležel *et al*. (2007):

$$2C = \left(\frac{\text{sample G1 peak mean}}{\text{standard G1 peak mean}}\right) \times \text{standard 2C genome size (pg DNA)}$$

3.3.5. Data analysis

All statistical analyses were done using R software (version 3.3.0, R Development Core Team, 2013) in RStudio version 1.0.136 (RStudio, Boston, Massachusetts, USA). Normality and homoscedasticity of phenotypic and biochemical traits were examined with Shapiro–Wilk test ($\alpha = 0.05$). Correlations between the DNA content and eight traits of 29 lines were determined using the 'PerformanceAnalytics' package (Peterson *et al.,* 2018). 'Agricolae' package was used for one-way ANOVA to analyse 2C values and phenotypic and biochemical traits among different individuals (De Mendiburu & Simon, 2015). Fisher's least significant differences (l.s.d.) were used to test the differences between means.

Basic statistic for DArTseqLD marker analysis was calculated using the 'dartR' package in R (Gruber *et al.,* 2018). Nei's genetic diversity was calculated using the 'poppr' package (Kamvar *et al.,* 2014). For genetic relatedness analysis, the genomic pairwise relatedness between 31 Camelina lines was obtained by identity-by-state (IBS) using package 'SNPRelate' in R (Zheng *et al.,* 2012). The genetic population structure analysis was undertaken with a Bayesian model-based clustering algorithm implemented in STRUCTURE v2.3.4 (Pritchard *et al.,* 2000). Five runs were performed for each number of population (K) pre-sets from 1 to 10. The lengths of burn-in time and Markov Chain Monte Carlo replications were set to 10 000 and 500 000, with no prior information on the individuals' origin. To determine the most suitable value of K, STRUCTURE results were used and collated in STRUCTURE HARVESTER (as per Earl, 2012).

3.4. Results

3.4.1. Genetic diversity in Camelina

The germplasm used in this study was derived from that used in Ghamkhar *et al*. (2010) and Campbell *et al*. (2013) through three generations of single-seed descent. In total, 30 lines of Camelina were characterised using Diversity Arrays Technology at low density (DArTseqLD) for whole-genome profiling. The sequence data was assessed for quality to establish a draft set of 1929 SNP markers. Of these 32.9% had less than 30% missing data points and were used for the analyses. The distribution

of informative SNPs across the DH55 reference genome reflects the draft SNP set (Figure 3.1). A similar degree of polymorphism between the lines was observed in the Camelina subgenomes: CsG1 (38%), CsG2 (31%) and CsG3 (31%). The average polymorphism information content (PIC) of these SNP markers was 0.42 (range 0.23–0.50). The call rate of these markers, indicating individual marker quality, ranged from 0.8 to 1.0, with an average of 0.96.

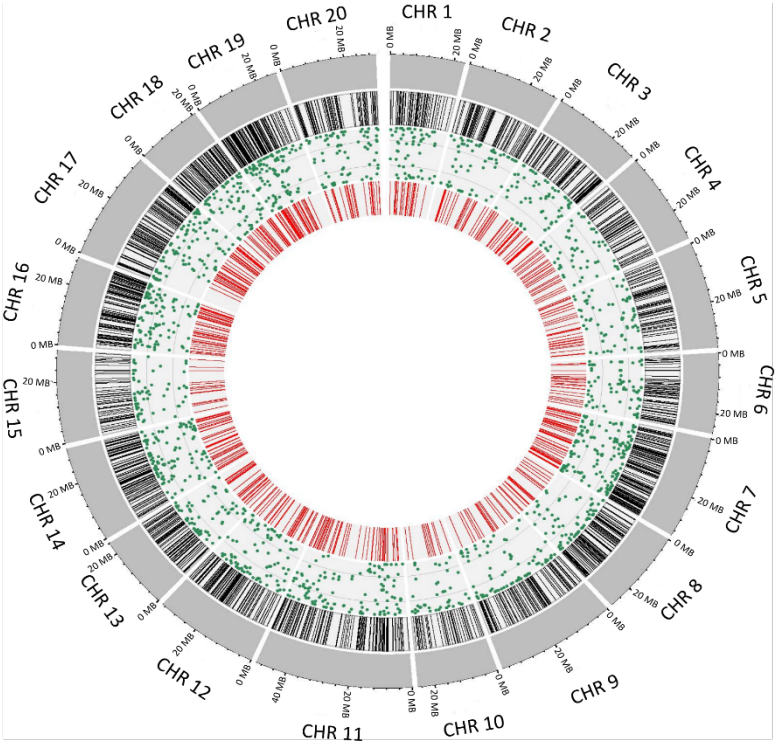

Figure 3.1: Landscape of SNP markers generated by whole-genome DArTseqLD analysis in Camelina genotypes mapped to the reference genome sequence of DH55. Moving inward from the outer ideogram are chromosome DH55 (grey), markers derived from DArTseqLD genome analysis in each chromosome (black lines), segregated value of each marker (dot point) and informative markers filtered for polymorphism and missing data points (red lines).

These markers were used to evaluate genetic diversity, relatedness and genetic structure in our Camelina panel. Nei's genetic diversity (H_S) ranged from 0.003 to 0.036, with a mean of 0.017 (Table 3.1). Genetic pairwise relatedness of Camelina genotypes was calculated among all pairs of Camelina lines based on the degree of allele sharing by IBS (Figure 3.2). Most pairs of lines had an IBS value of 0.5 to 0.6 (Figure 3.2A). The pairs of lines with the highest relatedness values (>0.95) were: C370–DH55, C235–C253, C339–C344, C349–C3347 and C2495–C2292, indicating high similarity between these pairs. There were 50 pairs of lines with the lowest relatedness values (<0.5), indicating low similarity between them. Genetic structure analysis was measured using the Bayesian clustering model implemented in STRUCTURE (Figure 3.2B). The highest value of delta K was observed at K=2 based on Evanno's test. With a value of K=2, Camelina genotypes were grouped into two

subpopulations. Most of the lines (0.58) were assigned to G2, and the reminder to G1; both groups contained admixtures (Table 3.1).

Table 3.1: Origin, nuclear DNA content and Nei's genetic diversity of 31 *Camelina sativa* lines. Genetic groups based on the STRUCTURE result and IBS value. Genetic group 1 represents the G1 and group 2 represents the G2. *P* indicates no-mixture, and *a* indicates admixture. Asterix followed by a number indicates potential duplicates based on the IBS value.

Genotype	Latitude	Longitude	Country	Nuclear DNA amount (2C value) ± SD	H_S	Genetic group
C235	N/A	N/A	Russia	1.438 ± 0.004	0.005	1p*[1]
C253	53.09 N	45.00 E	Russia	1.445 ± 0.004	0.008	1p*[1]
C339	N/A	N/A	Russia	1.411 ± 0.007	0.007	1a*[2]
C344	N/A	N/A	Russia	1.440 ± 0.006	0.010	1a*[2]
C349	N/A	N/A	Russia	1.440 ± 0.003	0.008	2p*[3]
C370	N/A	N/A	Russia	1.448 ± 0.002	0.008	1p*[4]
C403	63.30 N	44.00 E	Russia	1.429 ± 0.019	0.031	1a
C430	56.00 N	40.30 E	Russia	1.428 ± 0.007	0.015	2a
C1330	51.44 N	36.11 E	Russia	1.423 ± 0.017	0.017	1a
C1811	47.00N	3.00 E	France	1.435 ± 0.003	0.013	2p
C1993	N/A	N/A	Russia	1.438 ± 0.001	0.018	2a
C2292	53.20 N	83.45 E	Russia	1.434 ± 0.003	0.020	1p*[5]
C2305	56.00 N	93.00 E	Russia	1.446 ± 0.008	0.003	1a
C2495	58.36 N	49.38 E	Russia	1.438 ± 0.006	0.018	1p*[5]
C2504	N/A	N/A	Russia	1.420 ± 0.003	0.028	2a
C3347	51.02 N	31.53 E	Ukraine	1.436 ± 0.003	0.005	2p*[3]
C3364	49.04 N	33.25 E	Ukraine	1.442 ± 0.002	0.028	1a
C4059	N/A	N/A	Russia	1.425 ± 0.008	0.018	2a
C4068	N/A	N/A	Russia	1.461 ± 0.015	0.018	2a
C4074	N/A	N/A	Russia	1.414 ± 0.026	0.010	2p
C4077	N/A	N/A	Russia	1.424 ± 0.005	0.008	2p
C4111	N/A	N/A	Russia	1.423 ± 0.012	0.026	2a
C4112	N/A	N/A	Russia	1.386 ± 0.002	0.034	2p
C4130	N/A	N/A	Ukraine	1.410 ± 0.006	0.020	2a
C4138	N/A	N/A	Russia	1.379 ± 0.009	0.036	2a
C4139	51.00 N	40.15 E	Russia	1.412 ± 0.008	0.028	2a
C4164	N/A	N/A	Sweden	1.405 ± 0.017	0.011	2a
C4177	N/A	N/A	Russia	1.400±0.008	0.016	2a
C4182	55.19 N	89.48 E	Russia	1.414 ± 0.009	0.012	1a
C4183	N/A	N/A	Russia	1.420 ± 0.028	0.024	2a
DH55*	N/A	N/A	Former USSR	1.442 ± 0.002	0.011	1p*[4]
Mean				1.426 ± 0.020	0.017	

* DH55 is a double haploid derived from Camelina genotype SRS 933 (Kagale *et al.*, 2014)

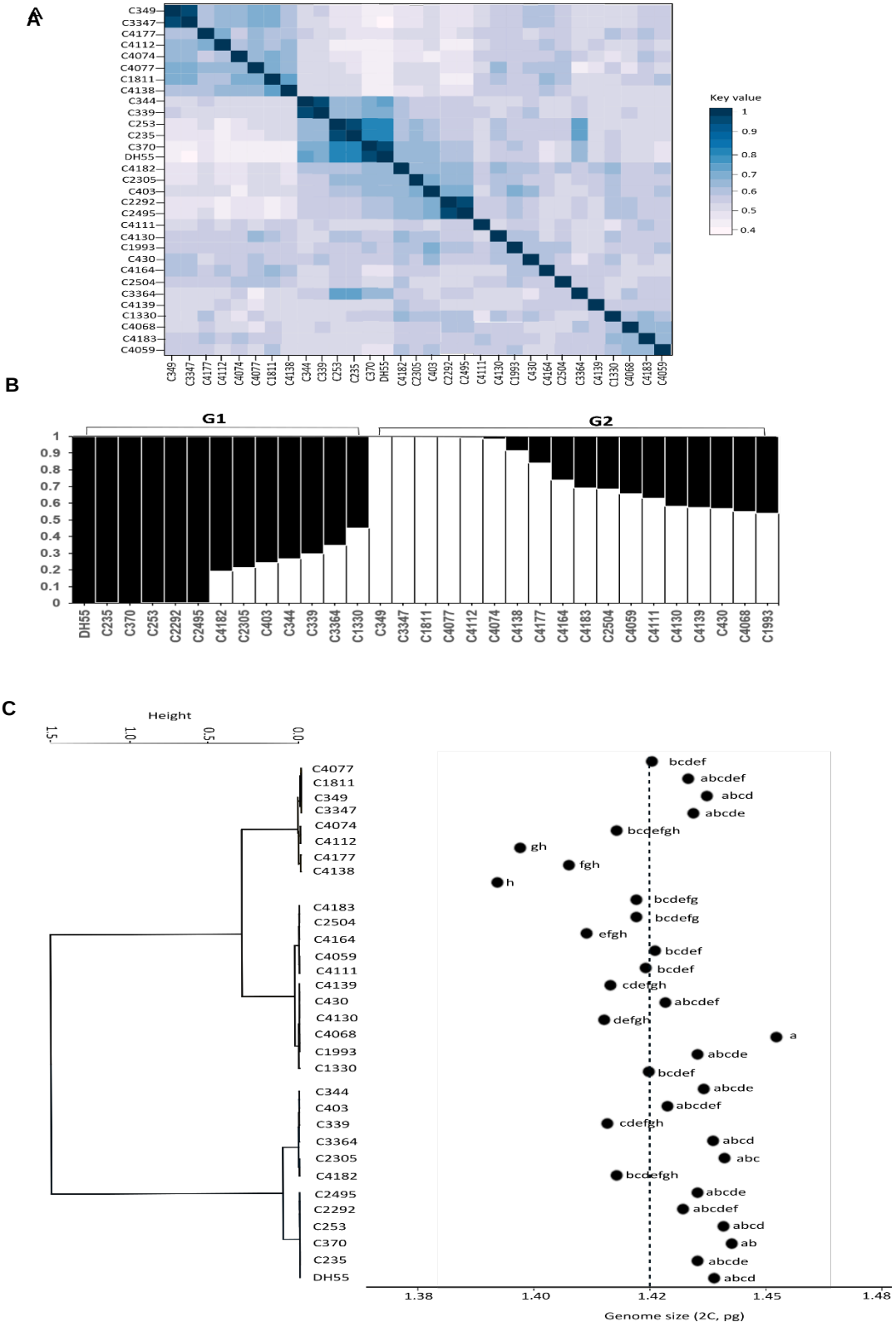

Figure 3.2: Relationships among 31 genotypes of *Camelina sativa* using DArTseqLD markers and nuclear DNA content. **A**, Genomic pairwise relatedness matrices of 31 sequenced individuals using identity-by-state (IBS) analysis. The colour intensity represents the similarity between two individuals. **B**, STRUCTURE analysis of Camelina genotypes based on DArTseqLD markers. **C**, Tree topology is based on the STRUCTURE result for nuclear genome size (2C) data represented by the accession's mean, calculated based on measurements from three replicate plants per accession, sampled each in three different days. Same letter next to dot points indicates means are not significantly different (P<0.05) using a Tukey–Kramer comparison test.

3.4.2. Intraspecific genome size variation of Camelina

To determine the genome size of 30 Camelina lines and the Camelina genome reference genotype DH55, we measured the 2C DNA amount using flow cytometry (Table 3.1). The evaluation of genome size revealed low but significant diversity among Camelina, which varied 1.06-fold among lines (P<0.05, Figure 3.2C). The 2C DNA amounts ranged from 1.379 pg in C4138 to 1.461 pg in C4068, with an overall mean 2C DNA content of 1.426 pg. The CVs and standard deviations for all samples were below 5%, indicating that the observed peaks had good quality and high precision (as recommended by Doležel *et al.*, 2007). From 31 lines, only two had less than 1.400 pg, and 22 lay within 1.400–1.440 pg.

3.4.3. Phenotypic variation of Camelina

The phenotypic and biochemical traits for 29 Camelina lines were measured to investigate whether genetic diversity and/or genome size affect the variation of key agronomic traits in this crop. Significant differences were observed among lines for all morphological traits tested in this study (Table 3.2). Flowering time ranged from 41 to 59 days post-planting ($\overline{X}$=50 days). The seed weight ranged from 38.9 to 186.4 mg ($\overline{X}$=84.6 mg) and seed size from 1.694 to 2.446 mm ($\overline{X}$=2.043 mm). The seedling vigour index (SVI) ranged from 0.7 to 2.9 ($\overline{X}$=1.5). In terms of fatty acids, polyunsaturated fatty acids (PUFAs) comprised the highest oil fraction, contributing 66–73% to total oil content, followed by monounsaturated fatty acids (MUFAs) (18–32%) and saturated fatty acids (SFA) (8–13%). The profile of fatty acid contents for Camelina lines obtained in this study was comparable to those generated for sister lines in Ghamkhar *et al*. (2010) and Campbell *et al*. (2013). For example, C4139 had the highest fraction of PUFAs in this study and Campbell *et al*. (2013), and more than 30% alpha-linolenic acid in Ghamkhar *et al*. (2010). Phenotypic corelations were estimated as Pearson correlations between each pair of traits. Seed weight and seed size were significantly correlated (r=0.59, n=29, P<0.01) (Table 3.3). Seed weight positively correlated with MUFAs and negatively correlated with SFAs. Seed size had a negative correlation with the composition of PUFAs and a positive correlation with MUFAs. There was a negative correlation between the percentage of PUFAs and MUFAs (r= –0.82, n=29, P<0.01). For example, genotype C4130 had the second-highest PUFA composition but the lowest percentage of MUFA.

Table 3.2: Phenotypic characteristics and fatty acid contents of 29 *Camelina sativa* lines.

Genotype	Flowering time (days)	100-seed weight (mg)	Seed size (mm)	Seedling vigour index	MUFAs (%)	PUFAs (%)	SFA (%)
C235	51	39	1.918	0.77	19	68	13
C253	53	107	1.915	2.30	22	69	9
C339	53	57	2.043	0.83	19	69	12
C344	59	85	2.045	0.77	22	69	9
C349	50	67	2.015	2.17	20	72	8
C370	55	50	1.919	1.84	19	68	13
C403	53	94	1.972	1.76	22	68	9
C430	51	106	1.964	1.81	24	67	9
C1330	43	95	2.297	1.50	21	70	9
C1811	52	65	1.825	0.72	21	71	8
C1993	43	126	2.249	2.63	25	66	9
C2292	43	186	2.411	1.35	26	66	8
C2305	56	86	2.175	0.70	19	72	9
C2495	49	146	2.466	2.81	32	59	9
C2504	57	123	2.062	1.03	21	70	9
C3347	49	48	1.947	1.91	19	71	10
C3364	49	88	1.935	2.05	21	70	10
C4059	51	69	2.116	0.84	21	70	9
C4068	49	66	1.888	1.20	20	67	13
C4074	52	49	1.694	2.63	20	69	11
C4077	56	70	1.916	1.60	24	65	11
C4111	51	76	2.220	0.88	20	71	9
C4112	48	59	1.752	0.83	19	69	12
C4130	54	96	2.115	1.83	18	72	10
C4138	45	78	1.875	1.15	22	70	8
C4139	51	103	1.858	0.69	19	73	8
C4164	54	65	2.249	0.91	21	68	11
C4182	41	84	2.334	2.90	20	67	12
C4183	48	69	2.086	1.83	20	68	12
***F*-pr**	0.026	<2e-16	<2e-16	<2e-16	1.49E-05	0.01	<2e-16
l.s.d *P*=0.05	6.57	3.91	0.12	0.43	4.34	5.42	1.31

Table 3.3: Correlation between phenotypic traits and fatty acid contents of 29 *Camelina sativa* lines. Mean values of each accession were used to analyse Pearson correlation coefficients (*r*), * indicates $P<0.05$; ** indicates $P<0.01$.

Traits	2C value (pg)	Seed weight	Seed size	Flowering time	MUFAs	PUFAs	SFA
Seed weight (mg)	0.11						
Seed size (mm)	0.12	0.59**					
Flowering time (days)	0.19	–0.26	–0.36				
MUFAs (%)	0.19	0.67**	0.51**	–0.22			
PUFAs (%)	–0.18	–0.33	–0.39*	0.2	–0.82**		
SFA (%)	–0.013	–0.58**	–0.22	0.04	–0.34	–0.26	
Seedling vigour index	0.38	0.26	–0.013	–0.04	0.35	–0.26	–0.21

3.4.4. Association between genome size, genetic variability and phenotypic variation

The tree-based approach of cluster analysis in STRUCTURE was used to examine the relationship between genetic diversity based on DArTseqLD markers and 2C value (Figure 3.2C). The graph revealed no association between genetic variability and genome size, as no genome size estimates

formed congruent groups. Further inspection of the data using PCA and ANOVA showed similar results. No significant correlation was observed between 2C values and morphology traits based on Pearson correlations of a subset of 29 lines Camelina (Table 3.3).

3.5. Discussion

Diverse germplasm is needed for any genetic improvement program. In this study, we characterised the genome size and DArTseqLD SNP of 31 Camelina lines originating predominantly from the centre of origin of this species. This germplasm included the reference genome line DH55. Nei's genetic diversity within the 31 Camelina lines indicates low genetic diversity, as supported by previous Camelina diversity studies (Vollmann *et al.,* 2005; Gehringer *et al.,* 2006; Singh *et al.,* 2015; Brock *et al.,* 2018; Luo *et al.,* 2019; Chaudhary *et al.,* 2020). The STRUCTURE analysis indicated that the 31 lines could be divided into two subgroups. Similar optimal K values were obtained by Singh *et al*. (2015) for 175 genotypes and Luo *et al*. (2019) for 213 Camelina genotypes. Thus, despite the relatively small number of lines in our study, our germplasm accurately represents the genetic diversity of Camelina as predicted by Ghamkhar *et al*. (2010). Chaudhary *et al*. (2020) postulated that lines originating from the Russia–Ukraine border add variation to Camelina due to the substructure contained within this population. We have incomplete passport data regarding our germplasm's exact geographical origin; however, accessions collected from this geographic region represent a possible explanation for the substantial variation in the genetic structure of our collection, as reported by Ghamkhar *et al*. (2010). The characterisation of of the genetic relationships and phenotypic traits provides additional information to guide strategies in breeding programs and research studies. For example, C2495 (largest seed size; Genetic group: 1p*[5]) and C4074 (smallest seed size; Genetic group: 2p) could be used to explore the genetic architecture responsible for increased seed size, an important breeding goal for this crop (Malik *et al.,* 2018).

Genome size measurements of 31 Camelina lines in this study revealed low yet significant intraspecific variation (1.06-fold) among individuals, as the differences were beyond the significance threshold (1.04-fold) (Doležel & Göhde, 1995). The genome size of Camelina has been determined by flow cytometry (*e.g.*, Martin *et al.,* 2015; Martin *et al.,* 2017; Brock *et al.,* 2018), but no comprehensive evaluation had been done on intraspecies variation. For example, Brock *et al.* (2018) found insignificant genome size variation (~1.02-fold) among five Camelina lines. Therefore, this is the first study to report genome size variation in Camelina. Such intraspecific genome size variation has been reported in other plants, such as *Glycine max* (L.) Merr., *Zea mays* L., *Cocos nucifera* L. and *Pisum sativum* L. (Greilhuber & Ebert, 1994; Rayburn *et al.,* 2004; Gunn *et al.,* 2015; Realini *et*

al., 2016). It remains unclear whether chromosomal structural variation or repetitive elements cause intraspecific genome size variation in Camelina.

Intraspecific genome size variation can indicate micro-evolutionary differentiation and be taxonomically significant, as found in *Juncus biglumis* L. and *Lagenaria siceraria* L. (Schönswetter *et al.,* 2007; Achigan-Dako *et al.,* 2008). However, the genome size estimates for our Camelina panel did not form consistent groups when combined with a phylogenetic tree, which might be caused by some mechanism in Brassicaceae (or other polyploid plant) that suppresses the proliferation of repetitive elements and diversification, thus supporting the concept of punctuated equilibria (Wicker *et al.,* 2018; Bird *et al.,* 2019; Beric *et al.,* 2020). Such a suppression mechanism might maintain the relatively high stability of DNA content in Camelina genotypes. Furthermore, genome size variation is sometimes accompanied by phenotypic consequences, as it defines the minimum or optimal cell size and acts as a constraint on carbon gain (Greilhuber & Leitch, 2013). The processes used to generate genome size variations in plants have been linked to differences in nuclear volume, cell volume, cell cycle, seed mass, photosynthetic rate, leaf cell size, stomatal density and tolerance to abiotic conditions (Beaulieu *et al.,* 2007; Hodgson *et al.,* 2010; Bilinski *et al.,* 2018). Nevertheless, we found no relationship between genome size and the eight key agronomic traits we measured. This is not surprising, as other studies reported similar results when analysing intraspecific variation in other species (Realini *et al.,* 2016; Oney-Birol & Tabur, 2018). For example, Basak *et al*. (2019) did not detect any link between genome size and morphological traits in turnip (*Brassica rapa* var. *rapa* L.). We suggest that the genome size variation in Camelina is related to non-coding regions and therefore does not affect phenotypic variation.

3.6. Conclusion

Intraspecific variation was found for genome structure, size and phenotypic traits in our Camelina germplasm. However, we observed that genome size does not reflect the phylogenetic relationships among Camelina lines or impact the variation observed at a phenotypic level. Despite the low genetic diversity detected between the 31 lines, we could divide the germplasm panel into two groups, providing a potential avenue for studying Camelina phenotype–genotype associations and assisting breeding efforts. It will also help to generate the species's pangenome through resequencing efforts to select subjects with a diverse genome size.

3.7. References

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CHAPTER 4

In the dark—characterising phenotypic plasticity in response to shade: A comparative analysis between oilseeds Camelina, canola and Arabidopsis

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4.1. Abstract

The ratio of red to far-red (R:FR) light in the available light spectrum changes in plants shaded by neighbouring plants and can significantly affect plant fitness. Plants cope with such changes in light by expressing phenotypic plasticity, defined as a genotype's ability to adapt to different environments. The breadth of such plasticity varies between and within species. For *Camelina sativa* (L.) Crantz, an emerging biofuel crop, the plastic response to changes in R:FR and associated underlying genetic diversity are largely unknown. To characterise Camelina plasticity to R:FR, we assessed multiple phenotypic traits and undertook a comparative phenotypic analysis with two Brassicaceae species: the well-established model plant, *Arabidopsis thaliana* (L.) Heynh, and domesticated oilseed crop, canola (*Brassica napus* L.). The reaction norms of 12 morphological traits at the reproductive stage were measured under a range of R:FR (0.2–10) among seven lines of Camelina, ten lines of Arabidopsis and three lines of canola. Camelina exhibited plasticity to R:FR, which varied depending on genotype. We observed no genotype by light environment interaction (G×E) pattern in Camelina plasticity, as was the case for Arabidopsis plants. Furthermore, the 20 lines of Brassicaceae species could be separated into three groups based on their plasticity to shade. All Camelina lines clustered within Group 3, together with early flowering Arabidopsis lines. Leaf area was identified as the most important plastic trait for maintaining Brassicaceae species biomass under competition. Information on *C. sativa* plasticity to R:FR and biological traits related to relative fitness under shade are valuable for breeding shade-resilient lines. We provide herein a framework to use the model species to reveal the pattern of phenotypic and genotypic diversity in related crop species.

4.2. Introduction

Light is the primary energy source for plant photosynthesis, playing a central role in plant growth and development. Changes in light quality, *i.e.,* wavelength composition, under shade directly affect plant fitness. Being shaded is a common problem that plants encounter in the field, especially under high-density planting and intercropping systems (Yang *et al.,* 2015). When a plant is shaded, neighbouring leaves absorb red (R; 600–700 nm) and blue (B; 400–500 nm) light and transmit unused far-red light (FR; 700–800 nm), resulting in a low ratio of photon irradiance R to FR (R:FR). The ability of plants to detect low R:FR (<1, which is the ratio for sunlight) is crucial for exhibiting phenotypic plasticity, or an individual genotype to express different phenotypes in response to environmental conditions (Bradshaw, 1965; Sommer, 2020). After detecting a low R:FR signal, plants exhibit two alternative plasticity strategies: 1) shade sensitive, in which an individual shows sensitivity to shade by accelerating their growth and/or promoting vertical growth to compete with their neighbour, together known as shade-avoidance syndrome (SAS); or 2) shade tolerance, in which an individual develops conservative resource-use strategies, such as slowing down their growth rate, and sometimes shows little or no morphological changes (Martinez-Garcia *et al.,* 2014; Gruntman *et al.,* 2017). Differences in plasticity strategy under low R:FR conditions are reported at the species and genotype level (Valladares *et al.,* 2014; Lorts & Lasky, 2019).

Therefore, determining the plasticity of crops is important to improve plant success in competing for light in high-density planting or mixed cropping systems. This study investigates plasticity to varying R:FR in an understudied crop, *Camelina sativa* (family *Brassicaceae*, tribe Camelineae). This oilseed crop is a promising sustainable source for food, industrial and biofuel production (Campbell *et al.,* 2013). There are no published reports on the effect of R:FR on *C. sativa*. To understand *C. sativa*'s response to R:FR, we use comparative analysis with two related species, *Arabidopsis thaliana* and canola. The model plant Arabidopsis is closely related to *C. sativa*—both belong to lineage 1 of Brassicaceae (Kagale *et al.,* 2014). Using the extensive information on Arabidopsis lines whose responses to R:FR vary, we can compare these related species to identify phenotypic plasticity in *C. sativa*. Also, comparing phenotypic plasticity to light quality in a more distantly related but important oilseed crop, canola (lineage 2 of Brassicaceae), will provide light-stress strategy information for a domesticated crop that is more tolerant to shade (Fellner *et al.,* 2003; Wang *et al.,* 2016).

Knowing which plastic traits reduce biomass and seed yield could improve breeding for shade-tolerant crop varieties by identifying which genotypes are suitable for high-density planting. However, analysing the consequences of plastic traits on plant fitness is a complex task, as multiple interacting functional traits and environmental factors affect plasticity (Anten *et al.,* 2009; Ta *et al.,*

2020). For example, Weinig (2000) showed that leaf elongation under shade indirectly affects plant biomass and fitness. Furthermore, Ta *et al*. (2020) used path analysis to characterise co-localising QTL for four important SAS traits, highlighting the importance of studying the relationships among traits to understand complex developmental responses. Therefore, multiple traits must be examined when understanding plant plasticity, considering that organisms are integrated complex phenotypes (Forsman, 2015).

This study aimed to characterise the Camelina plasticity pattern to varying R:FR levels in the available light spectrum from R:FR 0.2 (deep shade) to 10 (full light) and to observe the consequences of such plasticity to crop fitness. Multiple phenotypic traits were measured to achieve this goal, and comparative analysis was undertaken with Brassicaceae species: Arabidopsis and canola. To compare the variation in 12 plastic traits of three Brassicaceae species, we adopted the conceptual framework of Bakhtiari *et al*. (2019), which categorises plastic traits into five contrasting reaction norm patterns (Figure 4.1B). Reaction norm is commonly used to understand the degree of phenotypic plasticity, as the expression of plasticity, comprising genotype, environment and genotype by environment interaction (G×E) can be examined. Our approach established the Camelina response to varying R:FR, its plasticity patterns and plastic traits related to biomass under shade and compared the plastic response of two related species with different life history. A better understanding of plant plasticity to shade is expected to improve predictions related to crop responses to intense competition under dense vegetation and/or mixed cropping systems.

4.3. Materials and methods

4.3.1. Plant material

Seven lines of Camelina, ten lines of Arabidopsis and three lines of canola were used in this study (Table 4.1). The Camelina lines were obtained from the N.I. Vavilov Research Institute of Plant Industry, Russia and pure-lined at The University of Western Australia (UWA). Arabidopsis seeds were obtained from the Arabidopsis Biological Resource Center at Ohio State University (Columbus, OH, USA). Nuseed Australia kindly provided the AV-Jade and Tarcoola seeds of spring-type canola, and the Australian Grain Genebank, Horsham, provided the Topas seeds. These lines were selected based on their flowering time, reports on their response to R:FR and seed availability (Table 4.1).

Table 4.1: Description and sources of plant material used in this study.

Genotype	Species	Country of origin	Latitude	Longitude	Flowering time (days)*	Light quality response	Reference
C235	Camelina	Russia	NA	NA	53	NA	NA
C370	Camelina	Russia	NA	NA	58	NA	NA
C1811	Camelina	France	47	3	48	NA	NA
C2305	Camelina	Russia	56	93	51	NA	NA
C4138	Camelina	Russia	NA	NA	35	NA	NA
C4182	Camelina	Russia	55.19	89.48	33	NA	NA
DH55	Camelina	Former USSR	NA	NA	NA	NA	NA
Colombia (Col-0)	Arabidopsis	US	38.3	–92.3	30.4	Sensitive to low R:FR	Schwartz *et al.*, 2017
Ler-0	Arabidopsis	Germany	47.984	10.8719	30.33	Sensitive to low R:FR	Adams *et al.*, 2009
Shakdara (Sha)	Arabidopsis	Tajikistan	37.48	71.3	34.18	Adapt to different light quality	Köhl *et al.*, 2017
Bla-6	Arabidopsis	Spain	41.6833	2.8	56.8	Do not show accelerated flowering under low FR	Adams *et al.*, 2009
Ll-2	Arabidopsis	Spain	41.59	2.49	23	Weak response to low R:FR	Botto & Smith, 2002
Sf-2	Arabidopsis	Spain	41.7833	3.03333	37.67	Modest response to low R:FR	Adams *et al.*, 2009
Ge-2	Arabidopsis	Switzerland	46.5	6.08	68.78	Sensitive to low R:FR	Botto & Smith, 2002
Nok-3	Arabidopsis	Netherlands	52.24	4.45	41.27	Sensitive to low R:FR	Schwartz *et al.*, 2017
Br-0	Arabidopsis	Czech Republic	49.2	16.62	38.67	Insensitive to low R:FR	Schwartz *et al.*, 2017
Te-0	Arabidopsis	Finland	60.06	23.3	44.3	Adapt to high red light intensity	Köhl *et al.*, 2017
Topas	Canola	Canada	NA	NA	NA	NA	NA
AV-Jade	Canola	Australia	NA	NA	NA	NA	NA
Tarcoola	Canola	Australia	NA	NA	NA	NA	NA

*Arabidopsis flowering time data based on Lempe *et al*. (2005), Camelina based on a preliminary study. NA, not available

4.3.2. Plant growth condition and light treatments

Seeds were sown in 70 mm square plastic pots with a substrate of potting mix (UWA Mix, Richgrow) and perlite in a ratio of 3:1. Pots were placed in darkness at 4°C for one week to break dormancy before transferring to controlled-environment growth rooms for exposure to one of five R:FRs (0.2, 1.9, 3.4, 6.2, 10) as detailed in Table 4.2. Plants were watered daily and fertilised weekly with Thrive® all-purpose soluble fertiliser at 0.09 g pot^{-1}. Plants were grown at 22/18°C (day/night), 20 h photoperiod and air RH 70 ± 10%. Artificial lights for R:FR were provided by Valoya LED lights (Valoya Oy, Helsinki, Finland) and set at a light intensity of 329 ± 6 $\mu mol \cdot m^{-2} \cdot s^{-1}$. To achieve R:FR=1.9, we wrapped AP67 LED tubes with a single layer of filter 787 marius red (LEE Filters,

UK). Shade-mimicking conditions (R:FR=0.2) were obtained by adjusting the settings on a Heliospectra LX602C 630W LED Grow Light (Heliospectra, Sweden) to mimic the AP67 wavelength spectra with the addition of FR. The light intensity for R:FR=0.2 was set at 190 ± 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to mimic light intensity under canopy shade (Wan *et al.*, 2020). Light intensity and R:FR were measured with a Sekonic C7000 SpectroMaster spectrometer (Sekonic Corp., Tokyo, Japan). R:FR calculations followed the method described by Runkle and Heins (2001): photon irradiance between 655 and 665 nm divided by photon irradiance between 725 and 735 nm.

Table 4.2: Comparison of the light treatments used in this study.

Light treatment	Photon flux density ($\mu mol\ m^{-2}\ s^{-1}$)			B:R ratio	R:FR
	B (400–500 nm)	R (600–700 nm)	FR (700–800 nm)		
Heliospectra	10.51	19.61	45.08	0.7	0.2
AP67+ Filter	18.15	135.1	60.66	0.09	1.9
AP67	35.4	166.49	44.08	0.15	3.4
AP673	44.98	258.67	30.12	0.17	6.2
NS1	63.55	125.13	14.03	0.5	10

4.3.3. Phenotypic evaluation

Twelve traits were measured at the reproductive stage: flowering time, leaf number, internode length, leaf area, leaf width, leaf length, dry weight, leaf circularity, leaf perimeter, leaf compactness, specific leaf area and leaf index (Nakata & Lockhart, 1966; Tsukaya, 2006; Li & Kubota, 2009; Casal, 2012; Liu *et al.*, 2016; Klem *et al.*, 2019). Length and width of the youngest completely developed leaf (4th leaf) were measured using a ruler (Klem *et al.*, 2019). Internode length was calculated by dividing stem length by node number (Nakata & Lockhart, 1966). Immediately after sampling, the leaves were scanned, and leaf area, leaf circularity and leaf perimeter measured using LeafJ with ImageJ software (Maloof *et al.*, 2013). Specific leaf area (SLA) was calculated by dividing leaf area by leaf dry mass (Liu *et al.*, 2016). Leaf compactness was measured as the square of leaf perimeter divided by leaf area, and leaf index was measured as a ratio of leaf length to leaf width (Tsukaya, 2006). Root, shoot and fresh mass were measured with an electronic balance, before transferring the plant material to an oven set at 60°C. After four days, dry mass was quantified using an electronic balance.

4.3.4. Statistical analysis of phenotypic data

The experiment commenced on 14 March 2019 and was repeated on 23 April 2019. The experiments followed a split-plot design, with R:FR as the whole-plot factor and lines as the sub-plot factor. For each R:FR, five plants of each genotype were used, except for R:FR=0.2, where two plants per genotype were grown for each experiment due to limited space. All statistical analyses were carried out using the software R (R Core Team, 2013). Welch's Analysis of variance (ANOVA) was fitted

for all parameters to determine the effects of R:FR on plant morphological traits. PCA was calculated and visualised using 'FactoMineR' package (Lê *et al.*, 2008).

To evaluate phenotypic reaction for a single trait as a function of variation in R:FR, the reaction norms for each trait were calculated using mixed regression models with 'lme4' package (Bates *et al.*, 2015): random intercept model (RIM) or random slope model (RSM). R:FR was considered as fixed effect, while genotype was considered as a random effect. First, we tested the data of each trait and species using genotype as random intercept. Then, we included individual genotype as a random slope and tested the significance of random slope in the model using likelihood ratio tests. No models correlated consistently well across all traits and species; thus, different methods that gave best fits (highest AUC statistic) were used to explain the phenotypic pattern under different R:FR.

To quantify shade-avoidance responses of plants, phenotypic plasticity index (PPI) was measured for each trait as per Valladares *et al*. (2000):

$$PPI = \frac{Max\ (\bar{X}_c, \bar{X}_{nc}) - Min(\bar{X}_c, \bar{X}_{nc})}{Max\ (\bar{X}_c, \bar{X}_{nc})}$$

where $\bar{X}_c$ is trait mean under competition (low R:FR=0.2); $\bar{X}_{nc}$ is trait mean under no competition (R:FR=1.9). R:FR=1.9 was chosen as the environment with no light competition as: (1) The spectral pattern is the most similar with R:FR=0.2; (2) It was the closest R:FR to sunlight in our environments (Supplementary Figure 4.1). The PPI value was used to perform PCA, before clustering the lines using hierarchical clustering in the principal component (HCPC) function of the 'FactoMineR' package.

Phenotypic selection analyses for trait plasticity were performed to test for positive correlations between trait plasticity and fitness. Relative fitness was calculated by dividing dry biomass by mean biomass within each treatment environment (Bell & Galloway, 2007). Other studies highlight that biomass is a reliable predictor of plant fitness when reproductive outputs are unavailable (Liu *et al.*, 2016; Younginger *et al.*, 2017). The phenotypic selection was measured as standardised trait values based on regressions of average relative fitness of plasticity groups and group values of plasticity traits (as per Du *et al.*, 2017).

4.4. Results

4.4.1. Camelina plasticity pattern to R:FR

We used a multi-phenotypic approach—assessing the reaction norm of 12 morphological traits at the flowering stage—to determine Camelina variation in plasticity to R:FR (Figure 4.1, Supplementary Figure 4.2, Supplementary Figure 4.3). Camelina lines exhibited plasticity in all traits examined in response to different R:FR, except for leaf circularity (P>0.05, Figure 4.1A). Camelina responded to reduced R:FR by accelerating flowering time and increasing internode length by 65%, specific leaf area by 38%, leaf index by 15% and leaf compactness by 50% (Supplementary Figure 4.2). Dry weight, leaf perimeter and leaf size (width, length, area) decreased under reduced R:FR by 93%, 74% and 94%, respectively.

To better illustrate the direction and magnitude of plasticity in Camelina and its related species, we adapted framework from Bakhtiari *et al*. (2019), as follows (Figure 4.1B): (A) Species exhibits no trait plasticity and no genotypic variation to the different R:FR, (B) Species exhibits genotypic variation in traits, but no plasticity across treatments, (C) Species exhibits plasticity to varying light quality but shows no genotypic variation in the response, (D) Species exhibits phenotypic plasticity to varying R:FR accompanied by genotypic variation in plasticity and (E) Species exhibits genotype and plasticity interaction for the traits tested (G×E). Camelina lines exhibited variation and plasticity to R:FR for most traits and could be characterised as plasticity Pattern D (Figure 4.1A). Comparing the genotypic variation for plasticity with other Brassicaceae species revealed different response patterns to R:FR. Arabidopsis lines exhibited G×E for six traits (Pattern E, Figure 4.1A). This G×E was expected as we had selected Arabidopsis lines known to exhibit varied responses to R:FR (Table 4.1), including lines sensitive to FR (*e.g.*, Ge-2 and Nok-3) (Botto & Smith, 2002; Schwartz *et al.*, 2017), semi-sensitive to FR (*e.g.*, Ll-2) (Botto & Smith, 2002) and insensitive to FR (*e.g.*, Bla-6 and Br-0) (Adams *et al.*, 2009; Schwartz *et al.*, 2017). Our results confirmed previous results, demonstrating these varied responses to R:FRs in Arabidopsis lines, despite adopting different light treatments. For canola, nine traits exhibited plasticity and no genotypic variation (Pattern C), two traits showed plasticity with genotypic variation (Pattern D) and one trait displayed genetic variation but no plasticity (Pattern B).

Different patterns of plasticity between Brassicaceae species were demonstrated for flowering time in response to across all R:FR environment (Figure 4.2). Reduced time to flower was observed for all seven Camelina lines, with genotype C4182 exhibiting the highest sensitivity to R:FR (22% compression of flowering time; Pattern D; Figure 4.2A). Within Arabidopsis, a low R:FR significantly accelerated time to flowering for all lines except Bla-6 (Pattern E; Figure 4.2B). For

canola, accelerated flowering time was observed for three lines, with no genotypic variation in response to different R:FR (Pattern C; Figure 4.2C). These results indicate that the three Brassicaceae species have different genotypic variation and G×E patterns in their responses to varying R:FR.

4.4.2. Shading elicited a stronger plastic response than high R:FR environments

Principal component analysis (PCA) was used to determine the relationships between the flowering traits, species and R:FR light environment (Figure 4.3). The first two principal components (PCs) explained 77.6% of the trait variation in the three species. PC1 was mainly associated with leaf morphology traits (leaf perimeter, leaf width and length, leaf area, leaf circularity and leaf compactness), biomass, elongation traits and flowering time. PC2 separated the plants grown under the different R:FR treatments and was related to the number of leaves at flowering, flowering time, dry weight, leaf compactness, internode length, SLA, leaf area and leaf circularity. Plants grown under R:FR 1.9–10 were represented on the upper side of the graph. This result suggests that an R:FR>1, which is generally used to artificially light plant growth facilities, did not elicit a drastic plastic response, as the artificial light environment already provided enough R:FR for plants. In contrast, plants grown under extremely low R:FR (R:FR=0.2) were represented in the bottom two quadrants, indicating this treatment, which mimics shading, is the critical threshold for eliciting the strongest plastic response of the measured variables.

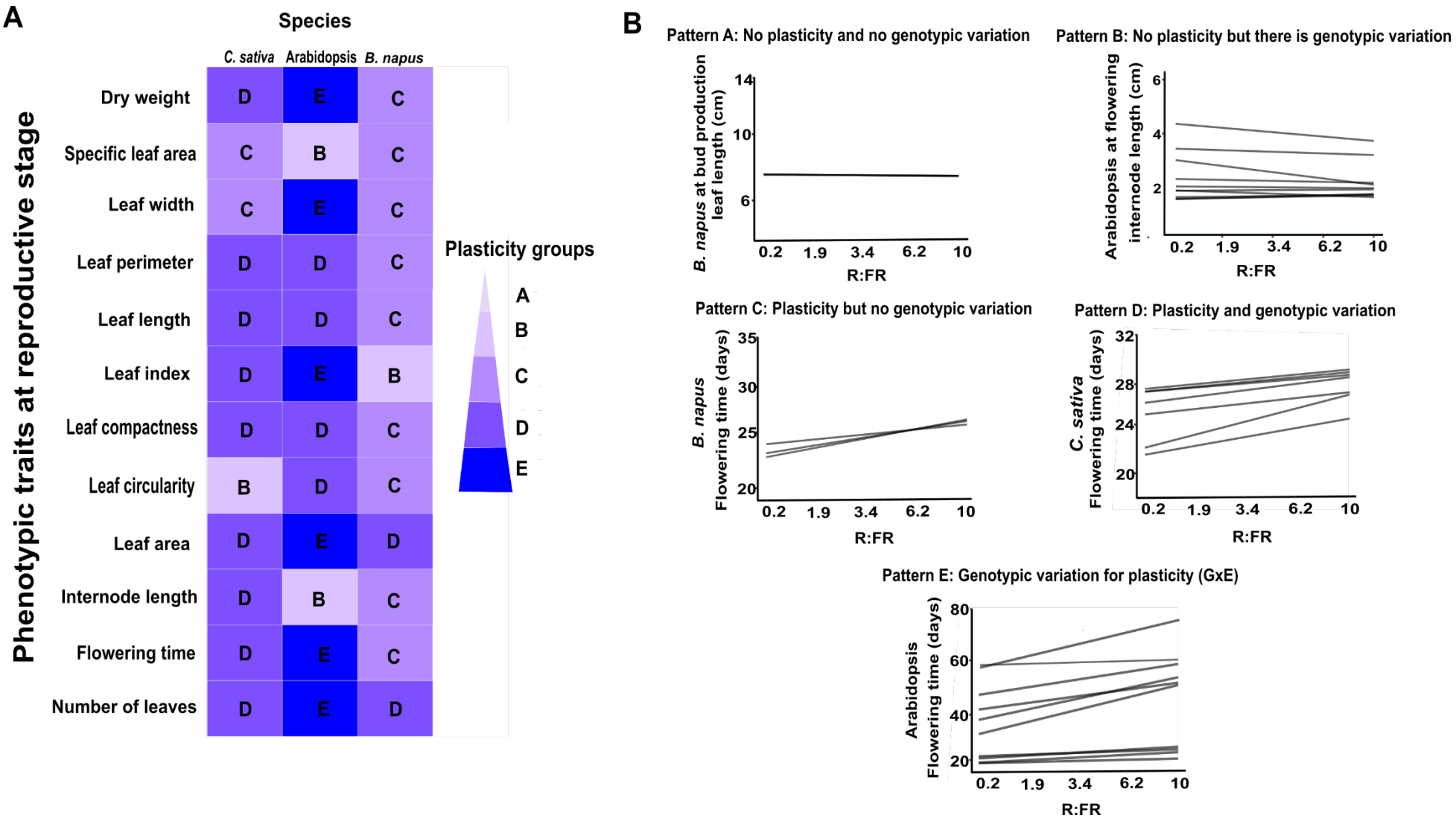

Figure 4.1: Phenotypic plasticity of three Brassicaceae species to varying R:FR measured at the reproductive stage (R:FR=0.2–10). **A**, The heat map summarises the plasticity pattern of 12 traits at the flowering stage in three Brassicaceae species. Plasticity patterns are shown by different colour bars and letters. **B**, Reaction norms of the five patterns of plasticity (A–E) adapted from Bakhtiari *et al.* (2019). Each point represents the average response to varying R:FR.

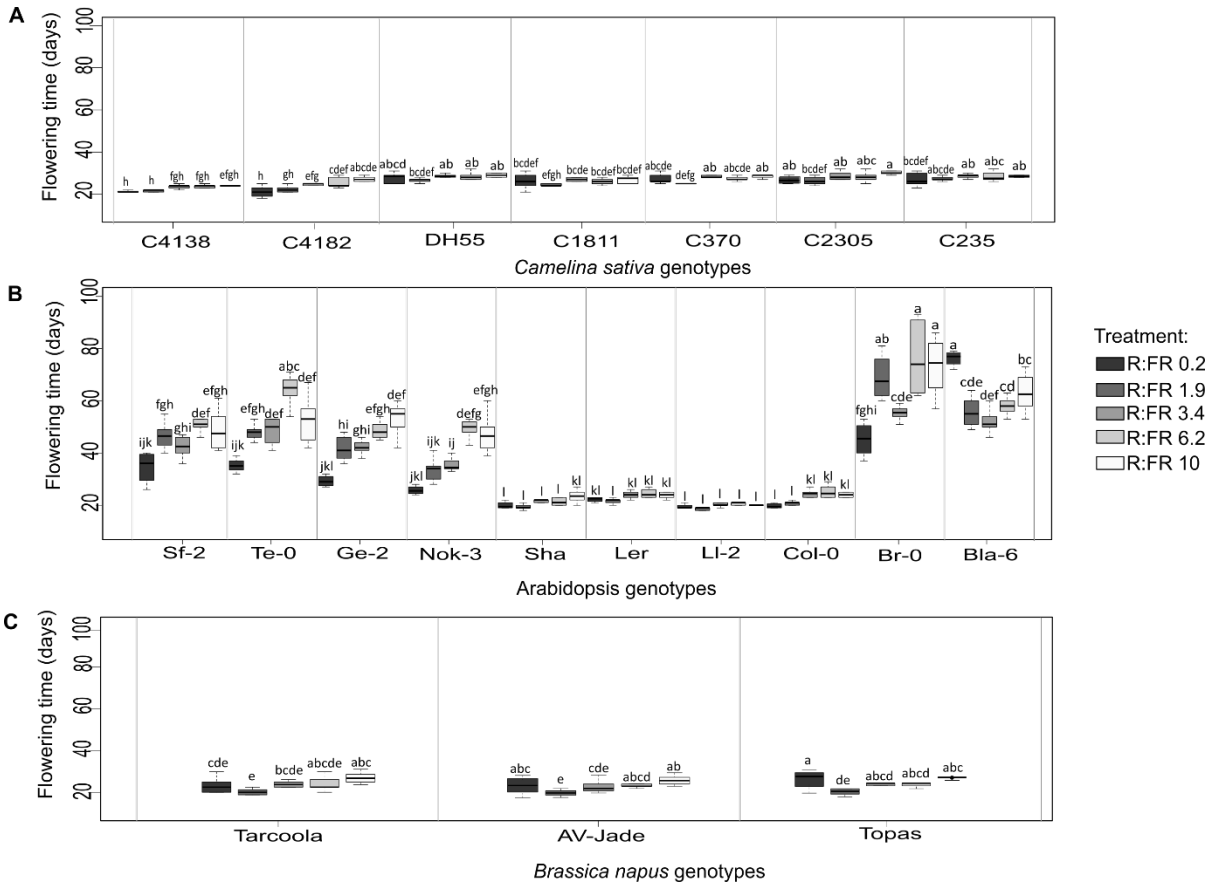

Figure 4.2: Flowering time response of three Brassicaceae species grown under different R:FRs. **A**, Camelina; **B**, Arabidopsis; **C**, Canola. Boxplot represents ten samples across two independent experiments for R:FR 1.9–10 and four samples for R:FR 0.2. Same letter in the boxplot indicates means are not significantly different between R:FR treatments (P<0.05, Welch ANOVA test).

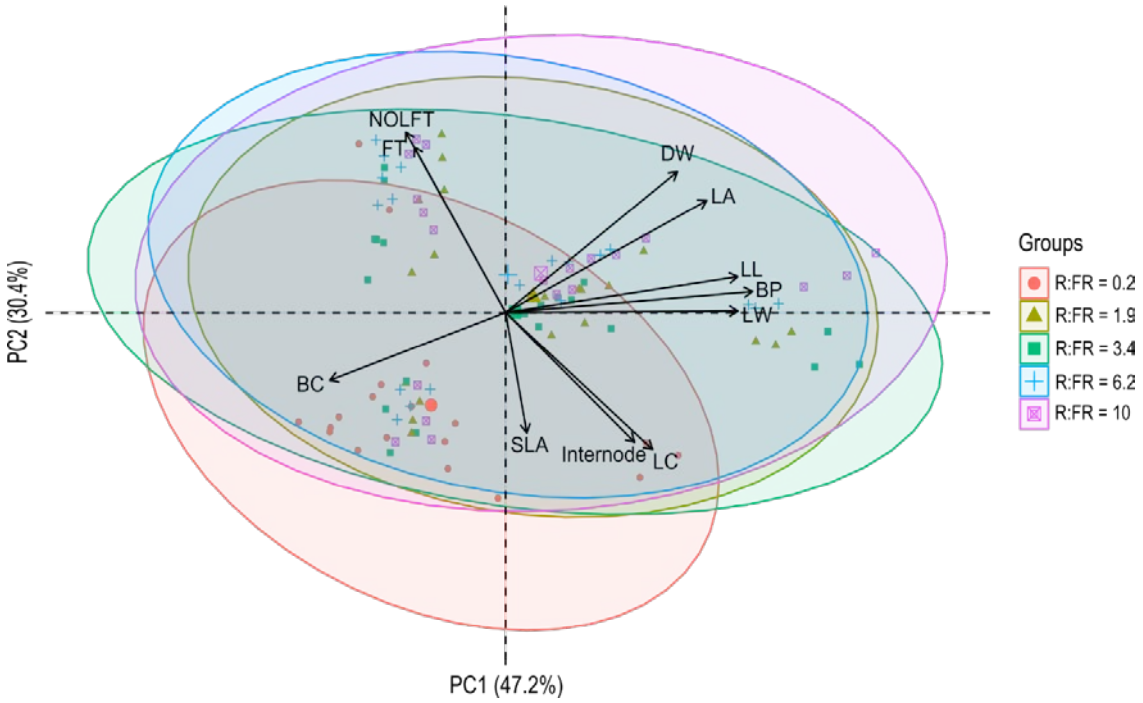

Figure 4.3: Multivariate analysis for flowering traits of three Brassicaceae species cultivated at different R:FRs. PCA plot shows two principal components estimated using 20 lines response in five different R:FRs.

All the measured traits were included in the analysis as the dependent variable and light treatments as classification factors represented by different symbols and colours. The 90% confidence eclipse intervals are drawn on traits that relate to different treatments. Abbreviations indicate traits names—FT: flowering time, NOLFT: number of leaves at flowering, DW: dry weight, LA: leaf area, LL: leaf length, LW: leaf width, Internode: internode length, BP: leaf perimeter, BC: leaf circularity, LC: leaf compactness, SLA: specific leaf area.

4.4.3. Three groups of shade response in Brassicaceae species

With evidence from PCA analysis that R:FR=0.2 (shade-mimicking condition) elicited the largest plastic response in Brassicaceae species, we evaluated the phenotypic plasticity index (PPI) for 14 traits to quantify shade-avoidance responses in the three Brassicaceae species (Table 4.3). This value varies from 0 for non-plastic to 1 for high plasticity to shade. For Brassicaceae species, the PPI value ranged from 0.02 to 0.98. The highest plasticity in response to shade was for biomass traits—root dry weight, shoot dry weight and total dry weight—while the lowest plasticity was observed for leaf index. There were no substantial differences between the three species for the number of leaves at flowering, dry weight, leaf area or leaf circularity, indicating similar shade-avoidance response mechanisms across the three Brassicaceae species. Differences in PPI between the three species were observed for flowering time, internode length, dry shoot weight, leaf width and specific leaf area. Camelina lines exhibited higher plasticity for internode length, dry shoot weight and leaf width when exposed to low R:FR than the other Brassicaceae species. Arabidopsis was more plastic in terms of flowering time and SLA.

Table 4.3: PPI value of 14 traits for three Brassicaceae species at the flowering stage. The PPI values are shown as means ± standard error (SE). Significant differences between species means are indicated by *P*-values from one-way ANOVA.

Plant traits	Parameters	Phenotypic plasticity index (mean ± SE)			*P*-value
		Camelina	Arabidopsis	Canola	
Phenological traits	Flowering time	0.12 ± 0.07	0.18 ± 0.04	0.18 ± 0.03	0.01
	Leaf number	0.51 ± 0.02	0.50 ± 0.08	0.31 ± 0.02	0.57
Biomass traits	Root dry weight	0.98 ± 0.002	0.82 ± 0.08	0.92 ± 0.01	0.24
	Shoot dry weight	0.91 ± 0.01	0.66 ± 0.08	0.71 ± 0.03	0.01
	Dry weight	0.92 ± 0.008	0.86 ± 0.06	0.75 ± 0.03	0.32
Elongation traits	Internode length	0.41 ± 0.02	0.25 ± 0.06	0.24 ± 0.02	<0.01
	Leaf length	0.69 ± 0.02	0.52 ± 0.09	0.2 ± 0.05	0.09
	Leaf width	0.69 ± 0.03	0.44 ± 0.09	0.22 ± 0.06	0.02
	Leaf area	0.93 ± 0.01	0.79 ± 0.1	0.53 ± 0.1	0.23
Leaf morphological traits	Specific leaf area	0.30 ± 0.07	0.69 ± 0.08	0.50 ± 0.04	<0.01
	Leaf perimeter	0.71 ± 0.02	0.55 ± 0.08	0.31 ± 0.06	0.08
	Leaf circularity	0.19 ± 0.02	0.25 ± 0.03	0.18 ± 0.07	0.20
	Leaf compactness	0.34 ± 0.09	0.44 ± 0.07	0.23 ± 0.03	0.20
	Leaf index	0.08 ± 0.03	0.18 ± 0.05	0.02 ± 0.02	0.11

PCA analysis of the PPI value was done to determine the relationship between traits and genotypes under shade (Figure 4.4A). The first PC in the PCA graph explained 43.2% of the variation, and the second PC explained 21.9% of the variation in the three species. PC1 is related to leaf area, leaf perimeter, leaf length, leaf width, dry weight and number of leaves. PC1 separated Arabidopsis Bla-6, Arabidopsis Br-0 and all *B. napus* lines from the rest of the lines examined. PC2 contains values for leaf compactness, number of leaves, flowering time, internode, leaf width and dry weight; and separated all *C. sativa* lines from the rest of the lines.

Cluster analysis based on PCA congregated the 20 lines of Brassicaceae into three groups based on their response to shade (Figure 4.4B). As expected from our selection criteria, Arabidopsis lines exhibited diverse reactions to shade and were categorised into all three groups. Arabidopsis lines Br-0 and Bla-6, known for their FR insensitivity, were grouped in Group 1 with three *B. napus* lines, Tarcoola, Topas and AV-Jade. Group 1 were associated with leaf compactness, leaf width, leaf area, dry weight, number of leaves, leaf length and leaf perimeter. Group 2 comprised of Arabidopsis lines Sf-2, Te-0, Ge-2 and Nok-3. This group was separated into the upper two quadrants associated with leaf circularity, dry weight, internode, leaf number and leaf compactness. Group 3 comprised early flowering Arabidopsis lines, including Ll-2, Col-0, Ler and Sha, and all *C. sativa* lines. Group 3 was clustered in the quadrant related to leaf perimeter, leaf length, leaf width, leaf area and flowering time.

4.4.4. Biological traits associated with biomass under shade

A standardised regression between each trait and biomass was performed to identify traits related to relative fitness during low R:FR (R:FR=0.2) and high R:FR (R:FR=1.9) within the three Brassicaceae species (Table 4.4). We found that leaf area were important traits for biomass under shade in three Brassicaceae species. For Camelina, biomass had a significant negative correlation with leaf area at low R:FR and with leaf index when grown under high R:FR. For Arabidopsis, leaf number, leaf length, leaf area and leaf perimeter had a significant negative correlation with biomass at low R:FR. Flowering time had a positive correlation with biomass at high R:FR in Arabidopsis, while leaf length and parameter had a negative correlation in this environmental condition. Leaf area had a negative correlation with biomass at low R:FR and leaf perimeter had a positive correlation with biomass at high R:FR in canola. These results identified important traits in each species for maintaining fitness under competition.

Table 4.4: Standardised regression coefficients of plasticity for traits under low and high R:FR in three Brassicaceae species. All values are based on mean genotypic values. *, ** and *** indicate significant differences at 0.05, <0.01, <0.001.

Traits	*Camelina sativa*		*Arabidopsis thaliana*		*Brassica napus*	
	Low R:FR	**High R:FR**	**Low R:FR**	**High R:FR**	**Low R:FR**	**High R:FR**
Flowering time	0.15	0.26	0.56	0.89*	-0.99	-0.004
Leaf number	-0.68	-0.39	-0.79*	-0.25	0.33	0.91
Internode length	0.59	0.66	0.51	0.38	-0.86	0.59
Leaf length	-0.33	-0.01	-0.69*	-0.93*	-0.66	0.81
Leaf width	-0.69	-0.28	-0.61	-0.84*	-0.59	0.86
Leaf area	-0.83*	-0.44	-0.95*	-0.79*	-0.99*	0.12
Specific leaf area	0.18	0.45	-0.58	-0.42	0.43	0.86
Leaf perimeter	-0.29	-0.08	-0.75*	-0.92*	-0.15	0.99*
Leaf circularity	0.33	0.24	-0.61	-0.35	0.77	0.56
Leaf compactness	-0.68	-0.47	-0.59	-0.07	-0.51	-0.81
Leaf index	-0.78	-0.78*	-0.44	-0.47	-0.76	0.72

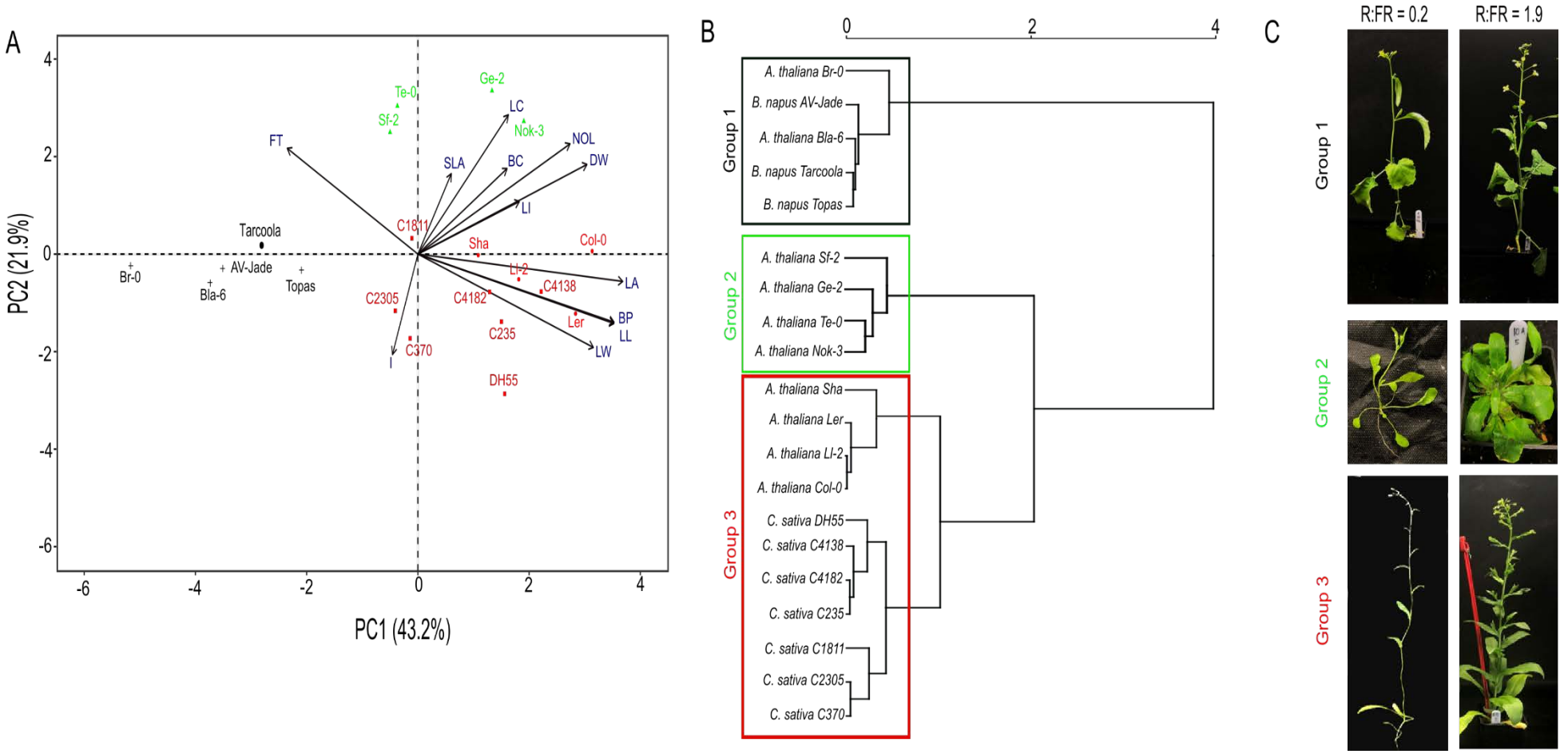

Figure 4.4: Three shade response groups of three Brassicaceae species based on their plasticity to shade-mimicking condition under contrasting light environments. **A**, PCA analysis for all traits' plasticity index (PPI values). PPI value of each trait measured according to Valadares *et al*. (2000). Abbreviations indicate traits names—FT: flowering time, NOLFT: number of leaves at flowering, DW: dry weight, LA: leaf area, LL: leaf length, LW: leaf width, I: internode length, BP: leaf perimeter, BC: leaf circularity, LC: leaf compactness, SLA: specific leaf area. **B**, HCPC cluster analysis further separated the 20 lines of Brassicaceae into three groups based on PPI value. Colour key indicates the different shade response groups. **C**, Plants from each group at the reproductive stage when grown under low and high R:FR.

4.5. Discussion

4.5.1. Does Camelina exhibit plasticity to different R:FR?

At the flowering stage, most morphological traits of Camelina exhibited a plastic response when exposed to various R:FR. Camelina displayed an SAS response typical of sun-loving plants—including internode elongation, altered leaf shape (leaf circularity, perimeter and compactness) and increased specific leaf area and leaf index—to escape low R:FR conditions and maximise light interception (Li & Kubota, 2009; Casal, 2012; Du *et al.*, 2017). Leaf area decreased in Camelina in response to low R:FR due to reduced leaf length and width. In other studies, the effect of R:FR on leaf growth ranged from inhibition to promotion, with variation possibly attributed to plant growth temperature, as higher temperatures like those used in this study (22°C) have shown similar results (Casal & Smith, 1989; Franklin *et al.*, 2003). Casal (2012) attributed the reduction in leaf growth in Arabidopsis to rapid promotion of auxin production to increase elongation, thus inducing cytokinin degradation and ultimately reducing cell proliferation. We also identified that low R:FR promoted early flowering in this species, evidenced by leaf number at flowering, one of parameters to measure flowering time in Brassicaceae species. However, this effect was less significant for time to flowering, as reported by others (Callahan & Pigliucci, 2002; Botto & Coluccio, 2007; Botto, 2015).

4.5.2. How does Camelina compare to the two other Brassicaceae species in terms of response and genetic variation?

The three Brassicaceae species shared the ability to perceive distinct light quality but exhibited some different plastic responses to those cues. For example, the three species exhibited different responses to varying R:FR at the reproductive stage for leaf circularity, internode length and leaf index. Furthermore, Camelina exhibited a different plasticity pattern to Arabidopsis, producing lines with similar slopes and shapes, indicating that the species differ on average in their phenotypes in an additive way but showed the same plastic response (no G×E pattern). It has been suggested that the plastic response to changes in R:FR can vary both among species due to differences in previous habitat experiences and the genetic variability of natural populations (Pratt & Mooney, 2013; Valladares *et al.*, 2014) and within species due to small-scale heterogeneity or natural selection among specific loci responsible for diversity (Botto & Smith, 2002; Botto, 2015; Bakhtiari *et al.*, 2019; Lorts & Lasky, 2019). The absence of G×E in Camelina could be related to 1) low genetic diversity in Camelina (*e.g.*, Singh *et al.*, 2015; Brock *et al.*, 2018; Luo *et al.*, 2019), 2) low sample size (seven lines from a similar location) and/or 3) strong stabilising selection of R:FR plasticity in this species. Further research involving additional lines is needed to accurately determine the reason for the low G×E in Camelina in the present study.

4.5.3. Could the life-history of the three Brassicaceae species explain their plasticity to shade?

We separated the 20 lines of Brassicaceae species into three groups based on their plasticity to shade, which could be explained by their evolution within their habitat. All three groups consisted of Arabidopsis lines that differ in their response based on the traits tested under shade. For example, Arabidopsis lines in Group 2 having a higher level of plasticity in flowering time and leaf compactness than other Groups. It is not known why these Arabidopsis lines have diverse plasticity to SAS. However, geographical variation may form gradients of selection across the species distribution, influencing their evolutionary dynamics and plasticity in changing environments (Gaitán-Espitia *et al.*, 2017). Botto (2015) found that shade plasticity for flowering and dry biomass was associated with altitude in structured populations in Northeast Spain. Although Schwartz *et al.* (2017) did not perform correlation analysis, their study indicated that most lines that do not rapidly accelerate in FR-enriched environments originate from high latitudes. However, when Botto & Smith (2002) observed plasticity to flowering traits under low R:FR in 100 genotypes of Arabidopsis, they could not quantify the relationship between the magnitude of the flowering response and latitude. They reasoned that the phylogeography of Arabidopsis has been affected by humans. At the molecular level, nucleotide polymorphism at photoreceptor genes, such as photoreceptor phytochrome B (phyB) (Filiault *et al.*, 2008), PHYTOCHROME-INTERACTING FACTOR 4 (PIF4) (Brock *et al.*, 2010) and protein EARLY FLOWERING 3 (gene: ELF3) (Jiménez-Gómez *et al.*, 2010; Coluccio *et al.*, 2011), underlie the plasticity of Arabidopsis genotypes to R:FR. The muted flowering time response of Bla-6 in Group 1 results from a single amino acid substitution in FY alleles that elicits a strong response of FLOWERING LOCUS C (FLC) and blocks the low R:FR induction of FLOWERING LOCUS T (FT) genes (Adams *et al.*, 2009). Moreover, Br-0 exhibits allelic variation in its CONSTANS (CO) and FT genes, contributing to its insensitivity to R:FR for flowering time (Schwartz *et al.*, 2017).

For canola, we suggest that its life history as a domesticated crop with an ecological range limited to agricultural production might be the main reason for grouped with FR insensitivity Arabidopsis genotypes. Several studies have shown that domesticated plants are less plastic than wild ones (Carriedo *et al.*, 2016; Sessa *et al.*, 2018). Domestication and subsequent breeding might indirectly select for SAS attenuation through selection for yield under high density conditions (Fellner *et al.*, 2003; Kebrom & Brutnell, 2007; Carriedo *et al.*, 2016). To date, little is understood about the molecular genetic mechanisms controlling plasticity to shade in domesticated crops, with auxin and PhyB-related genes seemingly responsible for low plasticity in domesticated plants (*e.g.*, Whipple *et al.*, 2011; Wang *et al.*, 2016). Just as suppressing SAS to breed high-density tolerant crops has been

a major breeding goal, different shade-sensitive groups in this study could be resources for future genetic research.

The lines of Camelina were clustered in Group 3, with the early flowering Arabidopsis lines. This group had the most substantial plastic responses to R:FR changes for leaf length, leaf width and leaf perimeter and the weakest plastic responses to flowering time compared to other groups. While the reason for this is unknown, a likely explanation is the evolutionary origins of Camelina. This crop has been suggested as a domesticated form of the wild species *Camelina macrocarpa* Andrz. ex DC, based on the absence of wild Camelina populations and identical genome structure and morphologies of Camelina and *C. microcarpa* (Brock *et al.,* 2018; Mandáková *et al.*, 2019). Thus, current Camelina lines might have been subjected to directional selection for SAS plasticity, depleting genetic variation in plasticity and causing the species to respond uniformly (as shown by the absence of G×E) (Saltz *et al.,* 2018), although not as much as the domesticated canola. Recently, Mandáková *et al*. (2019) identified possible parental genomes of Camelina species. Further studies on the phenotypic plasticity of these parental lines and its wild species *C. macrocarpa* under shade would provide genetic resources to better understand the evolution of shade plasticity in Camelina.

4.5.4. *Which trait is important for enhancing biomass under shade?*

The functionality of individual plastic traits depends on a plant's environmental settings, *e.g.,* stem elongation in plants—a well-established trait for SAS—increases reproductive structures in competitive settings but reduces fitness in non-competitive environments (Franklin & Whitelam, 2005; Casal, 2012). In settings where plants grow under deep shade (*e.g.,* forests, dense agriculture systems), less-plastic or shade-tolerant strategies are beneficial (Valladares *et al.,* 2000; Sanchez-Gomez *et al.,* 2006). In our environmental settings, leaf area was negatively associated with plant biomass under shade in all Brassicaceae species. Inhibited total leaf area has been one characteristic of SAS, which has been shown to correlate with the cell number for plants grown in low R:FR (Carabelli *et al.,* 2007). The higher concentration of auxin under shade, which is important for promoting stem elongation to escape shaded areas, has been suggested to be the reason for inhibition of leaf expansion and smaller leaves (Keller *et al.,* 2007; Wu *et al.,* 2017). Therefore, our result showed that the ability of Brassicaceae species to modify their growth—inhibit leaf size for elongation growth—is essential for this light-demanding species biomass under shade.

In addition, for Arabidopsis, leaf number, leaf length, and leaf perimeter (the parameter for the complexity of leaf shape) underwent negative selection for biomass in the shade. Thus, plants with a fast time to flower and a low leaf perimeter had more biomass at the flowering stage. Accelerated flowering—to complete the life cycle and reproduce before the canopy becomes overly shaded—is

one of the most dramatic SAS responses in Arabidopsis (Casal, 2012). The possible function of leaf shape in the light-adaptive response is connected to photosynthesis efficiency. Studies have shown that low dissection to the leaf margin increases surface area for photosynthesis (Karban, 2015). This finding provides a target trait for improving the biomass of shade-avoiding species under shade.

Understanding the responses of plants and their related species to changes in the light environment is an ongoing challenge. The findings presented in this study highlight the importance of comparative analysis and multi-phenotypic approaches for interpreting the function and evolution of complex traits. However, shade is not characterised only by changes in R:FR—changes in blue light, lower irradiance and micro-climatic changes (including changes in temperature and humidity) also affect plant fitness (Casal, 2013; Lee *et al.*, 2016). These factors were controlled in the present study. Therefore, experiments designed to observe the effects of individual plastic traits and their interactions, both in agricultural and natural system settings, would be valuable. Another challenge for future studies is determining the genetic architecture underlying plant plasticity to varying light conditions. Studies have shown that these traits have common and independent genetic control mechanisms (Gage *et al.*, 2017; Kusmec *et al.*, 2018; Diouf *et al.*, 2020). We propose that this goal be achieved by incorporating natural variations, as shown by the potential of the three groups of shade responses described in this study. Identifying these loci will provide a breeding strategy that can be used to develop high-yielding cultivars under monoculture at high density or crop systems with mixed species, such as intercropping or agroforestry.

4.6. Conclusion

This study offers a framework for exploiting light plasticity patterns from model plants and understanding plasticity in related understudied crops. Using multi-phenotypic traits and comparative analysis with closely related species (*i.e.*, Arabidopsis and canola), we determined that Camelina lines respond to R:FR changes. Genotype and plasticity variations were observed, but no evidence of G×E was found. The intermediate response of Camelina provides important information for breeders and farmers when choosing the optimum planting density for this crop. The natural variation in the three groups of shade response offers a useful resource for future molecular evaluations of the genetic architecture of plant responses to dense vegetation. Moreover, leaf area is an important determinant for increased biomass in Brassicaceae species under simulated shade conditions. This finding offers selectable traits for breeding programs aimed at improving crop photosynthesis under shade. Overall, this study provides valuable insight into the plasticity responses of Camelina and related species to shade. The findings are critical for developing crops that perform well at the community level.

4.7. References

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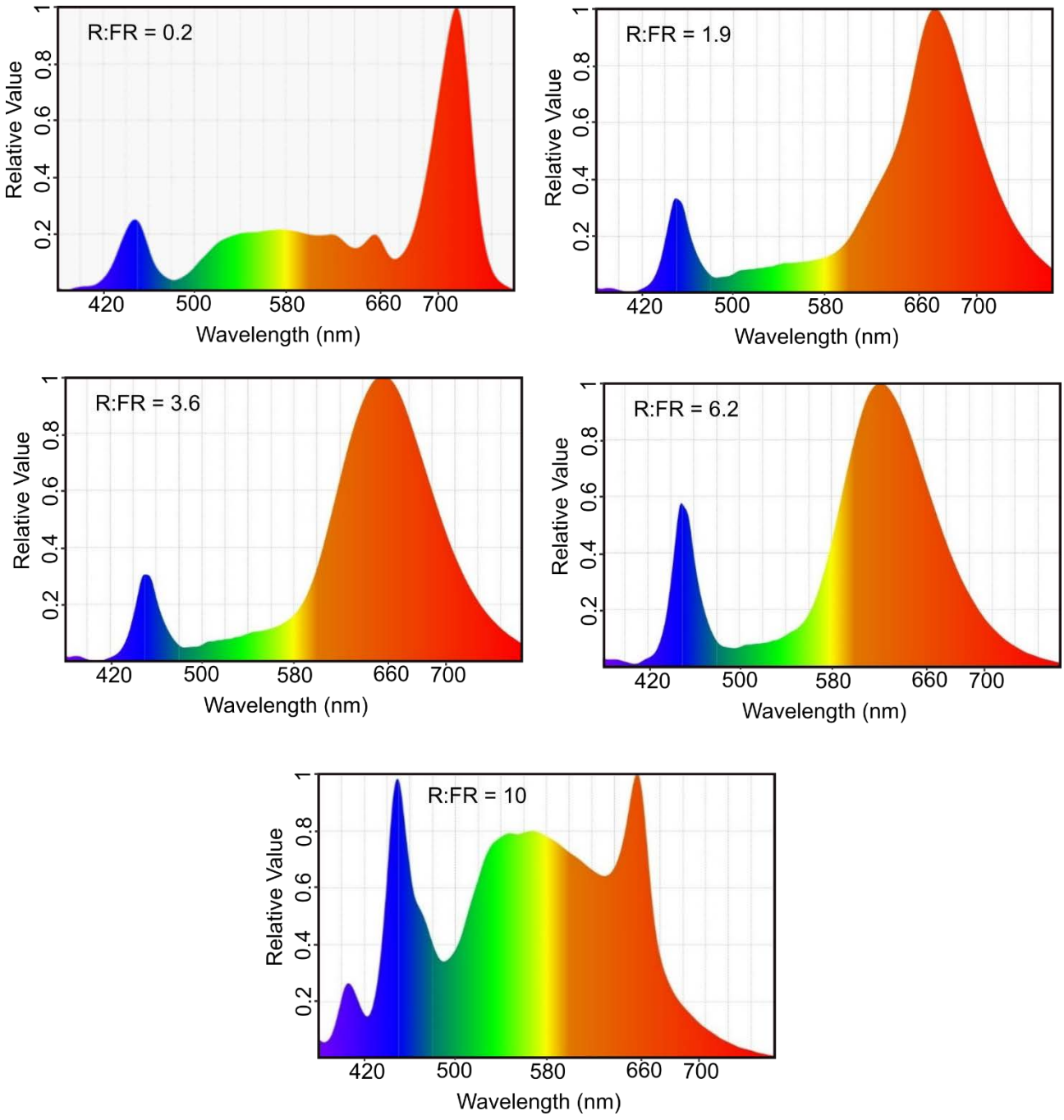
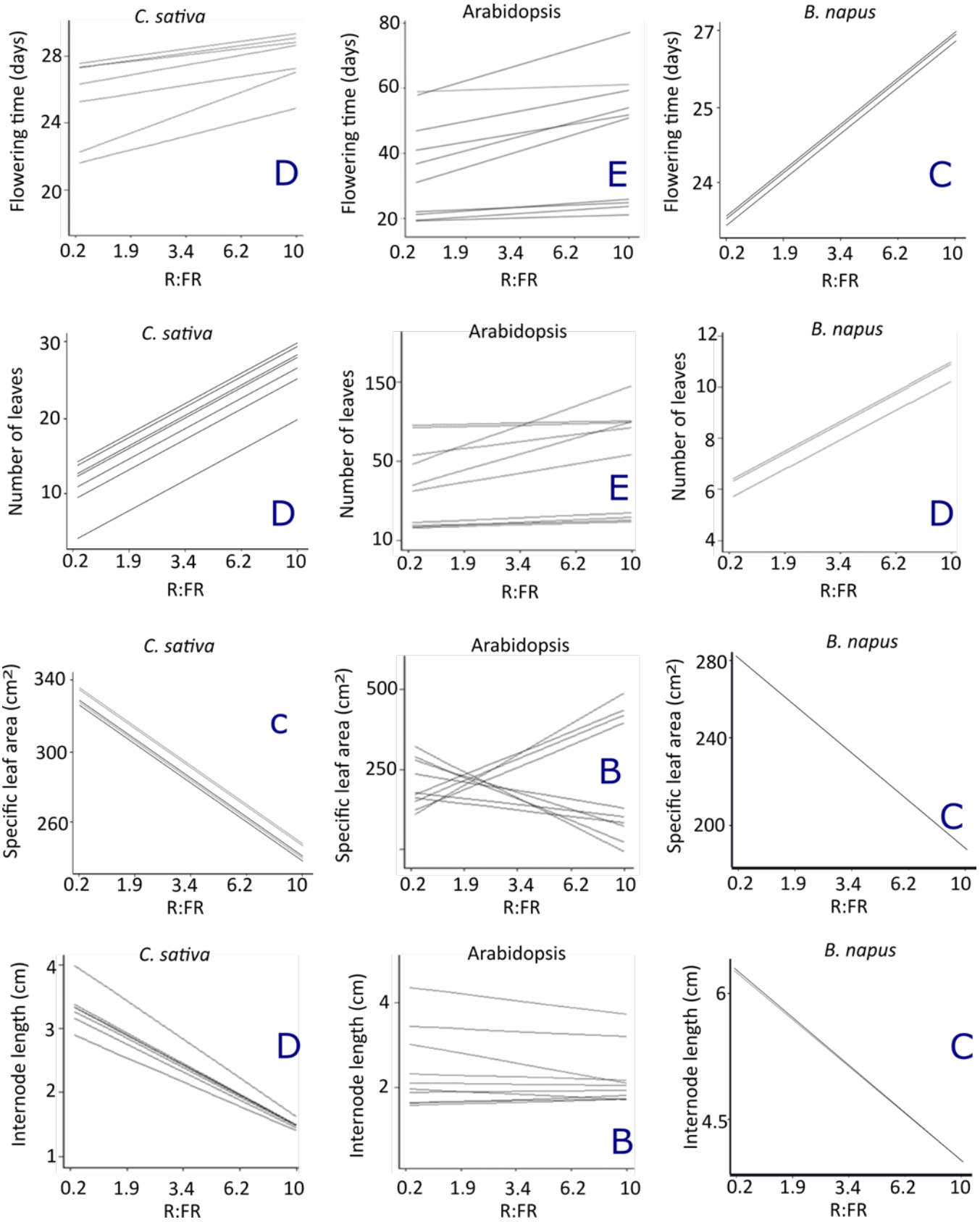
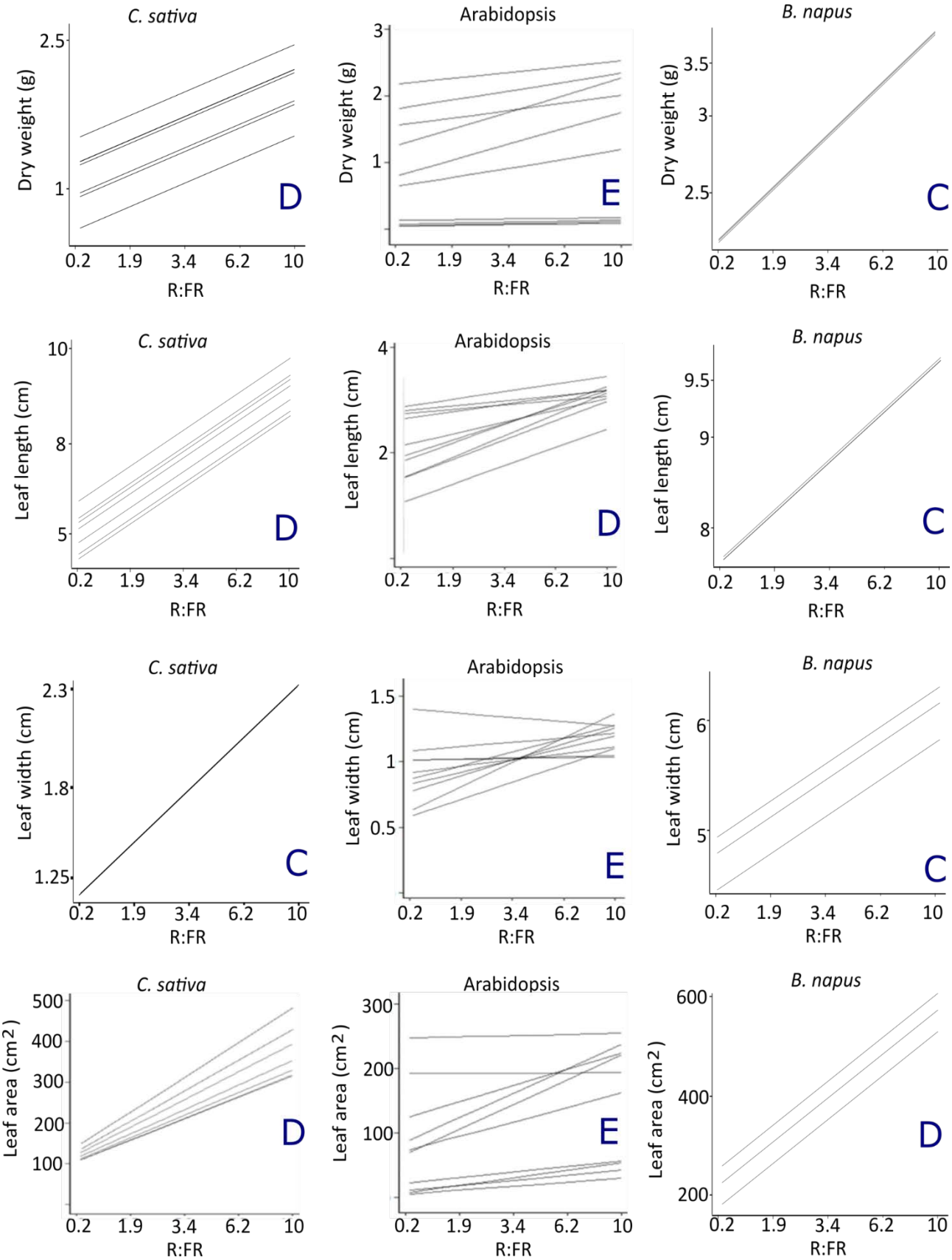
4.8. Supplementary figures

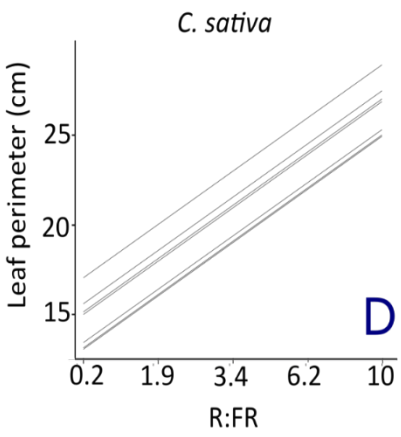
Supplementary Figure 4.1: Spectral composition of light environments used in this study.

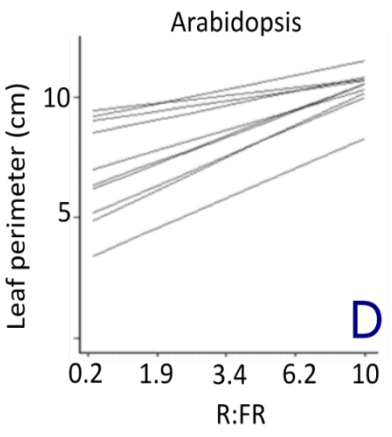


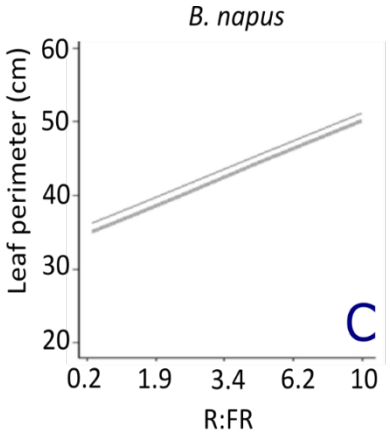
Supplementary Figure 4.2: The reaction norm to R:FR in three Brassicaceae species. The norms were calculated using mixed regression model. Lines represent accessions used in each species (Camelina n=7, Arabidopsis n=10 and canola n=3).

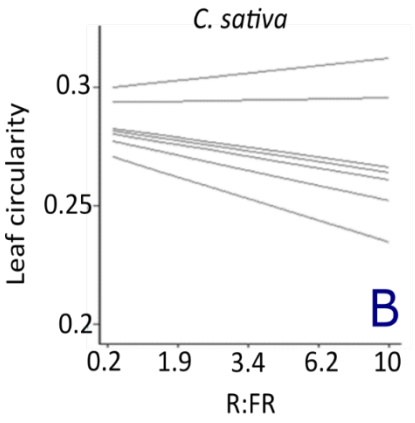


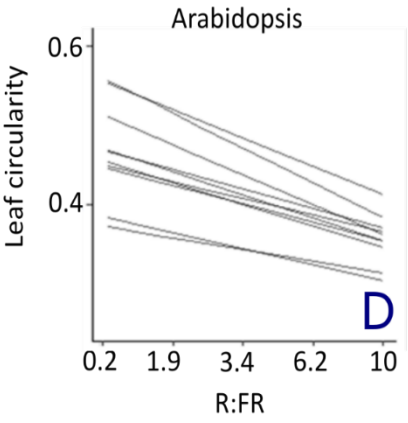

C. sativa
Dry weight (g)
2.5
1
D
0.2 1.9 3.4 6.2 10
R:FR
Arabidopsis
Dry weight (g)
3
2
1
E
0.2 1.9 3.4 6.2 10
R:FR
B. napus
Dry weight (g)
3.5
3
2.5
C
0.2 1.9 3.4 6.2 10
R:FR
C. sativa
Leaf length (cm)
10
8
5
D
0.2 1.9 3.4 6.2 10
R:FR
Arabidopsis
Leaf length (cm)
4
2
D
0.2 1.9 3.4 6.2 10
R:FR
B. napus
Leaf length (cm)
9.5
9
8
C
0.2 1.9 3.4 6.2 10
R:FR
C. sativa
Leaf width (cm)
2.3
1.8
1.25
C
0.2 1.9 3.4 6.2 10
R:FR
Arabidopsis
Leaf width (cm)
1.5
1
0.5
E
0.2 1.9 3.4 6.2 10
R:FR
B. napus
Leaf width (cm)
6
5
C
0.2 1.9 3.4 6.2 10
R:FR
C. sativa
Leaf area (cm2)
500
400
300
200
100
D
0.2 1.9 3.4 6.2 10
R:FR
Arabidopsis
Leaf area (cm2)
300
200
100
E
0.2 1.9 3.4 6.2 10
R:FR
B. napus
Leaf area (cm2)
600
400
200
D
0.2 1.9 3.4 6.2 10
R:FR

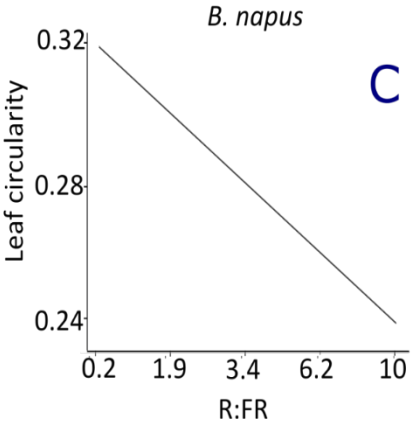

C. sativa
Leaf perimeter (cm)
25
20
15
D
0.2
1.9
3.4
6.2
10
R:FR

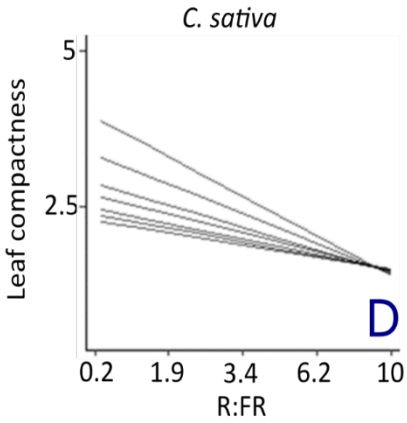

Arabidopsis
Leaf perimeter (cm)
10
5
D
0.2
1.9
3.4
6.2
10
R:FR

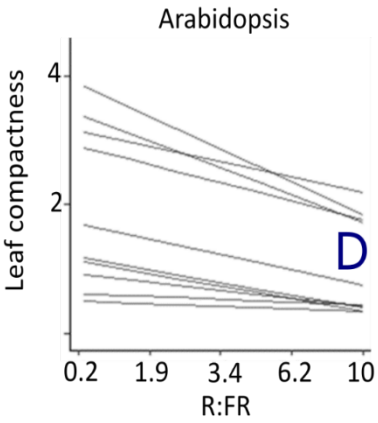

B. napus
Leaf perimeter (cm)
60
50
40
30
20
C
0.2
1.9
3.4
6.2
10
R:FR

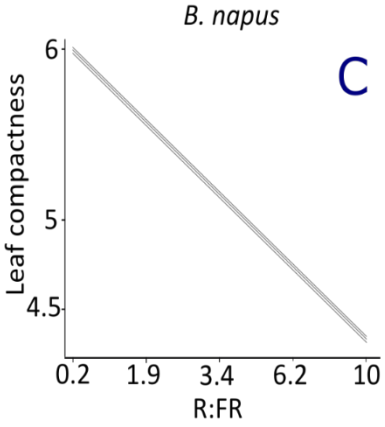

C. sativa
Leaf circularity
0.3
0.25
0.2
B
0.2
1.9
3.4
6.2
10
R:FR

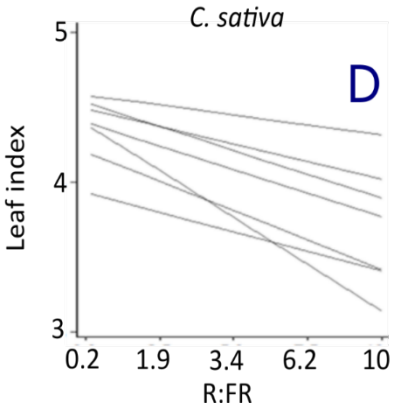

Arabidopsis
Leaf circularity
0.6
0.4
D
0.2
1.9
3.4
6.2
10
R:FR

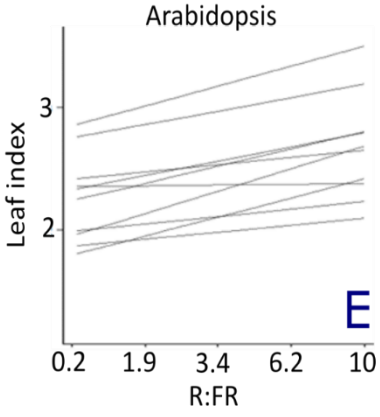

B. napus
Leaf circularity
0.32
0.28
0.24
C
0.2
1.9
3.4
6.2
10
R:FR

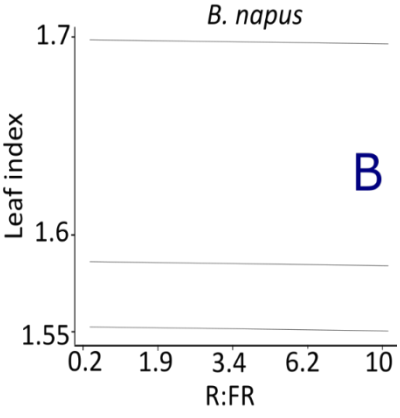

C. sativa
Leaf compactness
5
2.5
D
0.2
1.9
3.4
6.2
10
R:FR


Arabidopsis
Leaf compactness
4
2
D
0.2
1.9
3.4
6.2
10
R:FR

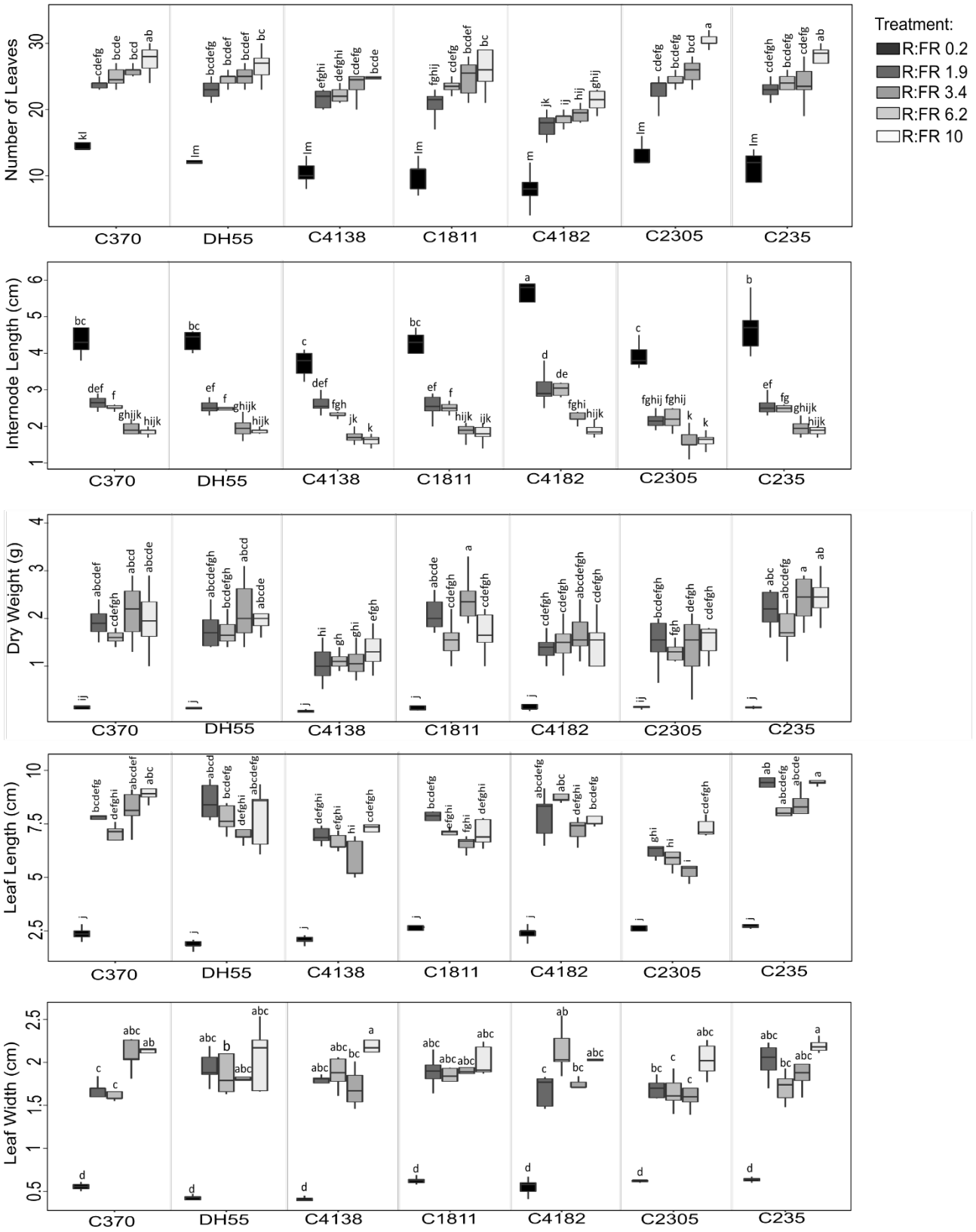

B. napus
Leaf compactness
6
5
4.5
C
0.2
1.9
3.4
6.2
10
R:FR

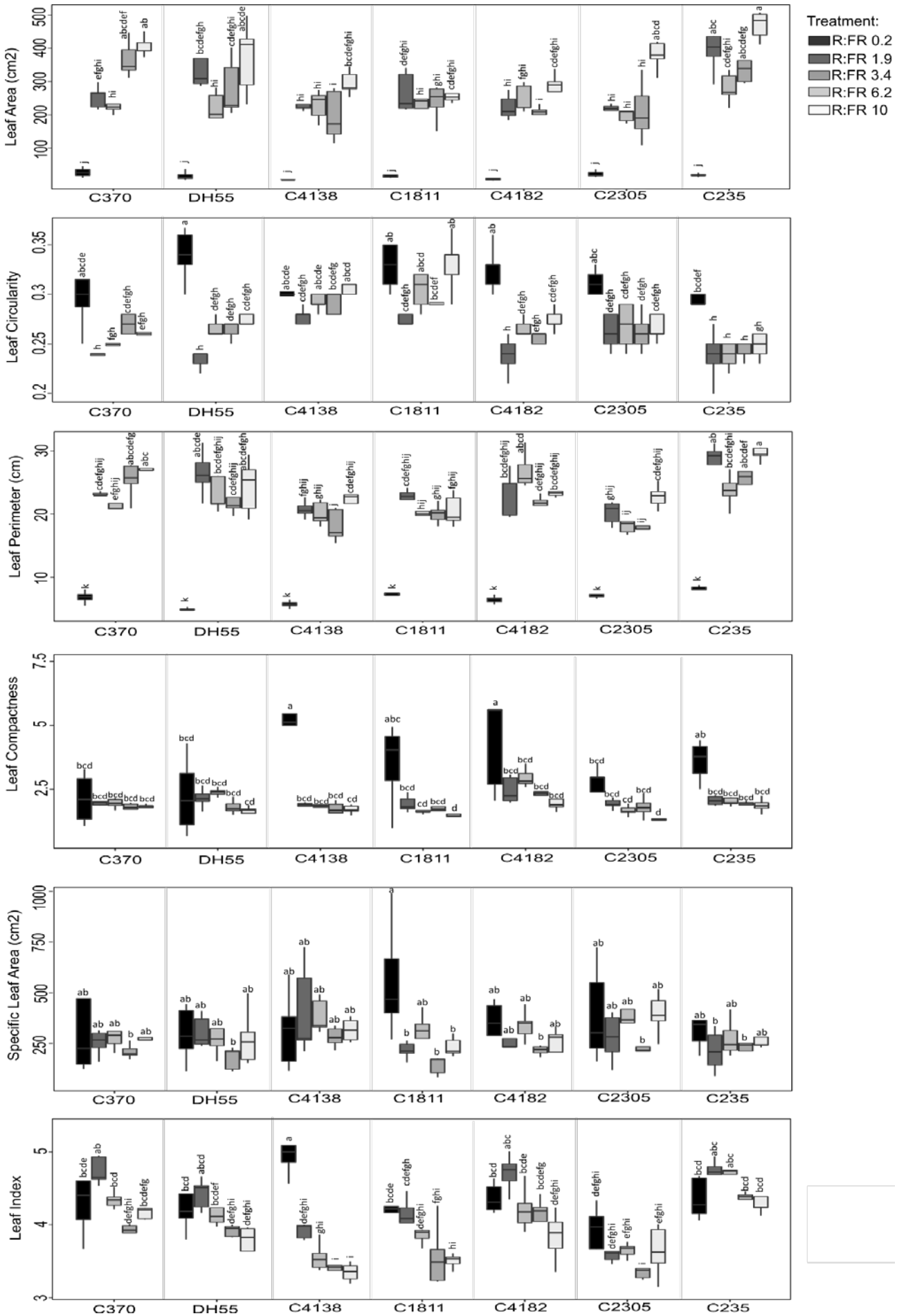

C. sativa
Leaf index
5
4
3
D
0.2
1.9
3.4
6.2
10
R:FR


Arabidopsis
Leaf index
3
2
E
0.2
1.9
3.4
6.2
10
R:FR

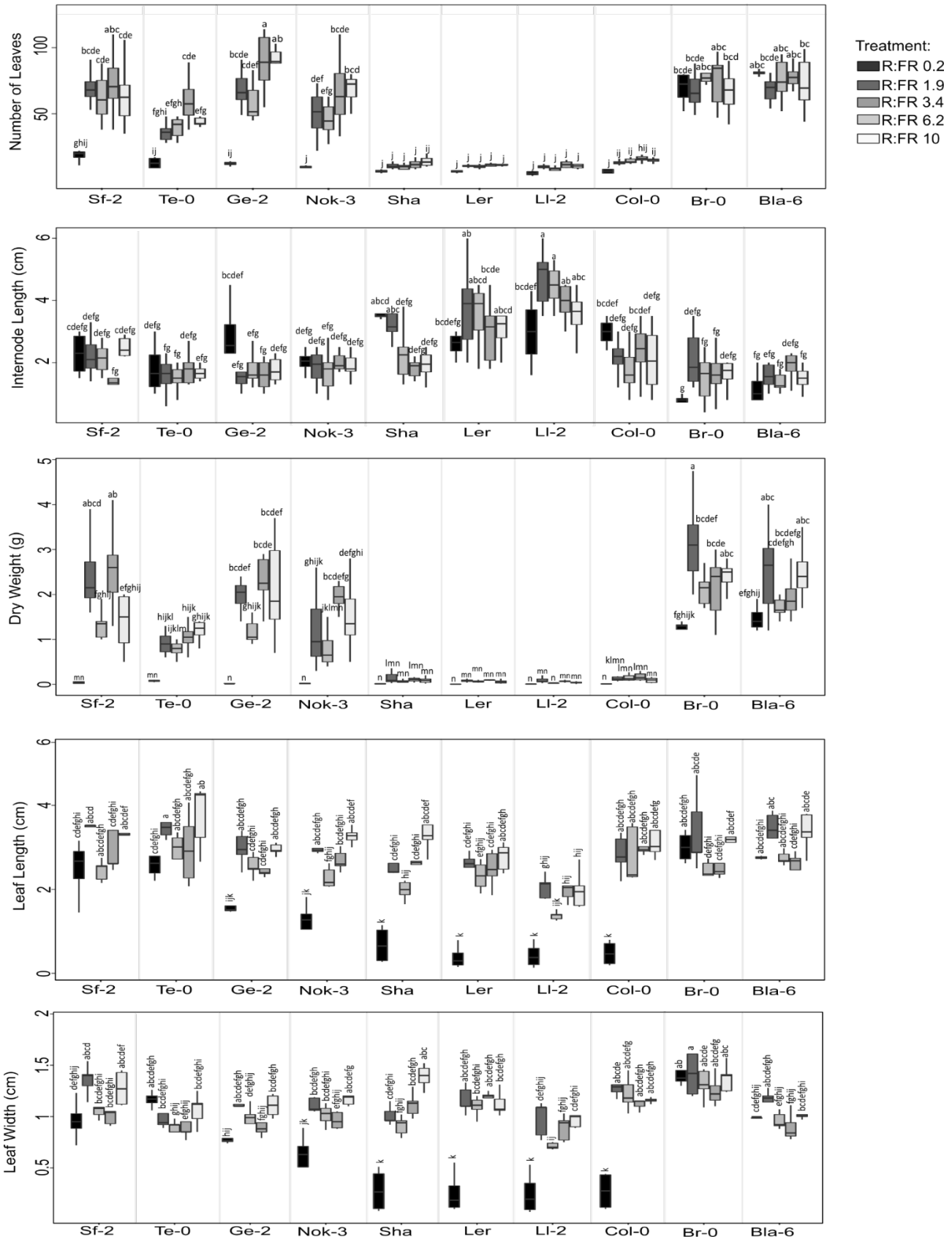

B. napus
Leaf index
1.7
1.6
1.55
B
0.2
1.9
3.4
6.2
10
R:FR

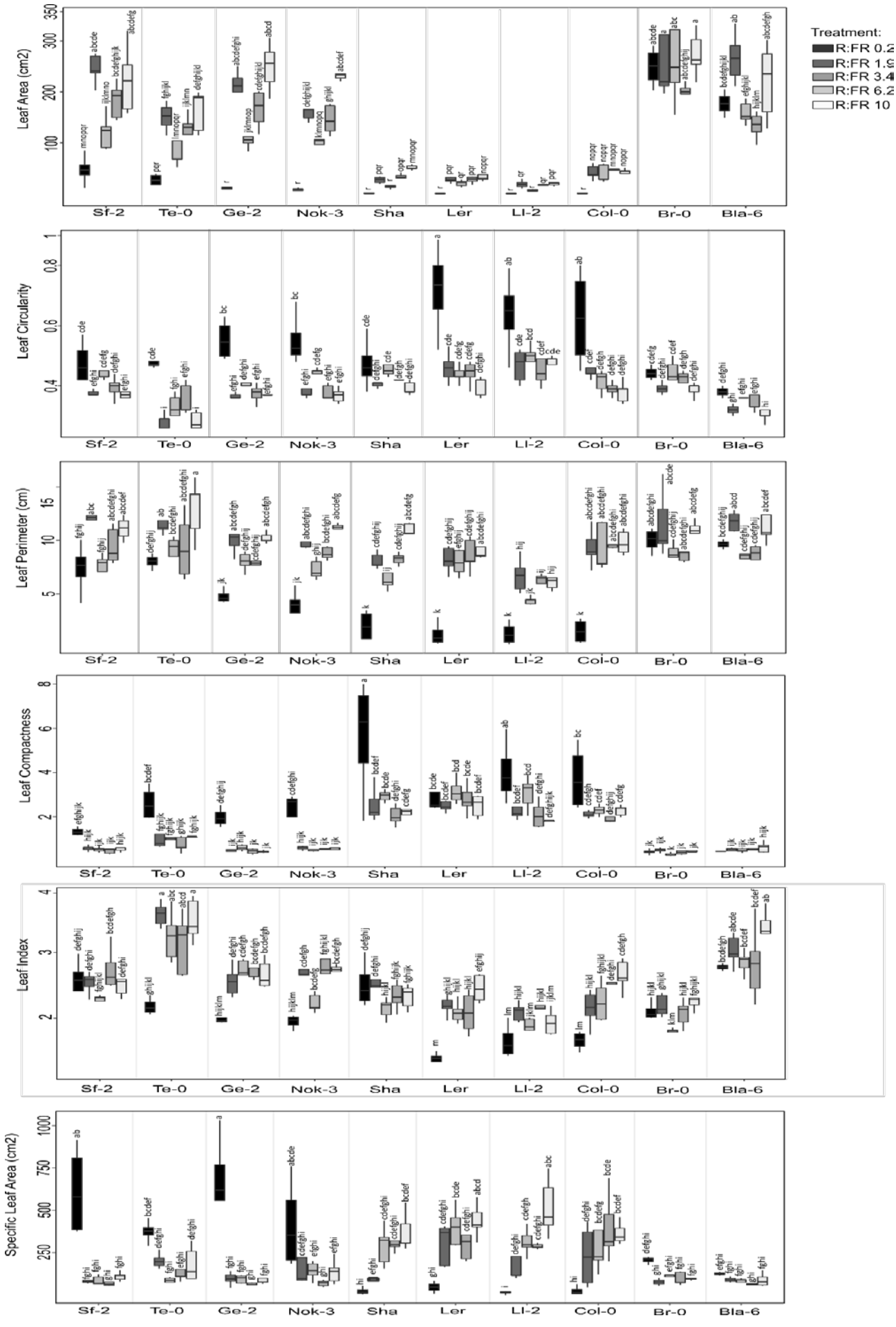
Supplementary Figure 4.3: Phenotypic responses of *Camelina sativa*. Boxplot represents ten samples across two independent experiments for R:FR 1.9-10 and four samples for R:FR 0.2. Same letter in the boxplot indicates similar means between R:FR treatments (P<0.05, Welch ANOVA test).



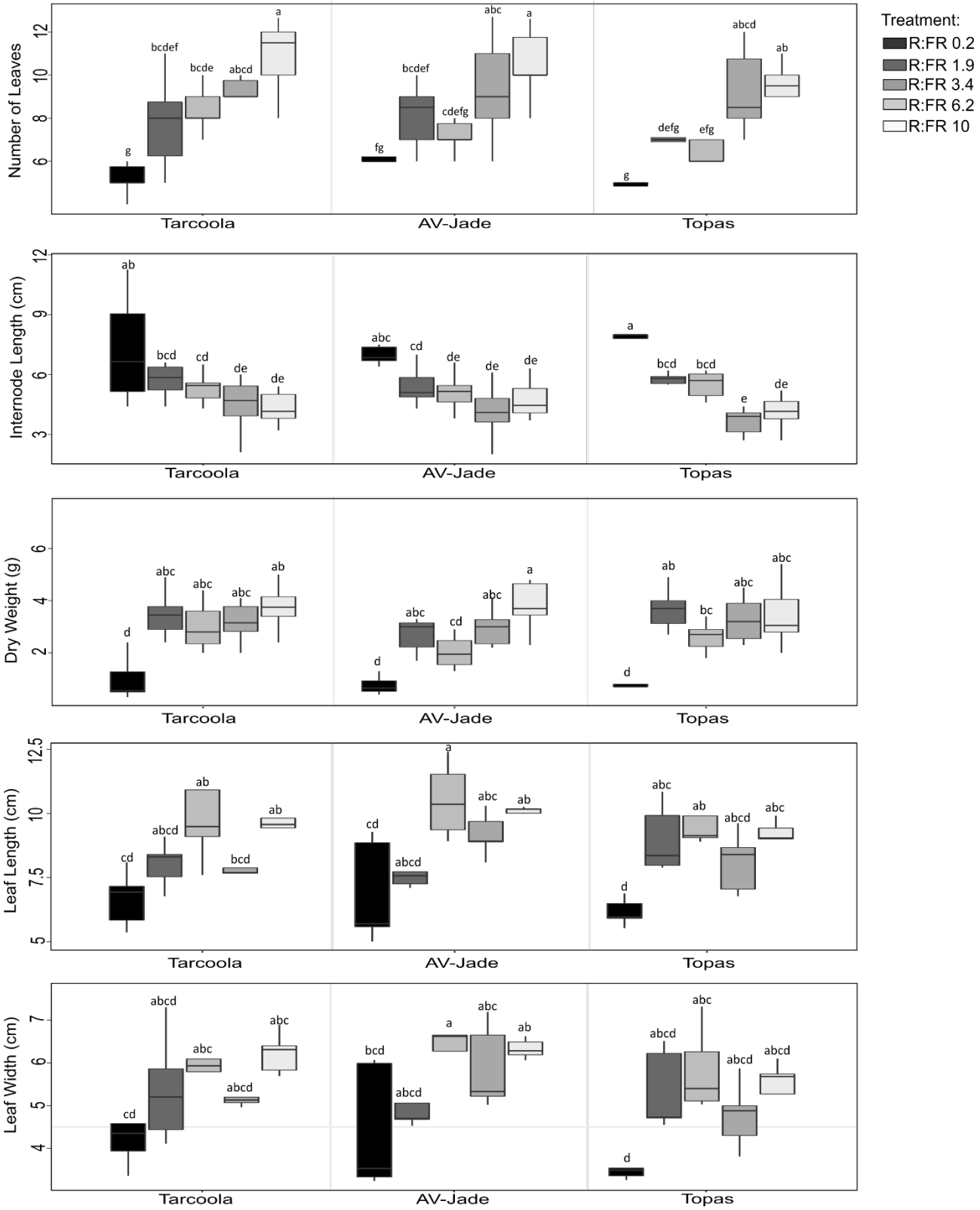

Leaf Area (cm2)
Leaf Circularity
Leaf Perimeter (cm)
Leaf Compactness
Specific Leaf Area (cm2)
Leaf Index
C370
DH55
C4138
C1811
C4182
C2305
C235
Treatment:
R:FR 0.2
R:FR 1.9
R:FR 3.4
R:FR 6.2
R:FR 10

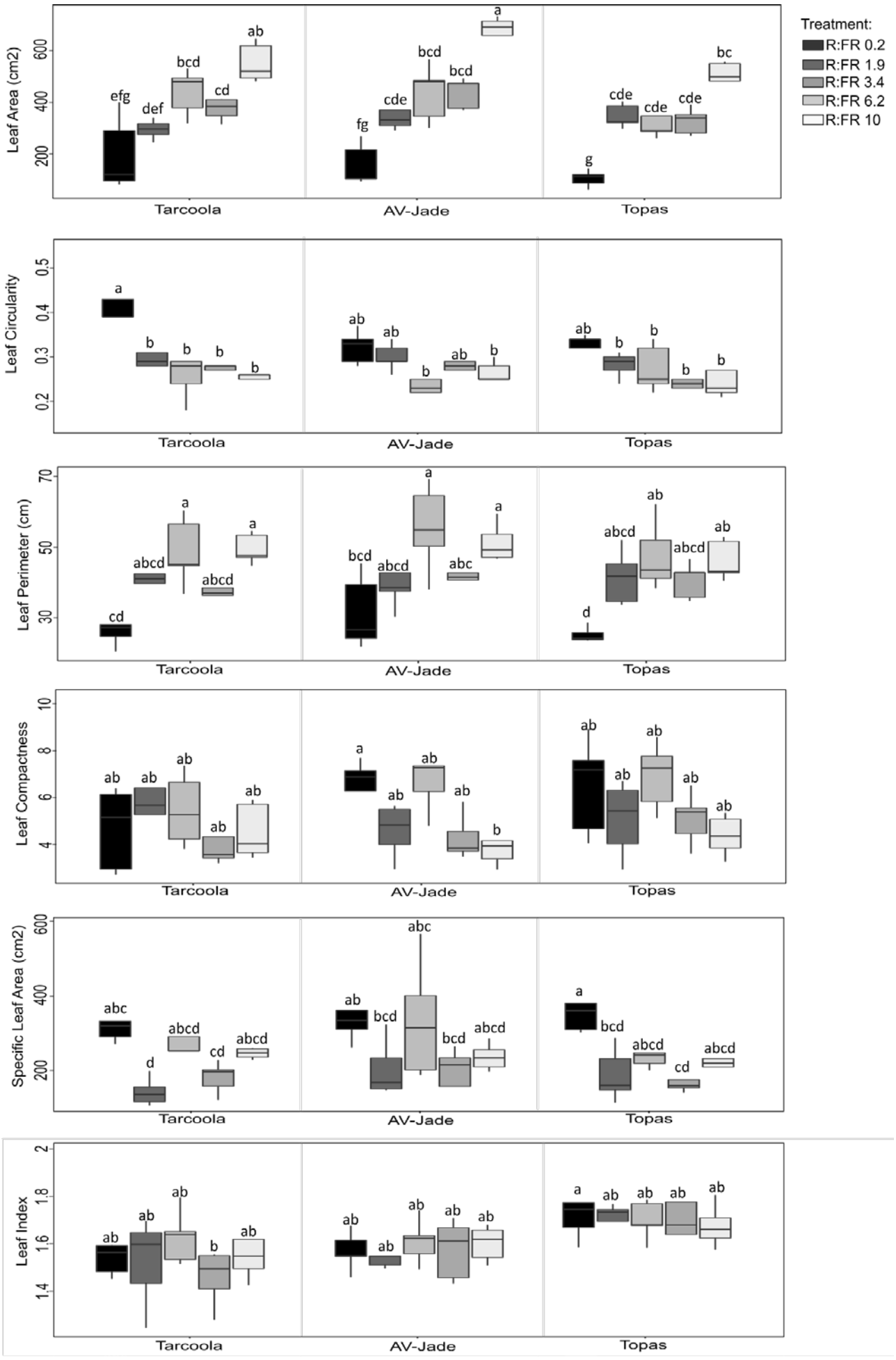
Supplementary Figure 4.4: Phenotypic responses of *Arabidopsis thaliana*. Boxplot represents ten samples across two independent experiments for R:FR 1.9-10 and four samples for R:FR 0.2. Same letter in the boxplot indicates similar means between R:FR treatments (P<0.05, Welch ANOVA test).




Leaf Area (cm2)
Leaf Circularity
Leaf Perimeter (cm)
Leaf Compactness
Leaf Index
Specific Leaf Area (cm2)
Sf-2
Te-0
Ge-2
Nok-3
Sha
Ler
Ll-2
Col-0
Br-0
Bla-6
Treatment:
R:FR 0.2
R:FR 1.0
R:FR 3.4
R:FR 6.2
R:FR 10

Supplementary Figure 5.5: Phenotypic responses of *Brassica napus*. Boxplot represents ten samples across two independent experiments for R:FR 1.9-10 and four samples for R:FR 0.2. Same letter in the boxplot indicates similar means between R:FR treatments (P<0.05, Welch ANOVA test).




Treatment:
R:FR 0.2
R:FR 1.9
R:FR 3.4
R:FR 6.2
R:FR 10
Leaf Area (cm2)
Leaf Circularity
Leaf Perimeter (cm)
Leaf Compactness
Specific Leaf Area (cm2)
Leaf Index
Tarcoola
AV-Jade
Topas

CHAPTER 5

Comparative reaction of *Camelina sativa* to *Sclerotinia sclerotiorum* and *Leptosphaeria maculans*

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5.1. Abstract

Sclerotinia sclerotiorum and *Leptosphaeria maculans* are two of the most important pathogens of many cruciferous crops. The reaction of 30 genotypes of *Camelina sativa* (L.) Crantz (false flax) was determined against both pathogens. Camelina genotypes were inoculated at seedling and adult stages with two pathotypes of *S. sclerotiorum*, highly virulent MBRS-1 and less virulent WW-1. There were significant differences (P<0.001) among genotypes, between pathotypes and a significant interaction between genotypes and pathogens in relation to percent cotyledon disease index (% CDI) and stem lesion length. Genotypes C370 (% CDI 20.5, stem lesion length 1.8 cm) and 253 (% CDI 24.8, stem lesion length 1.4 cm) not only consistently exhibited cotyledon and stem resistance, in contrast to susceptible genotype C2305 (% CDI 37.7, stem lesion length 7.2 cm), but their resistance was independent to *S. sclerotiorum* pathotype. An F_5-recombinant inbred line population was developed from genotypes C370×C2305 and responses characterised. Low broad-sense heritability indicated a complex pattern of inheritance of resistance to *S. sclerotiorum*. Six isolates of *L. maculans*, covering combinations of five different avirulent loci, were tested on Camelina cotyledons across two experiments. There was a high level of resistance, with % CDI<17, including the development of a hypersensitive reaction. This is the first report of the variable reaction of Camelina to different races of *L. maculans* and the first demonstrating comparative reactions of Camelina to *S. sclerotiorum* and *L. maculans.* This study not only provides a new understanding of these comparative resistances in Camelina, but highlights their potential as new sources of resistance, for crucifer disease resistance breeding in general and to enable broader adoption of Camelina as a more sustainable oilseed crop in its own right.

5.2. Introduction

Camelina sativa (L.) Crantz (false flax) has the potential to become an important cruciferous oilseed crop due to its high-value products and positive agronomic traits (Campbell *et al.,* 2013). Camelina oil has a unique fatty acid profile, particularly rich in *n-3* (omega-3) and has numerous potential markets in food, nutraceuticals, cosmetics, stock feeds and industrial products (*e.g.,* wax esters) (Nguyen *et al.,* 2013; Iven *et al.,* 2016; Lawrence *et al.,* 2016). However, the greatest potential for Camelina oil lies in second-generation biofuel, being a proven low-cost, non-food biodiesel feedstock in northern USA (Agusdinata *et al.,* 2011). In addition to its oil value, Camelina has agronomic features that make it an ideal crop *per se* for sustainable agriculture. Such qualities include its favourable response to low-input farming practices, pod shatter resistance and drought tolerance (Gugel & Falk, 2006; Campbell *et al.,* 2013; Waraich *et al.,* 2013). These traits, along with its short growth cycle, enable Camelina to be sown as an alternative to canola (*Brassica napus* L.), in rotation with cereals in semi-arid cropping systems and as demonstrated in The Great Plains region, USA (Obour *et al.,* 2015). An additional key trait of Camelina is its resistance to many common pests and pathogens that cause significant yield losses in canola (Séguin-Swartz *et al.,* 2009; Pavlista *et al.,* 2011).

Sclerotinia rot (SR, causal agent: *Sclerotinia sclerotiorum*) and blackleg (also known as phoma stem canker, causal agent: *Leptosphaeria maculans*) are the two most important diseases of crucifers worldwide, including canola and mustard (*Brassica juncea* L.) (Sivasithamparam *et al.,* 2005; Li *et al.,* 2008; Delourme *et al.,* 2012; Uloth *et al.,* 2013; Barbetti *et al.,* 2015). Both pathogens can infect at any stage of plant/crop development (Khangura & Barbetti, 2001; Li *et al.,* 2008; Uloth *et al.,* 2013, 2014, 2015). In Australia, yield losses from either disease have exceeded 50% (Sivasithamparam *et al.,* 2005; GRDC, 2013) and SR alone causes losses of AUD 23 million in Western Australia (DAFWA, 2015). Compared with canola and mustard, relatively few studies have addressed either disease in Camelina, despite Camelina genotypes known to show variation for resistance to SR (*e.g.,* Eynck & Séguin-Swartz, 2009). Using a high genetic diversity Camelina germplasm collection (Ghamkhar *et al.,* 2010), we previously showed variable response to SR across 30 Camelina genotypes at the seedling stage, observing a range of response from mildly susceptible to resistant (Purnamasari *et al.,* 2015). However, there can be inconsistency in the expression of resistance against SR depending upon the type of inoculation test adopted (Uloth *et al.,* 2013, 2014) and/or pathotype (Ge *et al.,* 2012; Barbetti *et al.,* 2014; Neik *et al.,* 2017; Willbur *et al.,* 2017). Previously, Ge *et al.* (2012) identified eight distinct *S. sclerotiorum* pathotypes from 53 isolates obtained from the agricultural regions of Western Australia, isolates taken from infested stems of canola and lupin that

showed severe Sclerotinia stem rot disease. Accordingly, it is important to define SR resistance in Camelina, use more than one type of inoculation test, and compare resistance expression using contrasting pathotypes. Therefore, there is a clear need to further explore resistance against *S. sclerotiorum* within this collection of Camelina and determine the resistance mechanism to SR. Towards this aim, subsequent to the earlier research of Purnamasari *et al*. (2015), we first developed a 141 genotype recombinant inbred line (RIL) population from resistant × susceptible parents identified in that study.

Camelina sativa reportedly exhibits very high resistance to *L. maculans* (Salisbury, 1987; Séguin-Swartz *et al.,* 2009). For example, Li *et al*. (2005) found Camelina R4175-01W2 developed no symptoms to 80 isolates of *L. maculans*. Similarly, Gregorich *et al*. (2009) found no disease symptoms in four Camelina varieties inoculated with two races of *L. maculans*. Field trials have confirmed Camelina to be very highly resistant or immune to blackleg disease (Séguin-Swartz *et al.,* 2009). Notably, these historical studies have only involved relatively few genotypes and/or *L. maculans* races. Hence, there was a clear need to evaluate a diverse Camelina collection for responses to inoculation with a wider range of *L. maculans* races.

Towards meeting these needs, studies were undertaken to 1) determine the reaction of 30 diverse genotypes of Camelina against two different pathotypes of *S. sclerotiorum* at cotyledon and adult stages and different races of *L. maculans* at cotyledon stages; 2) characterise (at the cotyledon stage) 141 $F_{5–6}$ RILs derived from crossing a resistant with a susceptible genotype of Camelina, for their responses to *S. sclerotiorum*. We discuss a new understanding of comparative resistances to these diseases in Camelina and highlight the value of potential new sources of resistance to both diseases for breeding across a range of oilseed and horticultural crucifers.

5.3. Materials and methods

5.3.1. Fungal isolates

The isolates of *S. sclerotiorum* and *L. maculans* used in this experiment are listed in Table 5.1. Criteria for selecting the isolates were based on their differences in pathogenic potential on Camelina. For *S. sclerotiorum,* two different pathotypes were chosen: 1) isolate MBRS-1, a very aggressive isolate belonging to the prevailing pathotype (pathotype 76) occurring in Western Australia (Ge *et al.,* 2012) and has been used extensively for screening crucifers (*e.g.,* Uloth *et al.,* 2013) and for Camelina (Purnamasari *et al.,* 2015); 2) isolate WW-1 (pathotype unknown) a less aggressive isolate that causes distinctly different relative resistance/susceptibility rankings across canola genotypes as compared

with MBRS-1 (Garg *et al.*, 2010). For *L. maculans*, the isolates chosen were: UWA192 (preliminary experiment), as it is a highly virulent isolate on canola genotypes containing single dominant gene-based resistance from *Brassica rapa* ssp. *sylvestris* (Li *et al.*, 2004a), and isolates UWAM3, UWAP11, WAC4028, WAC4094 and WAC7803 (second experiment) based on their avirulent (Avr) loci for which the isolate is avirulent and represented races Av1-4-5-7-8, Av1-5-6-7, Av3-5-6, Av1-3-5-6-8 and Av6-9 (Balesdent *et al.*, 2005). Furthermore, isolate UWAP11 is also known to be highly virulent on canola genotypes containing polygenic resistance (Li *et al.*, 2003).

Table 5.1: *Sclerotinia sclerotiorum* and *Leptosphaeria maculans* isolates used in this study.

Isolate code	Species	Date	Origin	Source (isolate located)
MBRS-1	*S. sclerotiorum*	2004	Mount Barker, Western Australia	Infected stem tissue of canola.
WW-1	*S. sclerotiorum*	2004	Walkaway, Western Australia	Infected stem tissue of canola.
LH192	*L. maculans*	2002	Mount Barker, Western Australia	Infected stem tissue of canola cv. Hyola 60
UWAM3	*L. maculans*	2001	Mount Barker, Western Australia	Infected stem tissue of *B. juncea* cv. Roy 394
UWAP11	*L. maculans*	2001	Wongan Hills, Western Australia	Infected stem tissue of canola cv. Pinnacle
WAC4028	*L. maculans*	1984	Mount Barker, Western Australia	Infected stem tissue of canola cv. Wesreo
WAC4094	*L. maculans*	1984	Western Australia	Infected stem tissues of canola cv. Wesway
WAC7803	*L. maculans*	1973	Western Australia	Infected leaf tissue of *Raphanus raphanistrum*

5.3.2. Plant material

Thirty genotypes of Camelina obtained from the N.I. Vavilov Research Institute of Plant Industry, Russia, originating from five countries (former Czechoslovakia, France, Russia, Sweden and Ukraine), were evaluated (Table 5.2). These genotypes are well characterised for agronomic performance, fatty acid analysis, molecular, ecogeographic analysis and their cotyledon resistance against *S. sclerotiorum* MBRS-1 (Ghamkhar *et al.*, 2010; Campbell *et al.*, 2013; Purnamasari *et al.*, 2015). Canola cv. Mystic and 06P712 were used as check comparisons in *S. sclerotiorum* studies and cv. Mystic was used as a check comparison in *L. maculans* studies. The response of canola cv. Mystic and 06P712 to *S. sclerotiorum* has been defined with isolates MBRS-1 and/or WW-1 (Li *et al.*, 2006; Garg *et al.*, 2010; Ge *et al.*, 2012; Uloth *et al.*, 2013, 2014, 2015; You *et al.*, 2016). In the current study, a 141-line F_5-RIL population was established by single seed descent from a cross between resistant C370 and susceptible C2305. The two parents for this population were chosen based on their response to *S. sclerotiorum* MBRS-1 using cotyledon inoculation (Purnamasari *et al.*, 2015). All plants were grown in 1 L pots in a pasteurised soil mixture composed of finely crushed pine bark/coco peat/sand at 2.5:1.0:1.5 (wt/wt). Plants were watered daily and fertilised weekly using Thrive™ all-

purpose soluble fertiliser at the recommended rate. All experiments were conducted within a growth room set to 18/14°C (day/ night) with a 16 h light/8 h dark cycle and light intensity of 320 µmol quanta m^{-2} s^{-1}.

5.3.3. Screening tests for *S. sclerotiorum*

A screening test was carried out using two different methods: 1) cotyledon inoculation for isolate WW-1 and 2) stem inoculation for isolates MBRS1 and WW-1. Inoculum preparation and cotyledon assay were performed as described by Garg *et al*. (2008). Briefly, two plants per pot were grown in 30-cell trays (85 mL Kwikpot Trays, each cell 55 mm in diameter) until cotyledons were fully expanded, equivalent to growth stage 1.00 the Sylvester-Bradley & Makepeace (1984) scale. Seven agar plug discs were cut from actively growing margins of 3-day-old colonies of *S. sclerotiorum* growing on potato dextrose agar at 20°C and used to inoculate 150 mL of sterilised potato dextrose broth containing peptone (potato dextrose broth 24 g, peptone 10 g, H_2O 1 L). Cultures were placed on a rotary shaker at 150 rpm at 20°C. After three days, colonies of *S. sclerotiorum* were collected and washed twice with deionised water. The fungal mats were then transferred to 125 mL of the same liquid medium and macerated using a hand-blender for 3 minutes. The mycelial suspension was filtered through four layers of cheesecloth to remove any large mycelial components, and the density determined using a haemocytometer and concentration adjusted to 1×10^5 fragments mL^{-1}.

For cotyledon inoculation, a single 5 µL droplet of WW-1 mycelium suspension was deposited on each lobe of each cotyledon of the 30 Camelina genotypes using a micropipette. The inoculum was shaken often to maintain a homogenous mycelial suspension. Inoculum suspension contained 0.002% Tween 20 wetting agent to help the cotyledons retain the droplets; Tween was included with deionised water used to inoculate control plants of all genotypes. Plants were kept in the dark in 35 L clear plastic storage boxes with a 2.5 cm depth of water at the bottom of the boxes to maintain high humidity conditions after inoculation. Disease development was assessed at 72 h post-inoculation for *S. sclerotiorum* on 0–9 disease severity scale as used by Purnamasari *et al*. (2015), where: 0 = no visible symptoms, 1 = necrotic hypersensitive, 2 = necrotic or water-soaked lesion (10% of total leaf area), 3 = necrotic or water-soaked lesion (20%), 4 = necrotic or water-soaked lesion (20–30%), 5 = necrotic or water-soaked lesion (30–40%), 6 = necrotic or water-soaked lesion (40–50%), 7 = necrotic or water-soaked lesion (50–60%), 8 = collapsing of cotyledon tissue and 9 = collapsing of cotyledon tissue with masses of mycelium. The disease scores were converted into a percent cotyledon disease index (% CDI) using the method of McKinney (1923), as follows:

$$CDI\ (\%) = \frac{[(a \times 0) + (b \times 1)\ + (c \times 2)\ + (d \times 3)\ + (e \times 4) + \ldots (j \times 9)] \times 100}{(a + b + c + d + \ldots j)\ \times 9}$$

where *a, b, c, d, e … j* are the number of plants with disease scores of 0, 1, 2, 3, 4, … 9, respectively. There were four replications arranged in complete randomised block design, and the entire experiment was repeated once.

Stem inoculation of *S. sclerotiorum* was carried out in a controlled environment using the method described by Li *et al*. (2006), which is a combination of the methods used by Li *et al*. (2004b) and Buchwaldt *et al*. (2005). About 5 mm diameter mycelial discs from an actively growing 48 h culture grown on glucose-rich medium (peptone 10 g, glucose 20 g, agar 18 g, KH_2PO_4 0.5 g, H_2O 1 L, pH 6.0 before autoclaving) was placed onto the stem above the first node by wrapping with Parafilm® tape. The same diameter disc of glucose-rich medium without fungal inocula was used for control-treated plants. Five plants of each genotype were used for inoculation when 50% of the plants had at least one open flower. Plants were irrigated immediately post-inoculation with overhead misting for 10 minutes and the misting repeated on each of the following three days to maintain conducive conditions for *S. sclerotiorum*. Stem lesion lengths were measured with a linear ruler three weeks after inoculation, as this timing provides disease data that is independent of plant maturity (Li *et al.*, 2007a). The experiment was arranged as a complete randomised block design and repeated once.

5.3.4. Screening tests for *L. maculans*

A screening test for *L. maculans* was carried out in two experiments. Experiment 1 was an initial experiment using 30 genotypes of Camelina with *L. maculans* UWA192; the results of this experiment enabled the selection of six genotypes rated as resistant (two genotypes), intermediate (two genotypes) and susceptible (two genotypes) based on the rank order of % CDI (LSD test, Table 5.4). These six genotypes were again challenged with five isolates covering five races of *L. maculans* to confirm the response of Camelina to these races (Experiment 2). Conidial suspensions for *L. maculans* were prepared as described by Li *et al*. (2005). Agar strips (0.5 × 1 cm) from actively growing cultures containing mature pycnidia were each transferred to 1 mL deionised water and left until a suspension of conidia was evident. V8 agar plates (V8 juice 150 mL, $CaCO_3$ 1.5 g, agar 15 g, H_2O 1 L) were spread evenly with 100 µL of the conidial suspension and incubated at 22°C under a single cool-white fluorescent light tube and a single black light tube. After 7 days, the culture was flooded with 10 mL of deionised water and gently rubbed with a glass rod. The conidial suspension was filtered with Mira cloth (Calbiochem, La Jolla, USA), density determined with a haemocytometer and the concentration adjusted to 1×10^7 spores mL^{-1}.

Fully developed cotyledons (equivalent to Sylvester-Bradley (1984) growth stage 1.00) of Camelina were inoculated using standard procedures as developed for canola (Li *et al.*, 2005). Cotyledons were punctured once with a stainless-steel needle before inoculation at the puncture point by deposition of

5 µL of the conidial suspension onto each half-cotyledon. Disease severity was scored 14 days post-inoculation using a scale modified from Williams (1985): 0 = no visible symptoms, 1 = necrotic hypersensitive, 2 = Small lesion expanding in inoculation spot (<0.5 mm), 3 = Small lesion expanding in inoculation spot (0.5–1 mm), 4 = Collapsed spot (1 mm), 5 = Collapsed spot (2–3 mm), 6 = Collapsed spot (3–4 mm), 7 = Collapsed spot (4–5 mm), 8 = Collapsed spot (>5 mm) and 9 = Cotyledon has died. The disease scores were converted into a percent cotyledon disease index (% CDI) as described above. There were four replications arranged in complete randomised block design for each experiment, with the experiment repeated once.

5.3.5. Statistical analyses

GenStat software (18th ed.; VSN International) was used for statistical analysis of data. The statistical *t*-test in GenStat were used to compare the disease data from the original and repeat experiments in each study. As there were no differences between the experiments detected (*i.e.*, $P>0.05$), data for both experiments in each study were pooled and analysed as a single dataset. The % CDI for *S. sclerotiorum* MBRS-1 (Table 5.3) was extracted from Purnamasari *et al.* (2015). The % CDI for cotyledons and lesion length for stems were analysed using analysis of variance (ANOVA). Fisher's least significant differences ($P<0.05$) were used to separate significant differences between means and calculate the genotype rank order. The unique relative ranking score for each genotype is provided within brackets, where 1 represents the most resistant genotype, and the largest relative genotype ranking score represents the most susceptible genotype. This rank order then was used to classify the genotypes into the most resistant, intermediate resistant and most susceptible genotypes. Regression analysis was undertaken using the regression function in Microsoft Excel to determine the relationship between cotyledon assays and stem inoculation. The broad-sense heritability (H^2) for % CDI was calculated from the ANOVA table using a method described by Fehr (1991), as follows:

$$H^2 = \frac{\left(\frac{MS_g - MS_r}{R}\right)}{\left(MS_r + \left(\frac{MS_g - MS_r}{R}\right)\right)} \times 100\%$$

where MS_r is mean square error, MS_g is mean square genotype and R is the number of replicates.

5.4. Results

5.4.1. Response of Camelina genotypes to *S. sclerotiorum*

Stem inoculation

For plants inoculated with *S. sclerotiorum* isolates MBRS-1 and WW-1, there were significant effects ($P<0.001$) of genotype for the severity of stem lesions (expressed as lesion length) at three weeks' post-inoculation (wpi) (Table 5.2, Figure 5.1). For MBRS-1, genotype C253 was the most resistant with mean stem lesion length of 1.62 cm, while genotype C2305 was the most susceptible with lesion length of 11.73 cm. For WW-1, genotype C2292 was the most resistant with stem lesion length of 0.4 cm, and genotype C4112 was the most susceptible with stem lesion length of 3.4 cm. There were differences in virulence between the two *S. sclerotiorum* isolates ($P<0.001$) across Camelina and canola genotypes. Isolate MBRS-1 was more virulent with a mean stem lesion length of 4 cm compared with WW-1 with a mean stem lesion length of 1.4 cm. Moreover, there was a significant interaction between genotype and isolates ($P<0.001$), indicating that the genotype response is influenced by isolate. For example, genotype C4412 was a middle-ranking genotype against MBRS-1 but ranked 32nd for WW-1 based on lesion length (LSD test, Table 5.2 and 5.3). However, some highly susceptible genotypes (*e.g.,* C4074 and C2305) were similarly susceptible against either isolate. In addition, canola cv. Mystic and 06P712 were among the most resistant genotypes from canola or Camelina, with mean stem lesion length approximately 2.3 for MBRS-1 and 1.1 for WW-1.

Camelina symptoms included leaf wilting as early as four days' post-inoculation (dpi) and a necrotic and bleached lesion by 1 wpi. At 3 wpi, the most resistant response showed a very small lesion, accompanied by hypersensitive-type reaction (Figures 5.1A and B). In contrast, stems of some other Camelina genotypes showed intermediate-sized necrotic lesions (2 to ≤6 cm length), demonstrating moderate resistance (Figure 5.1C–E). The most susceptible and severely affected plants were observed after inoculation with MBRS-1 and showed the lesion extending and girdling the stem (>6 cm), causing stem collapse and plant death (Figure 5.1F and G).

Table 5.2: Mean stem lesion length (cm) of 30 *Camelina sativa* and *Brassica napus* genotypes inoculated with different isolates of *S. sclerotiorum* (MRBS-1 and WW-1). Each genotype's unique relative ranking score is provided within brackets, where 1 represents the most resistant genotype, and the largest relative genotype ranking score represents the most susceptible genotype.

Genotype	Origin	Species	MBRS-1 Mean lesion length (cm)		WW-1 Mean lesion length (cm)	
C253	Russia	Camelina	1.6	(1)	1.2	(14)
C344	Russia	Camelina	1.7	(2)	1.3	(19)
C4059	Russia	Camelina	1.7	(3)	1.5	(25)
C2504	Russia	Camelina	1.8	(4)	1.9	(26)
C4177	former Czechoslovakia	Camelina	1.9	(5)	1.5	(23)
Mystic	Australia	Canola	2.3	(6)	1.1	(11)
06P712	China	Canola	2.4	(7)	1.1	(12)
C235	Russia	Camelina	2.5	(8)	0.8	(5)
C339	Russia	Camelina	2.5	(9)	1.3	(17)
C1993	Russia	Camelina	2.6	(10)	0.4	(2)
C370	Russia	Camelina	2.7	(11)	0.9	(6)
C3364	Ukraine	Camelina	2.8	(12)	1.0	(9)
C2292	Russia	Camelina	2.8	(13)	0.4	(1)
C4112	Russia	Camelina	2.8	(14)	3.4	(32)
C349	Russia	Camelina	2.9	(15)	1.4	(21)
C4182	Russia	Camelina	3.0	(16)	1.0	(10)
C4139	Russia	Camelina	3.2	(17)	1.5	(24)
C1811	France	Camelina	3.5	(18)	1.1	(13)
C430	Russia	Camelina	3.9	(19)	1.0	(8)
C4183	Russia	Camelina	4.1	(20)	0.5	(3)
C4138	Russia	Camelina	4.3	(21)	1.3	(20)
C4077	Russia	Camelina	4.4	(22)	2.2	(29)
C1330	Russia	Camelina	4.9	(23)	0.9	(7)
C3347	Ukraine	Camelina	4.9	(24)	1.2	(16)
C4130	Ukraine	Camelina	5.0	(25)	0.7	(4)
C4068	Russia	Camelina	5.1	(26)	1.3	(18)
C2495	Russia	Camelina	6.0	(27)	1.2	(15)
C4111	Russia	Camelina	6.1	(28)	2.0	(27)
C403	Russia	Camelina	6.4	(29)	1.4	(22)
C4164	Sweden	Camelina	7.4	(30)	2.0	(28)
C4074	Russia	Camelina	8.6	(31)	2.7	(30)
C2305	Russia	Camelina	11.7	(32)	2.7	(31)
Mean			*4.0*		*1.4*	

Significance of genotypes for MBRS-1 $P<0.001$; l.s.d ($P<0.05$)=3.7
Significance of genotypes for WW-1 $P<0.001$; l.s.d ($P<0.05$)=0.8
Significance of isolates $P<0.001$; l.s.d ($P<0.05$)=0.5
Significance of genotypes x isolates $P<0.001$; l.s.d ($P<0.05$)=2.7

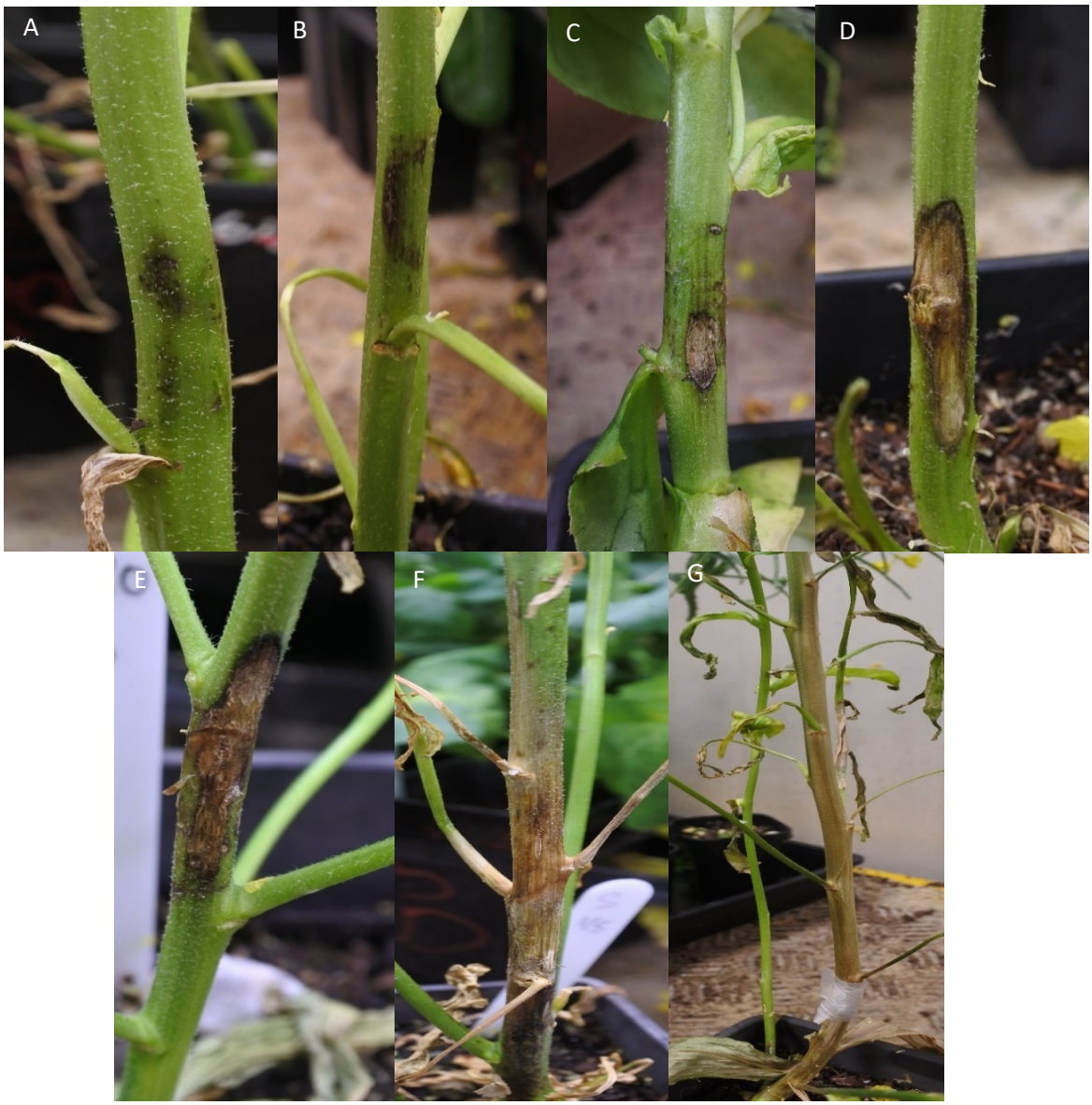

Figure 5.1: The range of stem lesion symptoms on *Camelina sativa* genotypes caused by *Sclerotinia sclerotiorum*. **A** and **B**, show a hypersensitive-type reaction, displaying a high level of resistance. **C** to **E**, show intermediate-sized stem lesions, representing moderate/intermediate resistance. **F** and **G**, show a large lesion girdling the stem, indicating extreme susceptibility.

Cotyledon inoculation: Response of 30 Camelina genotypes to two *S. sclerotiorum* isolates

Cotyledon lesion data for isolate WW-1 (current study) and, by way of comparison, isolate MBRS-1 (extracted from Purnamasari *et al.*, 2015) are presented (Table 5.3). There were differences between genotypes for % CDI at 3 dpi following inoculation with pathogenic *versus* less pathogenic isolates (P<0.001), as observed with stem inoculation. Based on the % CDI values, the most resistant genotypes were C370, C1993 and C253, with a mean % CDI ranging from 20.5–24.8, whereas C2305 was the most susceptible genotype, with a mean % CDI of 37.7. There were also differences between the two isolates (P<0.001), with a mean % CDI for MBRS-1 of 51.7 and WW-1 of 6.3. Furthermore, there was a significant host × pathogen interaction for cotyledon inoculation, similarly reported for

stem inoculation (*P*=0.002). In addition, canola cv. Mystic fell within the susceptible group, with a mean % CDI of 34.

Response of Camelina RILs population to *S. sclerotiorum* MBRS-1

The response of the set of 141 $F_{5–6}$ RIL population to *S. sclerotiorum* was evaluated along with resistant parent (C370) and susceptible donor (C2305). There were significant genotypic differences between the tested RILs and the parents for their resistance responses to cotyledon inoculation (*P*<0.001). The parents consistently differed in their reaction to *S. sclerotiorum*, with the resistant *versus* susceptible parent having % CDI values of 41.3 and 68.1, respectively. The % CDI of the RIL population lines ranged from 29.5 to 82, with the majority falling into the range of 50–70 (Figure 5.2). Of the RILs evaluated, 30 belonged to the resistant category. The broad-sense heritability of *S. sclerotiorum* resistance was 36%, indicating a low genetic variance for SR resistance in Camelina.

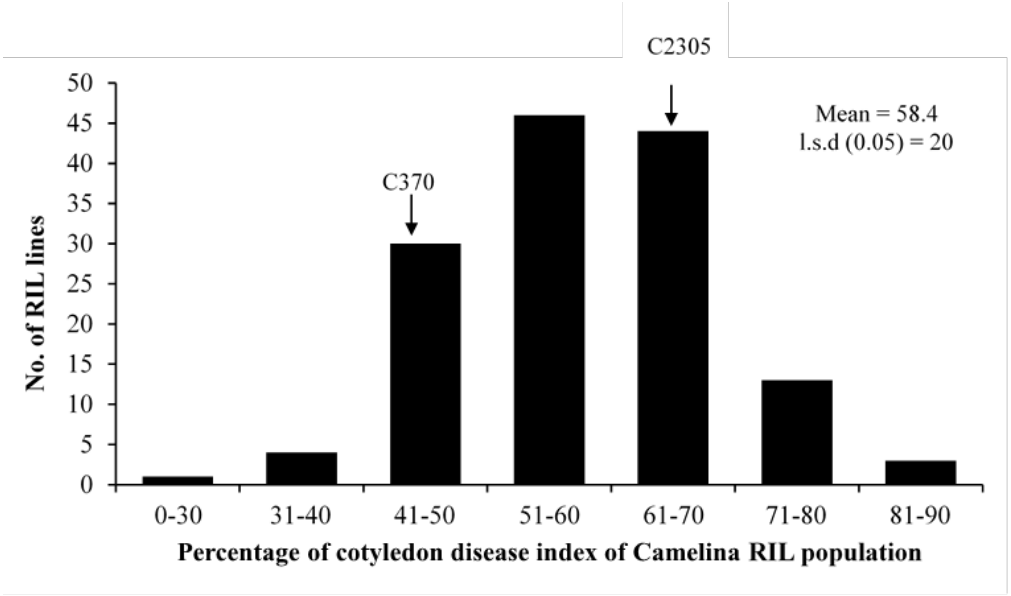

Figure 5.2: The proportion for 141 genotypes from the *Camelina sativa* C370 × C2305 RIL population against *Sclerotinia sclerotiorum.*

Table 5.3: Percent cotyledon disease index (% CDI) of 30 *Camelina sativa* genotypes and *Brassica napus* cv. Mystic inoculated with *Sclerotinia sclerotiorum* isolates (MRBS-1 or WW-1). Each genotype's unique relative ranking score is provided within brackets, where 1 represents the most resistant genotype, and the largest relative genotype ranking score represents the most susceptible genotype.

Genotype	MBRS-1[a] % CDI		WW-1 % CDI	
C370	38.9	(1)	2.1	(4)
C1993	41.7	(2)	5.9	(14)
C4068	42	(3)	9	(29)
C430	45.4	(4)	4.5	(6)
C349	46.2	(5)	5.2	(10)
C253	47.9	(6)	1.7	(3)
C4139	48.3	(7)	8.3	(23)
C4059	48.6	(8)	8.7	(28)
C4112	49.3	(9)	5.2	(9)
C344	50.4	(10)	7.6	(20)
C4130	51	(11)	6.9	(18)
C403	51.4	(12)	1.4	(2)
C4182	51.7	(13)	5.9	(12)
C4074	51.7	(14)	8	(22)
C3364	52	(15)	4.2	(5)
C235	52	(16)	1.4	(1)
C4183	52.8	(17)	6.3	(15)
C4164	52.8	(18)	8	(21)
C1330	52.8	(19)	7.6	(19)
C339	53.1	(20)	4.5	(7)
C3347	53.1	(21)	8.7	(27)
C2495	53.8	(22)	6.6	(16)
C4077	53.8	(23)	6.9	(17)
C1811	54.2	(24)	5.2	(11)
C2504	54.5	(25)	10.4	(30)
C4177	54.5	(26)	10.4	(31)
C4138	56.3	(27)	4.5	(8)
C4111	56.6	(28)	8.7	(25)
C2292	57.3	(29)	8.7	(26)
Mystic	59.7	(30)	8.3	(24)
C2305	69.4	(31)	5.9	(13)
Mean	*51.7*		*6.3*	

Significance of genotypes for MBRS-1 $P<0.001$; l.s.d ($P<0.05$)=9.9
Significance of genotypes for WW-1 $P<0.001$; l.s.d ($P<0.05$)=4
Significance of isolates $P<0.001$; l.s.d ($P<0.05$)=1.4
Significance of genotypes x isolates $P=0.002$; l.s.d ($P<0.05$)=7.9
[a]Data extracted from Purnamasari *et al*. (2015)

Comparison of stem and cotyledon inoculation assays

There was a significant quadratic relationship between mean stem lesion length and % CDI for MBRS-1 (r=0.57; P<0.001, n=31, Figure 5.3). The relative rankings of some genotypes were similar for mean stem lesion length and % CDI; examples include genotypes C253, C4111 and C2305 (Tables 5.2 and 5.3). In contrast, regression analysis showed no significant relationship between mean stem lesion length and % CDI where the less virulent *S. sclerotiorum* isolate WW-1 had been used (r=0.22).

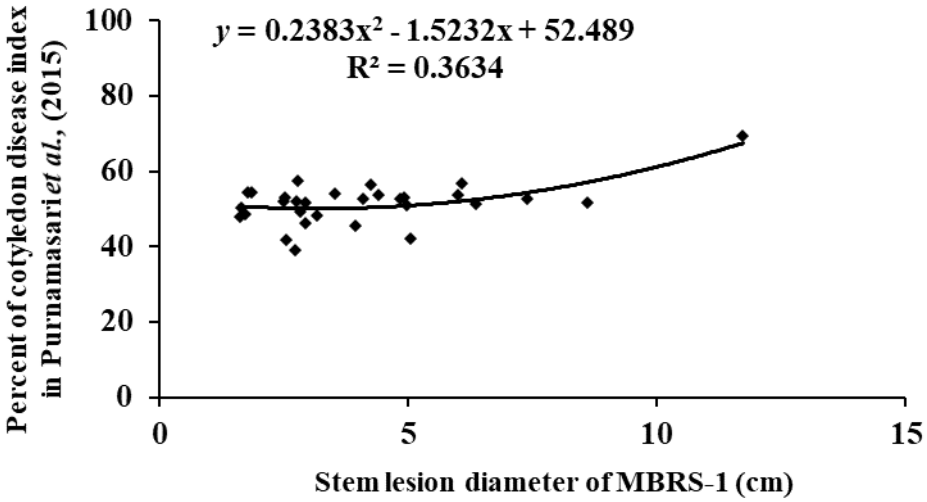

Figure 5.3: Correlation of data for stem lesion length 3 weeks after inoculation and % CDI 3 days after inoculation on 30 genotypes of *Camelina sativa* when inoculated with *Sclerotinia sclerotiorum* MBRS-1. Note: % CDI data has been extracted from Purnamasari *et al*. (2015).

5.4.2. Response of Camelina genotypes to *L. maculans*

Experiment 1

The response of 30 genotypes of Camelina screened to *L maculans* isolate UWA192 in the initial pathogenicity experiment included various symptoms, such as a small dark brown/ black necrotic area around the point of inoculation on cotyledons (Figure 5.4), and the lesions ranged from these necrotic hypersensitive lesions to larger brown necrosis (≤1 mm) around the inoculated area. Despite the relatively small differences in lesion size, these genotypic differences were significant (P<0.001) for % CDI by 14 dpi (Table 5.4). Canola cv. Mystic, the positive inoculation comparison, showed the greatest disease reaction with a % CDI of 61.5. Among Camelina genotypes, C2305 had the greatest % CDI (13.9 at 14 dpi), while C4139 had the lowest (7.6).

Table 5.4: Severity of disease on cotyledons (% CDI) of 30 *Camelina sativa* genotypes and *Brassica napus* cv. Mystic following inoculation with *Leptosphaeria maculans* UWA 192. Each genotype's unique relative ranking score is provided within brackets, where 1 represents the most resistant genotype, and the largest relative genotype ranking score represents the most susceptible genotype.

Genotype	% CDI	
C4139	7.6	(1)
C430	8.3	(2)
C1330	8.7	(3)
C370	9	(4)
C4183	9	(5)
C4130	9.4	(6)
C4177	9.4	(7)
C4068	10.8	(8)
C3347	11.1	(9)
C4077	11.1	(10)
C2504	11.1	(11)
C4112	11.5	(12)
C4164	11.5	(13)
C3364	11.5	(14)
C403	11.8	(15)
C4138	11.8	(16)
C339	11.8	(17)
C349	12.2	(18)
C1993	12.2	(19)
C253	12.2	(20)
C1811	12.2	(21)
C344	12.5	(22)
C4074	12.5	(23)
C4059	12.9	(24)
C4182	12.9	(25)
C235	12.9	(26)
C2292	13.2	(27)
C2495	13.2	(28)
C4111	13.2	(29)
C2305	13.9	(30)
Mystic	61.5	(31)

Significance of genotypes for % CDI: $P<0.001$; l.s.d. ($P<0.005$) = 4.04

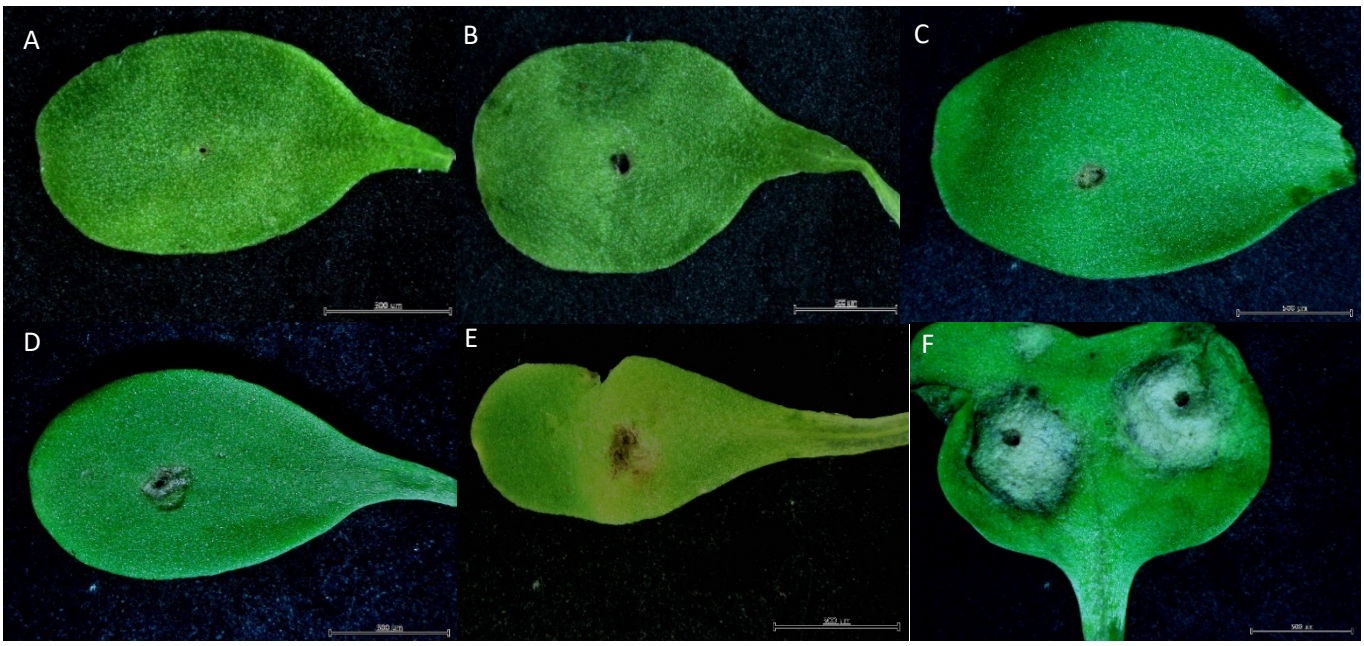

Figure 5.4: Responses on cotyledons of *Camelina sativa* and *Brassica napus* to inoculation with *Leptosphaeria maculans*. **A**, No symptoms formed on Camelina (disease score=0). **B**, Necrotic hypersensitive reaction (disease score=1). **C**, Very small necrotic tissue surrounding the inoculation point on Camelina (disease score=2). **D**, Small necrotic tissue around the inoculation point on Camelina (disease score=3). **E**, Necrotic tissue with diameter 1 mm on Camelina (disease score=4). **F**, Disease symptoms on canola cv. Mystic (disease score=7).

Experiment 2

To further investigate the variability in resistance to *L. maculans*, six Camelina genotypes (two each of 'resistant', 'intermediate' and 'susceptible' genotypes) were screened using five different *L. maculans* isolates, representing races Av1-4-5-7-8, Av1-5-6-7, Av3-5-6, Av1-3-5-6-8 and Av6-9. There was a significant effect of fungal isolate, genotype and an isolate × genotype interaction (all $P<0.001$) in terms of the disease responses across the genotypes and isolates tested (Table 5.5). Overall, all Camelina genotypes were highly resistant to all isolates, with mean % CDI <17. In comparison, canola cv. Mystic was moderately-to-highly susceptible to all test isolates, as evidenced by the development of large necrotic lesions, with a mean % CDI of 66.4. Furthermore, among the *L. maculans* isolates, UWAP11 caused the most severe disease symptoms, with a mean % CDI of 25.6, while WAC4094 caused least disease symptoms with a mean % CDI of 18. All *L. maculans* isolates caused a hypersensitive response (HR) on most Camelina genotypes categorised as a disease score of 1 (Figure 5.4B). Non-spreading lesions were also observed at some infection sites (Figure 5.4C and D). In some plants in some genotypes, all isolates except WAC4094 caused severe infection with a score of 4 (Figure 5.4E). The responses of some Camelina genotypes were isolate-dependent, such as

genotypes C370, C3364 and C4139. In contrast, genotype C2305 was consistently susceptible for % CDI against the four isolates used in this study.

Table 5.5: Response of six *Camelina sativa* genotypes for five different races of *Leptosphaeria maculans.* Each genotype's unique relative ranking score is provided within brackets, where 1 represents the most resistant genotype, and the largest relative genotype ranking score represents the most susceptible genotype.

Isolate	Race[a]	Host							
		C370	C430	C2305	C3364	C4111	C4139	Mystic	*Mean*
UWAM3	Av1-4-5-7-8	13.2 (4)	12.5 (3)	16.3 (6)	11.5 (1)	13.5 (5)	12.2 (2)	71.9 (7)	*21.6*
UWAP11	Av1-5-6-7	16 (3)	17 (5)	18.4 (6)	14.6 (2)	16.3 (4)	11.1 (1)	86.1 (7)	*25.6*
WAC4028	Av3-5-6	11.5 (1)	12.5 (2)	18.8 (6)	15.6 (5)	13.5 (3)	14.2 (4)	47.2 (7)	*19.1*
WAC4094	Av1-3-5-6-8	10.4 (1)	10.4 (2)	13.2 (6)	10.8 (4)	10.4 (3)	12.2 (5)	58.3 (7)	*18*
WAC7803	Av6-9	18.4 (6)	13.5 (3)	16.3 (5)	11.1 (2)	16 (4)	11.1 (1)	73.6 (7)	*22.9*

Significance of genotypes for UWAM3; P <0.001; l.s.d. (P<0.005)=8.6
Significance of genotypes for UWAP11; P <0.001; l.s.d. (P<0.005)=6.3
Significance of genotypes for WAC4028; P <0.001; l.s.d. (P<0.005)=10
Significance of genotypes for WAC4094; P <0.001; l.s.d. (P<0.005)=4.8
Significance of genotypes for WAC7803; P <0.001; l.s.d. (P<0.005)=7.3
Significance of *L. maculans* isolates; P <0.001; l.s.d. (P<0.005)=2.7
Significance of isolates x genotypes; P <0.001; l.s.d. (P<0.005)=7.1
[a]Race indicating the avirulence loci for which the isolate is avirulent and has been characterised as been proposed in Balesdent *et al.* (2005).

5.5. Discussion

Our study evaluates the relative resistances in a set of 30 genetically diverse genotypes of Camelina to two important diseases in the Brassicaceae, SR and blackleg. We confirmed the results of our cotyledonary testing against SR with stem inoculation. Confirming the resistance of Camelina genotypes against two distinct pathotypes provides new evidence of the high value of Camelina as a resistance source to this pathogen. We also confirmed the outstanding performance of Camelina against *L. maculans*, with genotypes displaying a highly resistant reaction to *L. maculans* (% CDI 7.6–18.8). The current study highlighted that the six isolates of *L. maculans* tested, representing a diverse range of Avr allele combinations, readily elicited an HR reaction. We believe this is the first report of variable Camelina response to *L. maculans* isolates; in contrast to other studies that found no Camelina symptoms following *L. maculans* inoculation (*e.g.*, Salisbury, 1987; Li *et al.*, 2005; Gregorich *et al.*, 2009).

For *S. sclerotiorum*, this study builds on our previous cotyledonary stage inoculation testing of Camelina (Purnamasari *et al.*, 2015). In the field, stems at the flowering stage are severely damaged by SR, and the stem inoculation method we used provided the first realistic measure for SR resistance on Camelina. Other studies have emphasised the importance of carrying out a range of tests with plants at different stages of maturity (Bradley *et al.*, 2006; Taylor *et al.*, 2015; Neik *et al.*, 2017), as

we have done. Our study highlights the importance of using distinct pathotypes of *S. sclerotiorum* with varying abilities to overcome particular host resistance(s) and/or associated resistance mechanisms of certain genotypes and identify pathotype-independent host resistance in Camelina.

Overall, we found Camelina genotypes expressed good resistance, with 18 genotypes having stem lesions 1.6 to 3.5 cm, equivalent to the top-ranked resistances for the two canola genotypes tested, cv. Mystic and 0P6712. Using the same stem inoculation technique, Mystic and 06P712 were the top-ranked resistant canola genotypes tested in Uloth *et al.* (2015) and You *et al*. (2016). Although the relative resistance rankings of Camelina genotypes varied depending on different inoculation techniques and/or isolates, some genotypes (highly resistant or highly susceptible) displayed consistent responses regardless of the inoculation technique or pathotype *S. sclerotiorum*. However, other genotypes with moderate level resistance, such as genotype C2292 and C4068, were less consistent across different screening studies. This inconsistency is expected for genotypes with moderate level resistance as environment influences resistance expression (Sun *et al.,* 2005; Uloth *et al.,* 2013). However, You *et al*. (2016) identified a few Chinese *B. oleracea* var. *capitata* genotypes that expressed extremely high-level combined stem and leaf resistance. Genotypes C370 and C253 will be particularly significant for developing new SR-resistant Camelina genotypes, as they have resistance mechanism(s) to *S. sclerotiorum* pathotypes and/or plant components (*i.e.*, cotyledon or stem) that appear to be effective independent of environmental settings.

There was noteworthy correlation between mean stem lesion length and the mean % CDI across Camelina genotypes for *S. sclerotiorum* MBRS-1, although not for WW-1. This is supported by Garg *et al*. (2008), who showed cotyledon resistance was well correlated with stem resistance for a set of canola genotypes. The correlation across different plant components (*e.g.*, cotyledon or stem) makes these highly resistant Camelina genotypes potential sources of resistance to target and exploit in developing new commercial *Brassicaceae* cultivars with more effective combined seedling and adult plant resistance. This outcome is particularly significant as many other studies have shown cotyledon, leaf and stem resistances are generally expressed differently across different plant components. For example, You *et al*. (2016) found no correlation between expressions of stem and leaf resistance, suggesting independent inheritance. Similarly, Uloth *et al*. (2013) found no correlation between seedling cotyledon and adult plant stem resistance following artificial inoculation or naturally occurring leaf infection across a similar diverse range of cruciferous genotypes in field studies. In the current study, the correlation between cotyledon and stem resistance in Camelina for highly virulent isolate of *S. sclerotiorum* suggests a common basal resistance mechanism that operates against this pathogen in both plant stages. Enyck *et al*. (2012) has shown monolignol biosynthesis is linked with

S. sclerotiorum resistance in Camelina, and strong induction of monolignol genes in resistant genotypes enhances lignin synthesis at the pathogen inoculation site, thus restricting the development and expansion of the pathogen within the plant. Similarly, Uloth *et al*. (2016) highlight the importance of lignin production in impeding *S. sclerotiorum* reaching the stem vascular and xylem tissues in highly resistant *B. carinata*, *B. juncea* and canola. Further studies to confirm the role of monolignol genes in the resistance of Camelina to *S. sclerotiorum* would be instructive.

When we challenged the susceptible × resistant RIL population by inoculating at the cotyledonary stage, the 141 F_5 RILs differed for % CDI following *S. sclerotiorum* inoculation. However, the estimate of broad-sense heritability for SR resistance in this population was 36%; lower than in other studies, such as the 67% found in canola by Zhao *et al*. (2006) for stem resistance (using petiole inoculation) or 61% found by Wu *et al*. (2013) for leaf resistance (at the seedling stage). Nevertheless, the high heritability values in those studies were not reflected when dissecting the genetic variance responsible for phenotypic variation of SR resistance. These and other studies found all QTL identified through biparental RIL mapping studies to be minor effect QTL, explaining ≤10% of the variance for SR resistance (Zhao *et al.*, 2006; Yin *et al.*, 2009; Wu *et al.*, 2013; Wei *et al.*, 2014). Similarly, genome-wide association studies (GWAS) for SR resistance identified only a few loci that collectively explain 16.5% of the phenotypic variance while the observed broad-sense heritability was 61.7% (Wu *et al.*, 2016). All of the available studies and low broad-sense heritability of resistance to *S. sclerotiorum* in Camelina suggest the resistance mechanism for SR in this species is a complex genetic trait determined by many genes each of little effect. One strategy to uncover the molecular mechanism for resistance to *S. sclerotiorum* in Camelina may be to identify candidate genes through the histological approach of transcriptomic sequencing followed by a candidate gene approach. For example, by using candidate gene-based association mapping strategy, Rana *et al*. (2017) found that marker-trait associations could explain 30% of the phenotypic variation in *B. juncea* and *B. fruticulosa* introgression lines to *S. sclerotiorum*.

The current study confirmed the outstanding resistance of Camelina genotypes to *L. maculans* (% CDI 7.6–18.8) and isolate independence of this reaction. Previously, as a distantly related species of canola, Camelina has exhibited high resistance to *L. maculans* in field trials (Salisbury, 1987; Séguin-Swartz *et al.*, 2009). Furthermore, Li *et al*. (2005) found that 80 isolates of *L. maculans* did not cause any symptoms on a single Camelina genotype. Similarly, Gregorich *et al*. (2009) found no disease symptoms in four varieties of Camelina inoculated with two specific races of *L. maculans* that are virulent to canola. Our study also highlighted that the six isolates of *L. maculans* tested, representative of races with diverse Avr allele combinations, readily elicited an HR. This outcome may be due to

the inherent genetic diversity present in our Camelina panel, a germplasm collection known to have higher genetic variability than previously reported for other Camelina germplasm (Ghamkhar *et al.,* 2010) or the relatively limited genotypes used in other studies. Bohman *et al*. (2004) found a single genotype of *Arabidopsis thaliana* (L.) Heynh. that showed evident disease symptoms to *L. maculans* out of 168 genotypes. Nevertheless, in the current study, as all Camelina genotypes showed a strong resistance reaction and the disease symptoms were greatly restricted compared with the canola control, it could be concluded that Camelina shows a non-host response to *L. maculans.* Furthermore, as there are different reports about the association between seedling and adult resistance response in canola (Li *et al.,* 2003, 2004a; Van de Wouw *et al.,* 2009; Long *et al.,* 2011), further study is required to explore the relationship between Camelina with *L. maculans*.

The genetic basis and mechanisms involved in the resistance of Camelina to *L. maculans* are not yet well defined or understood. The current studies showed that HR plays a critical role in this resistance reaction. Previously, the development of HR around the inoculation site has been known as the major characteristic of the resistance to *L. maculans*, both on cotyledons and stems, in Brassicaceae species such as canola, Arabidopsis, *Capsella bursa-pastoris* and *Diplotaxis muralis* (Chen & Séguin-Swartz, 1999; Li *et al.,* 2007b, 2008). This rapid 'suicide strategy' prevents further colonisation of *L. maculans,* and therefore the pathogen is confined to an area around the point of inoculation. It is possible that non-specific activation of defences in the early hours post-inoculation, such as camalexin production, has an essential role in the Camelina resistance mechanism against *L. maculans*. A similar mechanism has been reported in Arabidopsis, in which camalexin production partially contributes to resistance of Arabidopsis to *L. maculans* (Bohman *et al.,* 2004). As the current study confirmed the potential of Camelina as an important source of blackleg resistance, further studies to elucidate the resistance mechanism(s) will almost certainly identify novel R-genes, particularly as the complete genome sequencing data for Camelina is now available (Kagale *et al.,* 2014).

5.6. Conclusion

In conclusion, Camelina genotypes exhibited excellent resistance to these two economically devastating pathogens of crucifers. Most genotypes in Camelina showed a level of resistance at or greater than the top-resistance rank with canola cv. Mystic and 0P6712 when inoculated with *S. sclerotiorum* and significantly greater resistance than canola cv. Mystic when inoculated with *L. maculans*. *Camelina sativa* resistance to these diseases will be a highly valuable source for improving crucifers and should lead to the broader adoption of Camelina as a more sustainable oilseed crop in

its own right, especially in Australia and other countries where both diseases are devastating to canola and other Brassicaceae crops.

5.7. References

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CHAPTER 6

Genetic dissection of plant defence under shade: A case study from the *Camelina sativa–Sclerotinia* pathosystem

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6.1. Abstract

In natural and agricultural ecosystems, shady conditions can trigger shade-avoidance syndrome (SAS) and reduce disease resistance in shade-sensitive plants. While considerable progress has been made toward understanding the molecular mechanisms underlying plant defence under shade, no study has evaluated the genetic basis of natural variation for this trait. Here, two *Camelina sativa* (L.) Crantz lines, one resistant (Cs370) and one susceptible (Cs2305) to the broad-host recalcitrant pathogen *Sclerotinia sclerotiorum*, but with a similar response to shade, were evaluated for their genetic variation in plant defence under shade. We measured SAS and defence-related phenotypes at the cotyledon and flowering stages under full light (R:FR=10) and simulated shade (R:FR=0.39) within a 106 individual recombinant inbred line population derived from Cs370×Cs2305 and performed quantitative trait loci (QTL) analysis. Plant defence to *S. sclerotiorum* was compromised under shade at both plant developmental stages, with larger effects observed at flowering. At flowering, defence is controlled by at least two loci. First, RSR (Resistance to Sclerotinia Rot) was significantly reduced under shade compared to full light (PEV=14% under full light, PEV=0.1% under shade). Second, SIS (Susceptibility Induced under Shade) was detectable only under shade (PEV=16.7%). Under shade we were unable to observe an effect of RSR, while the effect of SIS on the response of Cs370 to SR was measurable. In experiments with non-diseased plants, we detected other loci with relatively low effects on plant phenology under shade, indicating different genomic regions regulating plant defence under shade and the SAS response in the offspring population. Using a similar approach, we evaluated two *Arabidopsis thaliana* (L.) Heynh. ecotypes, one with a strong reaction to shade (AtCol-0) and the other with low to nil reaction to shade (AtBla-6). We found AtCol-0 had reduced defence mechanism under shade, whereas AtBla-6 showed no reduction in its defence. The different genetic control of defence against SR under full light and shade could be integrated into breeding programs as a disease management tactic to protect plants cultivated as a dense monoculture or intercropped stands. The dissection of a genetic mechanism in the Camelina RIL population and different defence response among Arabidopsis ecotypes highlights how exploiting natural variation could decipher the complex genetic architecture of plant defences in broad-host pathogens under shade.

6.2. Introduction

Plants encounter various environmental factors that co-occur in natural and agricultural environments. To quickly perceive and adapt to these stimuli, plants have evolved sophisticated mechanisms (Lamers *et al.*, 2020; Saijo & Loo, 2020). This study focuses on the plant response to pathogen infection while growing under shade. When studied in isolation, shade and pathogen infection are strong triggers of specific molecular responses, yet little is known about the response to both factors despite being common in monoculture or intercropped cultivation systems. Therefore, understanding the defence mechanism under shade has major agronomic, ecological and economic implications.

A shady microclimate at low canopy levels provides conducive infection environments for many plant diseases in agroecosystems and natural ecosystems driven by relatively low temperatures, high humidity and shade (Rees *et al.*, 2007; Kazan & Manners, 2011; Lee *et al.*, 2016). Hence, for this study, where we sought to study shade independently from temperature and humidity, plants were grown indoors under a low red to far light ratio (R:FR<0.7) that serves to mimic shade. Under dense vegetation, shade-sensitive plants adapt to a reduction in the ratio by changing growth habit (*e.g.*, producing longer branches) and/or accelerating their phenology (*e.g.*, shortening flowering time). Plant phenotypes that respond to low R:FR are known collectively as the shade-avoidance syndrome (SAS; Grime, 1979; Smith & Whitelam, 1997). SAS favours growth responses that enhance a plant's ability to reach above its neighbours to compete for light resources and effectively complete their life cycle. However, such adaptive shade responses are thought to come at a cost, such as reduced effectiveness in responding to disease, generally referred to as a trade-off between growth and defence (reviewed in Ballaré, 2014; Ballaré & Pierik, 2017).

Due to its complexity, the regulatory circuits underlying the response to more than one stimulus have been identified through the study of single or multiple-gene mutant lines (*e.g.*, Cerrudo *et al.*, 2012; de Wit *et al.*, 2013; Chico *et al.*, 2014; Leone *et al.*, 2014; Nozue *et al.*, 2018; Liu *et al.*, 2019; Fernández-Milmanda *et al.*, 2020). However, there has been little investigation of natural variation in defence under shade (*e.g.*, Gommers *et al.*, 2017; Ranade & Garcia-Gil, 2020). Despite the power of mutants to elucidate the function of biological processes, information obtained from mutagenesis in the laboratory should be verified in wild or cultivated plants because 1) mutations can disrupt adaptive pathway(s); 2) the phenotypic effect may be an artefact and not directly result from mutation; 3) loci can show different phenotypic effects when mutated with the same method, known as the background effects phenomenon (Chandler *et al.*, 2013; Chow, 2016). Background effects can result in the mutants exhibiting enhanced or reduced phenotypic effects compared with wild types (Mullis *et al.*,

2018). Furthermore, natural variation of wild types would inform us of the evolutionary dynamics between plant–pathogen interactions in natural ecosystem.

We chose a crop, *Camelina sativa* (L.) Crantz, and a broad-host fungal pathogen, *Sclerotinia sclerotiorum* (Lib.) de Bary (causal agent of Sclerotinia rot (SR)), as subjects for this study. Camelina is an ancient Brassicaceae oilseed crop with high potential for biofuel production and sustainable agriculture. The Camelina genome draft has been published (Kagale *et al.,* 2014a) and several genetic tools have been developed since (*e.g.,* Kagale *et al.,* 2016; Jiang *et al.,* 2017; Na *et al.,* 2019; Yuan & Li, 2020). Camelina and the primary plant model species, *Arabidopsis thaliana* (L.) Heynh, belong to closely related genera (Kagale *et al.,* 2014b). As in many other Brassicaceae, the Camelina genome bears signatures of ancient polyploidisation events resulting in a paleo-allohexaploid genome (Kagale *et al.,* 2014a).

Sclerotinia sclerotiorum is one of the most successful plant pathogens, infecting more than 600 species, including nearly all oilseed crops. In the field, apothecia (fruiting body) are usually formed at crop canopy closure (Bolton *et al.,* 2006), which often coincides with the crop reproductive stage (Moellers *et al.,* 2017). Genetic studies into resistance to *S. sclerotiorum* have been hampered by the quantitative nature of the defence response to SR, *i.e.*, small gradual variation in response to disease among lines of the same species and inherent difficulties in establishing reliable inoculation methods (Garg *et al.,* 2008; Perchepied *et al.,* 2010; McCaghey *et al.,* 2019). In Camelina, the pathogen causes necrotic lesions resulting in collapsed leaves and stems and significant seed yield losses (Séguin-Swartz *et al.,* 2009). Little is known about the genetics of Camelina resistance to SR, except that upon infection, cell wall strengthening occurs due to increased synthesis of lignin monomers (Eynck *et al.,* 2012) and its resistance is independent of camalexin production (Purnamasari *et al.,* 2015).

Under a low R:FR, Camelina develops typical SAS phenotypes, including reduced time to flowering, leaf area and biomass and elongated stems (Purnamasari *et al*., unpublished; Chapter 4). A collection of Camelina lines showed low intraspecific diversity in response to different R:FR and clustered as moderately responsive to shade. Under the same conditions, Arabidopsis ecotypes had wider diversity in adaptive phenotypic plasticity in response to shade. Hence, the Arabidopsis germplasm collection offers an extraordinary opportunity to explore genetic variants under natural selection affecting plant defence under shade. Yet, to our knowledge, there has been no attempt to do so.

This study aimed to identify quantitative trait loci (QTL) with significant effects on crop response to pathogen infection under shade at cotyledon and reproductive developmental stages. For this purpose, we evaluated the Camelina response to SR under full light (R:FR=10) and shade-mimicking (R:FR=0.39) growth conditions. We used two Arabidopsis ecotypes with contrasting response to

shade as phenotypic references. To identify key genetic loci, we used a recombinant inbred line (RIL) population derived from a cross between a Camelina SR-resistant genotype, Cs370 and an SR-susceptible genotype, Cs2305. Our approach facilitated the discovery of two QTL with opposite effects under full light and shade-mimicking conditions. We believe this is the first genetic study on Camelina response to pathogen and the first to explore the interplay of plant responses to infection and shade. Our study provides crop-relevant information to complement the mutant-derived knowledge in plant defence under shade, a frequent and typical agricultural scenario for monoculture and intercropping practices.

6.3. Materials and methods

6.3.1. Plant and fungal material

The Camelina lines Cs370 and Cs2305 form part of a small collection obtained by researchers at The University of Western Australia from the N.I. Vavilov Research Institute of Plant Industry, Russia, genetically characterised by Ghamkhar *et al.* (2010) and Purnamasari *et al.*, unpublished (Chapter 3). Both lines have a similar moderate response to shade (Purnamasari *et al.,* unpublished, Chapter 4); Cs370 is resistant and Cs2305 is susceptible to SR (Purnamasari *et al.,* 2015, 2019; Chapter 5). These lines were crossed to generate a RIL population (*n*=141) (Purnamasari *et al.,* 2019); and 106 of 141 RILs were randomly chosen in this experiment due to space limitation. The RILs were genotyped and phenotyped using different individuals per F_5 family. The Camelina genome reference genotype, the double haploid line CsDH55, was kindly provided by Dr Isobel Parkin, Agriculture and Agri-Food Canada, Saskatoon, Canada. The Arabidopsis ecotypes AtCol-0 and AtBla-6 were selected as sensitive and insensitive to shade, respectively (Adams *et al.,* 2009; Purnamasari *et al.,* unpublished; Chapter 4) and obtained from the Arabidopsis Biological Resource Centre at Ohio State University (Columbus, OH, USA). AtCol-0 was described as susceptible to *S. sclerotiorum* (Ge & Barbetti, 2019).

In this study, *Sclerotinia sclerotiorum* isolate MBRS-1 was used for inoculation. MBRS-1 is an aggressive isolate grouped into pathotype 76 (Ge *et al.,* 2012), the prevailing type affecting crucifers in Western Australia (*e.g.,* Uloth *et al.,* 2013). The isolate is also very aggressive on Camelina (Purnamasari *et al.,* 2015, 2019).

6.3.2. Plant growth conditions and light treatments

All plants were grown in 70 mm square plastic pots with a pasteurised soil mixture composed of finely crushed pine bark/coco peat/sand at 2.5:1.0:1.5 (wt/wt). Plants were watered daily and fertilised weekly using Thrive™, an all-purpose soluble fertiliser at 0.09 g pot^{-1}. Plants were grown in

controlled temperature room at a temperature of 18/14°C (day/night), 20 h photoperiod and air RH 70 ± 10%. Artificial light was set at a light intensity of 320 ± 6 μmol·m^{-2}·s^{-1}. Full light (R:FR=10) was provided by NS1 (Valoya Oy, Helsinki, Finland). Shade-mimicking condition (R:FR=0.39) was obtained by adjusting the settings on a Heliospectra lightbox (Heliospectra, Sweden) to mimic NS1 wavelength spectra with the addition of FR, without altering the levels of photosynthetically active radiation (PAR) (Figure 6.1A). PAR, R/FR ratios and light intensity were measured with a Sekonic C7000 SpectroMaster spectrometer (Sekonic Corp., Tokyo, Japan).

6.3.3. Phenotypic evaluation

Response to SR at the cotyledon stage

Inoculum preparation and inoculation of cotyledon leaves were undertaken as described by Garg *et al*. (2008). The mycelia of *S. sclerotiorum* were cultured for three days in 150 mL of sterilised potato dextrose broth containing peptone (potato dextrose broth 24 g, peptone 10 g, H2O 1 L) at 25°C on a shaker at 150 rpm. The mycelial suspension was prepared by washing the fungal mat twice with deionised water and transferring them back to the liquid growth medium. The inoculum suspension was macerated with a hand-blender for three minutes and filtered through four cheesecloth layers to obtain mycelial fragments. The fungal concentration was determined using a haemocytometer and adjusted to 1×10^5 fragments mL^{-1}. The inoculum suspension contained 0.002% Tween 20 as a wetting agent. For inoculation, a single 5 µL droplet of *S. sclerotiorum* mycelium suspension was deposited on each lobe of every cotyledon using a micropipette. Mock-control inoculations were done with 5 µL droplets of 0.002% Tween 20 in deionised water. After inoculation, plants were covered with a clear plastic dome that had first been internally sprayed with a fine mist of DI water to maintain high humidity conditions. The experiment was performed in six replicates arranged in a complete randomised block design. A pot with two plants served as an independent experimental unit. Pots were held in 64-cell trays. Each tray included RILs (test units), Cs370, Cs2305, CsDH55 and canola cv. Mystic (checks units). Disease development was assessed at 72 h post-inoculation on a disease severity scale of 0–9 (Purnamasari *et al.,* 2015). Cotyledon disease severity (C-DS) was calculated using the indexing method based on McKinney (1923), as follows:

$$C-DS\ (\%) = \frac{[(a \times 0) + (b \times 1)\ + (c \times 2)\ + (d \times 3)\ + (e \times 4) + \ldots (j \times 9)] \times 100\}}{(a + b + c + d + \ldots j)\ \times 9}$$

where *a, b, c, d, e … j* are the number of plants with disease scores of 0, 1, 2, 3, 4, … 9, respectively. Plasticity index in defence response (C-DPI) was calculated as the slope '*b*' between the C-DS means at R:FR=0.39 and R:FR=10.

To inoculate Arabidopsis, we followed the procedure described by Ge & Barbetti (2019). Five µL of *S. sclerotiorum* mycelia, as described above, was applied to each leaf lobe on up to 10 leaves per plant. The plants were placed in a tray and sealed with a clear plastic lid and internally sprayed with a fine mist of DI water to maintain high humidity. The two ecotypes were inoculated when there were >10 mature leaves of similar size (approximately five weeks after sowing). There were five replicates per ecotype in a completely randomised design. Disease severity on leaves was recorded three days after inoculation, as described above, and converted to leaf disease indices using McKinney's method (1923).

Response to SR at the reproductive stage

The inoculum preparation and inoculation at the flowering stage were performed using the method described by Li *et al*. (2006). In brief, 5 mm diameter mycelial discs punched from an actively growing 48 h culture grown on glucose-rich medium (peptone 10 g, glucose 20g, agar 18 g, KH_2PO_4 0.5 g, H_2O 1 L, pH 6.0 before autoclaving) were wrapped onto the stem above the first node using Parafilm®. Mock-control inoculations were done using a disc of glucose-rich medium. For the three lines of Camelina—CsDH55 (genome reference line), Cs370 (resistant parent) and Cs2305 (susceptible parent)—and canola cv. Mystic, five plants of each genotype were used across all test environments. These plants were inoculated when 50% of the plants had at least one open flower, well within the reproduction stage and after floral induction. For the RILs, three plants of each genotype were used for inoculation at R:FR=10 and one plant of each genotype was used for inoculation at R:FR=0.39. It is important to note that as inoculation was performed at the base of the main stem, the R:FR received at the inoculation site for full light was below 10. However, the space between pots was adjusted to minimise any drop in R:FR. The humidity was maintained by overhead misting immediately post-inoculation for 10 minutes, with misting repeated daily thereafter. The lesion length along the stems was measured in centimetres three weeks after inoculation (Li *et al.*, 2007). For comparative purposes, disease severity at flowering time (F-DS) was calculated as the percentage of lesion length from plant total length. Plasticity index in defence response (F-DPI) was calculated as the slope '*b*' between the F-DS means at R:FR=0.39 and R:FR=10.

Pathogen growth under different light conditions

To investigate whether different light spectra affect the growth of *S. sclerotiorum*, a plug of mycelium was deposited in the centre of potato dextrose agar plates. Five inoculated plates were incubated in full light or shade for one week until sclerotia formed. The grown mycelium diameter was measured using a linear ruler three days after inoculation.

6.3.4. Shade-avoidance syndrome

All experimental units described in sections 6.3.1 and 6.3.2 served to determine the plant SAS phenotype before inoculation. Time to flowering was recorded as the calendar days from sowing to the first open flower. Phenotypic plasticity index (PPI) was calculated for each genotype following Valladares *et al*. (2000):

$$PPI = \frac{Max\ (\bar{X}_c, \bar{X}_{nc}) - Min(\bar{X}_c, \bar{X}_{nc})}{Max\ (\bar{X}_c, \bar{X}_{nc})}$$

where $\bar{X}_c$ is trait mean under competition (R:FR=0.39); $\bar{X}_{nc}$ is trait mean under no competition (R:FR=10).

6.3.5. Statistical analysis of phenotypic data

Descriptive statistics, analysis of variance (ANOVA) and normality tests were performed with the statistical software R version 4.0 (R Core Team, 2013). One-way ANOVA using the Welch test for unequal variance was carried out using JMP® software version 15.1.0 (SAS Institute Inc., Cary, NC). General linear models and LS means were obtained for each genotype using R package 'emmeans' (Lenth *et al.*, 2018). Slope values 'b' to obtain plasticity indexes at cotyledon and reproductive stages (C-DPI and F-DPI) were derived from the 'emtrends' function in the 'emmeans' package. Pearson correlation coefficients and plots between the various SR resistance and flowering time variables were done using JMP. The 'aov' function was used to obtain heritability (H^2) variance components in ICI mapping software version 4.2 (Meng *et al.,* 2015). Statistical analysis of F-DS (normalised and non-normalised) and F-LL did not significantly differ. Hence, our analysis depict non-normalised F-LL and QTL effects are shown in centimetres.

6.3.6. Genotyping and construction of genetic linkage map

The RILs (*n*=106 F5 families) and parental lines (Cs370 and Cs2305) were grown under controlled conditions at the UWA plant growth facility. At the cotyledon stage, ~1 g of young leaf tissue was excised, immediately frozen in liquid nitrogen and freeze-dried. Freeze-dried samples were sent to Diversity Arrays Technology Pty. Ltd. (University of Canberra, Bruce, ACT, Australia) for DNA extraction and genotyping-by-sequencing at low sequence density, using the DArTseqLD platform (Noyszewski *et al.,* 2019). Marker sequences were aligned against the CsDH55 genome assembly (Kagale *et al.,* 2014a). The DArTsoft marker extraction pipeline identified 1929 SNPs and 2213 silicoDArT markers for this population.

A linkage map was constructed using ICI mapping software version 4.2 (Meng *et al.,* 2015), R/ASMap (Taylor & Butler, 2017) and the 'R/qtl' package (Broman *et al.,* 2003). SNPs and

silicoDArT markers were filtered to exclude markers with missing values for Cs370 and Cs2305, missing in more than 30% of RILsand non-polymorphic markers, resulting in 322 SNPs and 454 silicoDArTs reliable markers. Cluster analysis was performed to identify markers with identical segregation patterns, create marker bins and select a representative marker using the ICI mapping bin option. This step resulted in the construction of a marker bin map comprising 112 bins from these polymorphic markers, with the number of SNP markers per bin varying from 2 to 432. After binning, R/qtl and R/ASMap were used to check and ensure marker quality by inspecting the markers for duplicate lines, segregation distortion, switched alleles, single and double cross-overs. A total of 515 loci (*i.e.,* sites represented by single marker or marker clusters) were used for preliminary linkage map construction using the MSTmap functionality available in R/ASMap. For these initial linkage maps, heat map, recombination rates of lines and double recombination analysis in R/ASMap were used to assess marker clustering and ordering accuracy. Markers within linkage groups were ordered a final time using ICI mapping. The ordering of markers distributed over 20 chromosomes was performed using k-Optimality, rippling to fine-tune the marker order with a window size 7. Genetic distances between loci were calculated using the Kosambi mapping function. The RILsfor the 515 loci in the linkage map are provided in Supplementary Table 6.3 and Supplementary Figure 6.5.

6.3.7. QTL analysis

For each RIL, the means of the following variables served the QTL analysis:

Cotyledon stage: Disease

C-$DS_{0.39}$ and C-DS_{10}: Disease Severity calculated based on 0–9 disease indexing at R:FR=0.39 and R:FR=10, respectively, and

C-DPI: Disease Plasticity Index.

Flowering stage: Disease

F-$LL_{0.39}$ and F-LL_{10}: Non-normalised lesion length at R:FR=0.39 and R:FR=10, respectively, and

F-DPI: Disease Plasticity Index.

Reproductive stage: Phenology

$TF_{0.39}$ and TF_{10}: Time to flower at R:FR=0.39 and R:FR=10, respectively, and

TFPI: Plasticity index for time to flower

QTL analysis was done using the composite interval-mapping algorithm in ICI mapping (Meng *et al.,* 2015). A 1000-permutation test of shuffling the phenotype means with the genotypes was performed to estimate a significant logarithm of the odds (LOD) threshold for a QTL using a Type I error set at $P<0.05$. Epistatic interactions among loci were estimated using the ICIM-EPI map

function in ICI mapping with a significant threshold LOD=5 and a probability value (PIN) of 0.0001 (Li *et al.,* 2008). The coefficient of determination (R^2) for the marker most tightly linked to a QTL was used to estimate the proportion of the total phenotype explained variation (PEV) by the QTL. QTL regions associated with resistance to different traits in the F_5 Camelina RIL population were considered the same if their LOD support intervals overlapped.

6.3.8. Identification of candidate genes based on intra-genome synteny

Due to whole-genome polyploidisation events, the Camelina genome comprises three copies or subgenomes (Kagale *et al.,* 2014a; Chaudhary *et al.,* 2020). Subgenome 1 (CsG1) contains six chromosomes (CsDH55 4, 7, 8, 11, 14 and 19), Subgenome 2 (CsG2) contains seven chromosomes (CsDH55 1, 3, 6, 10, 13, 16 and 18) and Subgenome 3 (CsG3) contains seven chromosomes (CsDH55 2, 5, 9, 12, 15, 17 and 20) (Chaudhary *et al.,* 2020). To identify the microsynteny of QTL regions of interest, we first compared three homologous chromosomes. The RSR region has homologues on chromosomes 4 and 6, while the SIS region has homologues on chromosomes 1 and 19. Gene models within the QTL intervals in different chromosomes were extracted from the NCBI Genome Data Viewer (https://www.ncbi.nlm.nih.gov/genome/gdv/?org=Camelina-sativa&group=brassicales) and are listed in Figures 6.2C and 6.3D. To analyse synonymous (K_S) and nonsynonymous (K_A) substitution rates between the subgenome paralogs, the functional protein-coding exons were downloaded from NCBI and aligned using Geneious R10.2.5 (Kearse *et al.,* 2012). The calculation of the ratio of K_A to K_S was estimated in DnaSP version 5.10 (Librado & Rozas, 2009). Orthologs in Arabidopsis were obtained from OrthoDB (Kriventseva *et al.,* 2019). Gene function analysis was then carried out using the Protein ANalysis THrough Evolutionary Relationships (PANTHER) Classification System (http://pantherdb.org/) (Thomas *et al.,* 2003). For each gene model and GO term, a comprehensive literature search was undertaken, which is summarised in Supplementary Tables 6.6 and 6.7.

6.4. Results

6.4.1. Shade affects the defence against SR in Camelina susceptible and resistant lines

Camelina defence against *S. sclerotiorum* significantly diminished under simulated shade conditions at the reproductive stage (Figure 6.1B–E). At the cotyledonary stage, R:FR had no significant effect on Camelina response to SR (Figure 6.1C). In other studies under full light, we reported the occurrence of relatively high degrees of tolerance to SR in a few Camelina lines. Here, we revisited those findings and added CsDH55, the genome reference genotype. Under full light (R:FR=10), Cs370 and CsDH55 had significantly lower (P<0.05) disease severity than Cs2305 at the cotyledon

(Figure 6.1B and C) and reproductive stages (Figure 6.1D and E). Similar results were obtained in our earlier studies (Purnamasari *et al.*, 2015, 2019). However, at the reproductive stage, both resistant and susceptible lines had higher disease severity under shade (R:FR=0.39) than under full light. The different responses to disease between the two lines did not significantly differ from zero.

6.4.2. RIL segregation in response to disease under shade and full light

The Camelina resistant genotype Cs370 was crossed with the susceptible genotype Cs2305 to generate a population of RILs by single seed descent (n=106 F5 families). The RILs were phenotyped for response to *S. sclerotiorum* inoculation at the cotyledon and flowering stages under shade-mimicking and full light. Unlike the parental lines, shading significantly diminished the defence against *S. sclerotiorum* in the RILs at both developmental stages (Figure 6.1E). At the cotyledon stage, the mean disease severity for the RILs was mild but significantly increased under shade compared to full light (DS=65.5% vs 59.3%, P<0.05) (Supplementary Table 6.1). At the reproductive stage, the shade effect was significantly larger (DS= 16.1% vs 70.7%, P<0.05, for full light vs shade, respectively). Under full light, the RIL frequency distribution for disease severity was significantly skewed towards tolerance (low disease severity), whereas under shade, the skewness was towards susceptibility (Figure 6.1F). Weak positive correlations were detected between C-$DS_{0.39}$ and C-DS_{10} (r=0.24) and C-$DS_{0.39}$ and F-DS_{10} (r=0.23) among RILs(Supplementary Figure 6.1). We also analysed the disease plasticity index (DPI; difference between the mean response to disease under shade and full light) for each RIL (Figure 6.1G). We measured this parameter because slope measures the rate and direction of the changes in response. At the cotyledon stage, the mean DPI did not significantly differ from zero (DPI= –0.6, P>0.05). However, at the flowering stage, the mean DPI significantly differed from zero (DPI= –3.1, P<0.05).

In all cases, the frequency distribution of the mean RIL response to disease was continuous (Figure 6.1E and F). The histogram shapes, represented by the violin plots, are quasi-normal at the cotyledon stage and quasi-bimodal at flowering time. Broad-sense heritability (H^2) for C-DS was 61.3% at low R:FR and 41.4% at high R:FR (n_i=6 per RIL, n=106 RILs) (Supplementary Table 6.2). At the cotyledon stage: 1) the difference between parental lines in response to disease was small; and 2) frequency distributions of response to disease in RILs were continuous and quasi-normal—indicating that the response is controlled by several loci with relatively low effects. To detect numerous loci with small effects, more RILs and repeats would be required, such as—population of size 5000 based on calculations of population size and loci effects for Arabidopsis (Klasen *et al.*, 2012). At flowering time, the distribution frequency was quasi-bimodal, with larger parental lines differences in response

to disease than the earlier plant growth stage, indicative of sufficient population size to identify QTL with larger effects on the response.

6.4.3. Relationship between defence response and SAS-related phenology

In a previous study (Purnamasari *et al.*, unpublished, Chapter 4), we showed no significant difference between Cs370 and Cs2305 or any other Camelina genotype in various SAS traits. Here, we measured time to flower, one of the SAS phenotypes, to evaluate whether the lack of difference was maintained when alleles are recombined in the RIL population and ensure that inoculation occurred in each RIL at the same physiological stage. The progeny's flowering time and respective plasticity index means across the RILs were intermediate to those of the parental lines (Supplementary Figure 6.2A). The time to flower plasticity index ranged from 0.007 for RILs less responsive to shade to 0.27 for the most responsive lines, in what seems a quasi-bimodal distribution (Supplementary Figure 6.2B). Shade also impacted the morphology of RILs, such as reduced stem diameter (data not shown). However, there was no correlation between disease severity and any SAS trait. The Pearson correlation indices for DS at flowering time and time to flower for mean RIL responses is r=0.14 under shade and r=0.05 under full light (n=106, P>0.05). Therefore, there was no apparent confounding effect in the segregation of SAS-related phenotypes and response to disease in the RILs population.

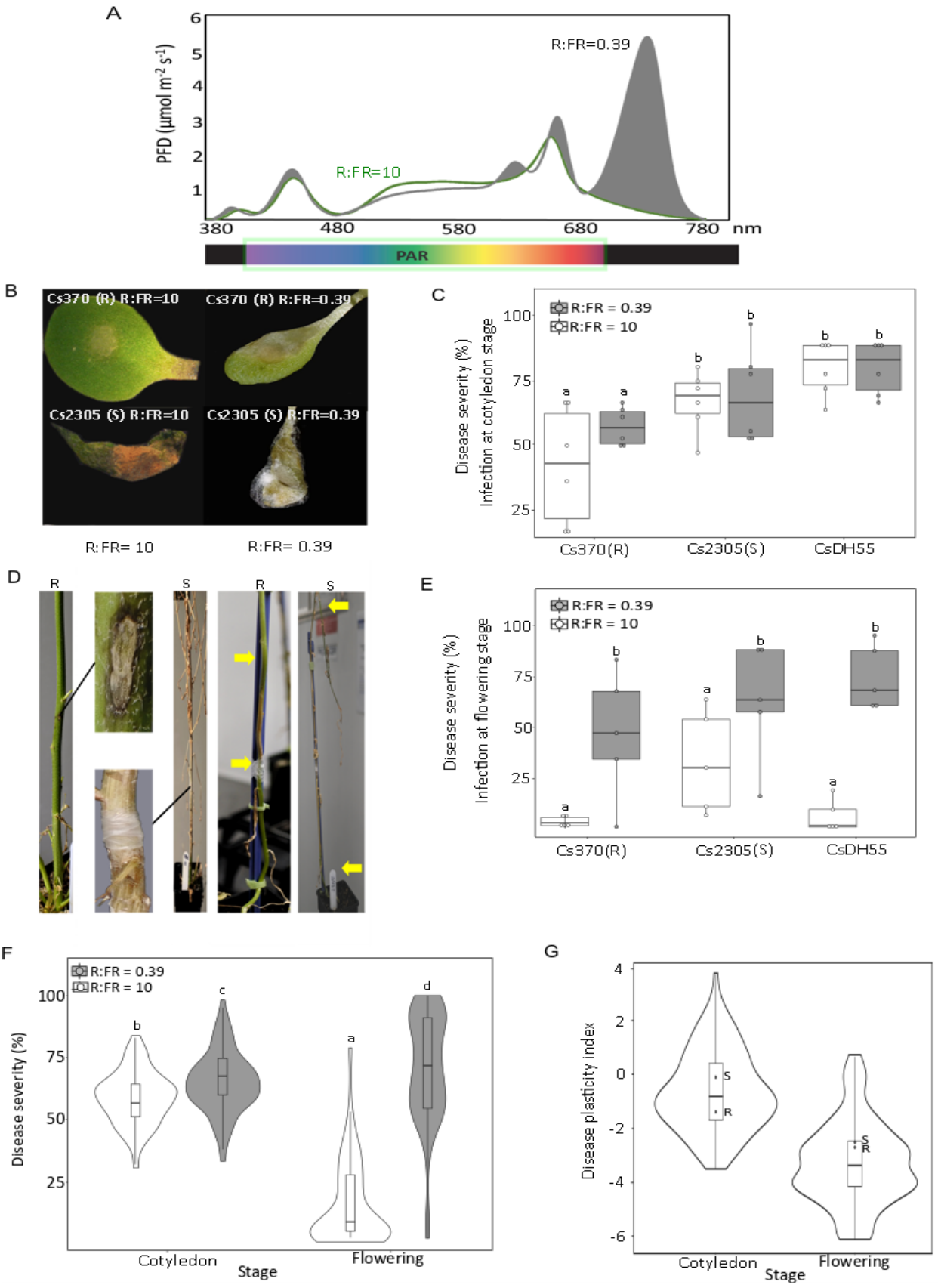

Figure 6.1: Shade effect for the *Camelina sativa* response to *Sclerotinia sclerotiorum* infection. **A**, Red to far-red spectra ratio (R:FR). An R:FR=0.39, to mimic shade conditions (shown in grey), is achieved by manipulating Heliospectra to mimic NS1 with the addition of FR spectrum. Full light (R:FR=10) is simulated using the NS1 emitting light spectrum (white). **B**–**C**, Camelina infected at the cotyledon stage nine days post-planting (dpp). **B**, Representative photos under shade (top) and full light (bottom) for Cs370 (resistant, R) and

Cs2305 (susceptible, S). Top-right and clockwise, leaves rated 30%, 60%, 100%, 100% for C-DS. **B**, Disease severity three days post-inoculation (dpi) for each genotype n=6. Box plots represent C-DS value (%) of three Camelina lines. Same letter in the boxplot indicates accessions' means are not significantly different (P<0.05, Welch ANOVA test). **D–E**, Camelina primary stem infected at the flowering stage, 48±5 dpp. **D**, Stem reaction of R and S under full light and shade-mimicking conditions at 21 dpi. **E**, DS at the flowering stage for each genotype n=6. Box plots represent F-DS value of three Camelina lines. Same letter in the boxplot indicates light treatment's means are not significantly different (P<0.05, Welch ANOVA test). **F**–**G**, Violin and box plots for recombinant inbred lines (RIL) F_5 derived from a cross between Cs370 (R) and Cs2305 (S). **F**, DS of RILs at different plant developmental stages grown under shade and full light. Violin plots represent the mean response per RIL (n_i=6 at the cotyledon stage, n_j=3 at the flowering stage; n=106 RILs). Box plots represent DS value of RILs. Same letter in violin plot indicates means for the plant stage × light treatment interaction are not significantly different (P<0.05, Welch ANOVA test). **G**, Disease plasticity index (DPI) calculated as the slope between the disease response under low R:FR and high R:FR. The plasticity index value for R and S parental lines are indicated with black circles.

6.4.4. RSR - First discovered resistance locus against SR in Camelina is compromised under shade

We identified a novel QTL called **R**esistance to **S**clerotinia **R**ot (RSR) with significant effects on Camelina response to *S. sclerotiorum* under full light (RSR effect=0.2; LOD=4.1). The locus was located on CsLG9 (equivalent to CsDH55 CHR9) and explained 14% of the phenotypic variation (Table 6.1, Figure 6.2A). The defence-enhancing allele RSR^{+} was derived from the resistant parent, Cs370. The average lesion length in the homozygous resistant group ($RSR^{+/+}$) was 0.8 cm, significantly shorter than the 1.7 cm recorded for the homozygous susceptible group ($RSR^{-/-}$) (Figure 6.2B). Based on the response of heterozygous individuals ($RSR^{+/-}$, n=6), resistance appeared to be additive. Interestingly, RSR had no significant effect on the Camelina response to SR under shade, *i.e.*, the effect of the RSR locus was compromised under shade (Figure 6.2A).

Table 6.1: QTL affecting *Camelina sativa*'s response to SR and time to flower, under shade and full light in the Cs370×Cs2305 RIL population. Loci presented passed the significance threshold (Permutation test n= 1000).

Loci	Traits[a]	Location	Position (cM)	Flanking markers	LOD[b]	R^2 (%)	Mean response[c]	QTL Effect[d]
RSR	$F\text{-}LL_{10}$	CsLG9	40	M030-M952A	4.1	14	1.4	–0.45
SIS-1	$F\text{-}LL_{0.39}$	CsLG15	51	M985-M998	4.6	16.1	3.6	0.2
SIS-2	F-DPI	CsLG15	58	M998-M969	3.9	16.7	–3.3	–0.68
SAS-TF	TFPI	CsLG11	91	M844-M240	3.9	18.9	0.1	0.03

a) LS = lesion length at R:FR=0.39 or R:FR=10 indicated by acronym subscripts. LS-PI = lesion length plasticity index. TFPI = time to flower plasticity index
b) LOD: logarithm of the odds ratio
c) Mean response: mean of trait value
d) QTL effect: additive effect per-allele, half of the difference between the means for genotype$^{+/+}$ and genotype$^{-/-}$

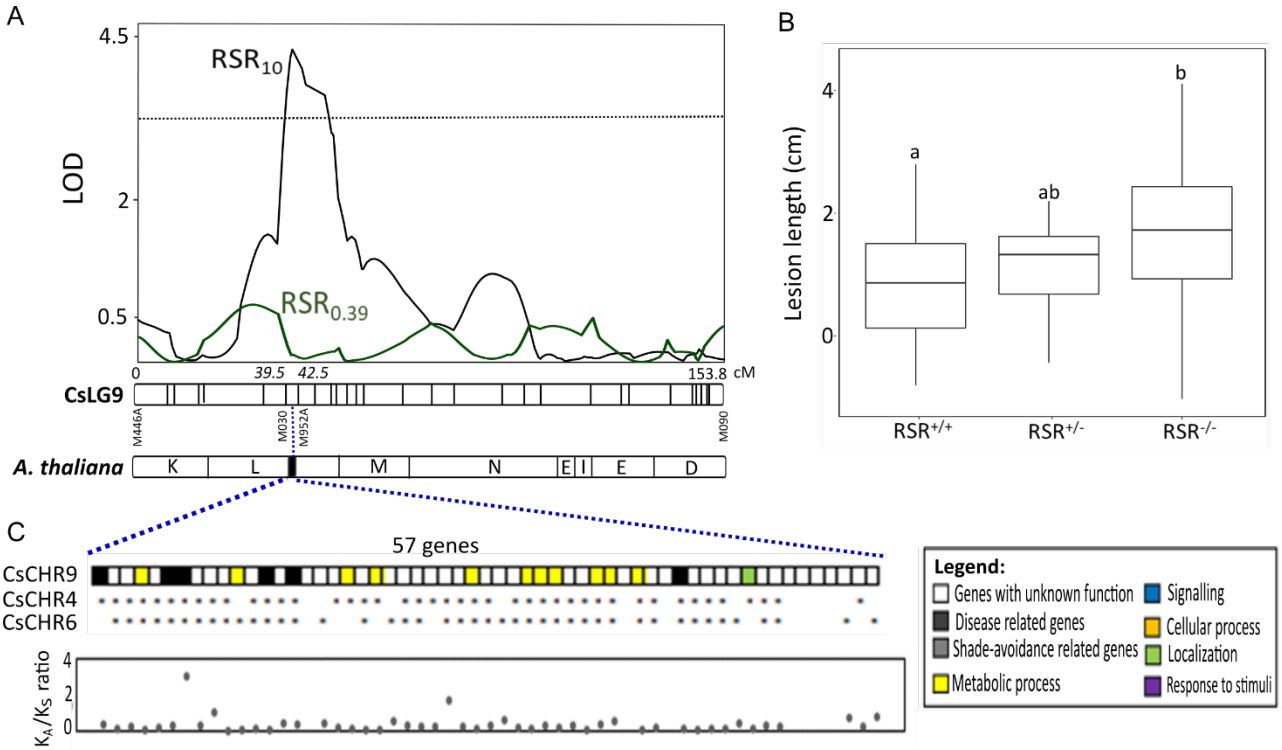

Figure 6.2: *Camelina sativa* Resistance to Sclerotinia Rot (RSR) QTL. **A**, LOD scores against the marker order of CsLG9 (equivalent to CsDH55's CHR9) obtained for lesion length under full light on the Cs370×Cs2305 RIL population, indicating the putative position of RSR. Genetic distances (cM) are provided above the CsLG9 bar, with the marker indicators below. Distance numbers in italics indicate the confidence intervals of the QTL. The corresponding synteny blocks in Arabidopsis chromosome positions are labelled in capital letters K–D. **B**, RSR effects by genotype groups in RIL population (*n*=106). Box plots indicate lesion length under full light for each genotype group. Same letter above the box-plots' whiskers indicates means are not significantly different ($P<0.05$). **C**, Genic order and respective GO terms on the corresponding sequence of the reference genome CsDH55 CHR9 followed by the paralog regions in the genome. The presence of paralogs on the other subgenomes is denoted by (*) and average K_A/K_S ratios are provided for the present paralogous gene pairs.

6.4.5. SIS enhances Camelina susceptibility to SR under shade

Under shade, in addition to RSR's effect on defence being compromised, a novel **S**hade **I**nduced **S**ensitivity (SIS) QTL further compromises defence in Camelina (Table 6.1, Figure 6.3). SIS had the largest effect in this study. SIS was located on CsLG15 (equivalent to CsDH55 CHR15) and had significant effects on lesion size at the flowering stage at low R:FR and disease plastic index, denoted as SIS-1 and SIS-2, respectively. M998, the right flanking marker of SIS-1, was co-located with SIS-2. SIS-1 explained 16.1% and SIS-2 explained 16.7% of the observed variation. In both cases, the source of the susceptible allele, $SIS^{+/+}$, was the parental genotype that is overall tolerant to SR. For SIS-1, the homozygous susceptible group ($SIS^{+/+}$) had significantly longer mean lesion length (10.5%) than the homozygous resistant group ($SIS^{-/-}$) (Figure 6.3B). For SIS-2, the homozygous susceptible group ($SIS^{+/+}$) had significantly lower mean F-DPI (33%) than the homozygous resistant group ($SIS^{-/-}$) (Figure 6.3C). Importantly, RSR and SIS were located on chromosomes 9 and 15, which form part of the third subgenome in the ancient allopolyploid Camelina genome (Cs-G3;

Chaudhary *et al.*, 2020). This subgenome is more dominant than the other two subgenomes in its expression profile (Kagale *et al.*, 2016).

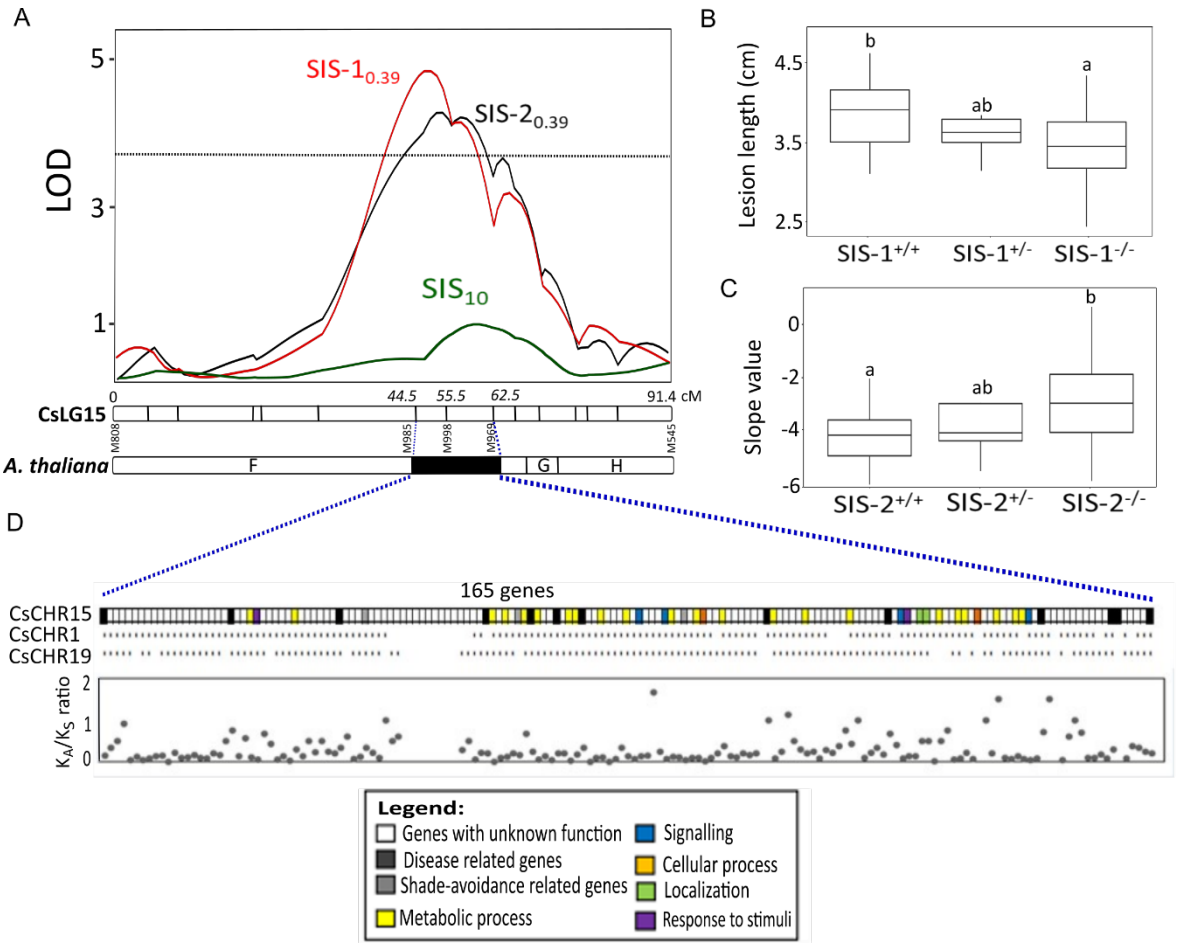

Figure 6.3: *Camelina sativa* Shade Induced Sensitivity (SIS) QTL. **A**, LOD scores against the marker order of CsLG15 (equivalent to CsDH55 CHR15) obtained for lesion length under shade (SIS-1) and slope value (SIS-2) on the Cs370×Cs2305 RIL population indicating the putative position of SIS; genetic distances (cM) are provided above the CsLG15 bar, with the marker indicators below. Distance numbers in italics indicate the confidence intervals of the QTL. The corresponding synteny blocks identified in Arabidopsis chromosome positions are labelled in capital letters F–H. **B**, SIS-1 effects by genotype group. Box plots indicate lesion length under shade for each genotype group. Same letter above the box plot indicates means are not significantly different (P<0.05). **C**, SIS-2 effects by genotype group. Box plots indicate F-DPI value for each genotype group. Same letter above the boxplot indicates means are not significantly different (P<0.05). **D**, Genic order and respective GO terms on the corresponding sequence of the reference genome CsDH55 CHR15 followed by the paralog regions in the genome. The presence of paralogs on the other subgenomes is denoted by (*) and the average K_A/K_S ratios are provided for the present paralogous gene pairs.

Transgressive segregation was observed among the RIL lines (Supplementary Figure 6.1). A few RILs could maintain their defence against SR better than the resistant parent, Cs370, under both light conditions; some only at the cotyledon stage (RIL70 and RIL75), others at the flowering stage (RIL43, RIL67, RIL116 and RIL126). Significant epistatic interactions were identified at the cotyledon (C-DS$_{0.39}$ and C-DS$_{10}$) and flowering (F-DS$_{0.39}$) stages (Supplementary Table 6.4). None of the loci pairs in epistasis involved RSR or SIS (Supplementary Figure 6.3). The loci we

characterised here are likely 'the tip of the iceberg', with other loci with varying effects also involved in the response to disease and phenology under shade. Meaningful evaluation of additional loci, including those in epistatic interactions, would require a larger RIL population size.

6.4.6. Loci affecting response to disease do not influence phenology under shade

RSR and SIS do not affect time to flower under shade or full light or the Camelina phenology plasticity index. No QTL affected time to flower under shade or full light. However, a QTL on CsLG11 had a significant effect on TFPI (*i.e.*, difference between shade and full light for time to flower)—**S**hade-**A**voidance **S**yndrome-**T**ime to **F**lower (SAS-TF; Supplementary Figure 6.2C). SAS-TF explains 18.9% of the phenotypic variation in time to flower. The allele driving the acceleration in time to flower under shade, SAS-$TF^{+/+}$, was derived from the susceptible parent Cs2305 (additive effect=0.03). Lines carrying the Cs2305 allele had a significantly shorter time to flower than those carrying alleles from Cs370, with flowering time accelerated by 30.8%. SAS-TF spans about ~5 MB in nucleotide sequence (20.5 cM). No significant epistatic interactions were observed with SAS-TF (Supplementary Table 6.4). So far, we have not found a genetic link between response to shade in phenology and defence under shade.

6.4.7. Candidate genes underlying RSR and SIS

In the absence of genome sequence for the parental lines, Cs370 and Cs2305, the evaluation of genes underlying RSR and SIS was limited to information available in the CsDH55 reference genome. The CsDH55 genotype resembled Cs370 in its response to disease under shade and overall genetic diversity among these lines was low (Nei's genetic diversity=0.017; Purnamasari *et al.*, unpublished, Chapter 3). Like the genomes of other Brassicaceae species, Camelina has undergone ancient polyploidisation events; as a result, three subgenomes (CsG1–G3) are clearly distinguished in the diploid 2n=40 chromosome genome of Camelina (Kagale *et al.*, 2014a). The QTLs were not detected in the CsG3 paralogous regions in CsG1 and CsG2. Assuming similarity between Cs370 and CsDH55 in their nucleotide sequence, the lack of effect on paralog loci could be explained by i) gene absence, ii) mutations in the coding sequences affecting gene regulation/expression or protein function and/or iii) differential expression profiles (Kagale *et al.*, 2016). Therefore, the selection criteria for generating a draft list of gene candidates were: 1) absence in syntenic chromosomes of Camelina subgenomes CsG1 and CsG2, 2) high K_A/K_S ratio (potential indicator of positive selection) in genes present in subgenomes other than CsG3, 3) gene ontology (GO), 4) synteny in Arabidopsis genome and 5) literature review (Figure 6.2C and 6.3D).

Based on the CsDH55 gene annotation information, the RSR loci span 1.3 Mb (57 genes) and SIS loci span 2.5 Mb (165 genes) (Figure 6.2C and 6.3D). Seven genes of RSR did not have homology

with other chromosomes; among these genes, only two have been characterised, putative F-box/FBD/LRR-repeat protein and transcription factor MYB98. Fifty RSR genes had homology with chromosome 4 (CsG1) and/or 6 (CsG2). For RSR, the average synonymous substitutions and nonsynonymous substitutions for the 50 paralog pairs were 0.14 and 0.04, respectively. Among the genes located in RSR, 42 paralogous pairs were under purifying selection with a K_A/K_S ratio from 0–0.49. There were six genes with K_A/K_S ratio values from 0.5–1, indicating these paralog pairs underwent relaxed selection. Two genes—MYB domain protein and an uncharacterised LOC104710593—had K_A/K_S values > 1, strong evidence of diversifying selection. Using GO enrichment analysis, these 57 annotated genes could be classified into eight groups based on their putative mechanistic biological processes, including six disease-related genes. The literature review showed that 10% of the genes have GO functions related to plant defence mechanisms under pathogen attack, such as Prohibitin-4, Glycosyltransferase protein RCOM, Pectate lyase 11, Zinc finger CCCH domain and MYB domain protein. The top RSR candidate genes are presented in Supplementary Table 6.5.

Of 165 genes, 13 SIS genes did not have homology with genes in other subgenomes, including eight cysteine-rich repeat secretory protein genes, CYP26-1 isomerase, auxin-responsive protein IAA2 and three uncharacterised genes. One hundred and fifty-two SIS genes had homology with genes in chromosomes 1 and/or 19, CsG1 and CsG2, respectively. The average synonymous substitutions and nonsynonymous substitutions for the 152 paralog pairs located in SIS were 0.1 and 0.03, respectively. Most of the genes in this region (120 of 152 genes) had an average K_A/K_S ratio ranging from 0 to 0.49, indicating that most genes were under purifying selection against deleterious mutations. K_A/K_S ratios ranging from 0.5 to 1 were found for 28 genes, including chitin elicitor receptor kinase 1, defensin-like protein 46, leucine-rich repeat extensin-like protein 6, calmodulin-like protein 11, receptor-like protein 12 and protein WVD2-like 3, representing relaxed selection. Four genes—cold-regulated 413 plasma membrane protein 1-like, uncharacterised LOC104748435, cytochrome P450 708A2-like and pentatricopeptide repeat-containing protein At3g23020—had K_A/K_S ratios > 1, indicating that they could have undergone some selective pressure in Camelina. GO enrichment analysis separated these genes into eight biological processes groups: disease-related genes (13 genes of SIS), SAS-related genes (two genes of SIS), genes related to the metabolic process (22 genes of SIS), genes related to signalling (three genes of SIS), genes related to the cellular process (two genes of SIS), genes related to localisation (two genes of SIS) and genes related to response to stimuli (two genes of SIS) (Figure 6.3D). The literature review further indicated that 9% of the genes within SIS were directly associated with the plant response to fungal attack and/or genes that are crucial for the maintenance of defence mechanisms under shade. These genes include Monothiol glutaredoxin,

Chitin elicitor receptor kinase 1, Zinc finger CCCH domain, BTB/POZ domain, FHY-3, JAZ-13, IQ-DOMAIN 1, PCC1, Leucine-rich repeat extension protein 6, Receptor protein 12, Defensin protein 46 and Ethylene-responsive transcription factor (Supplementary Table 6.6).

6.4.8. Defence response under shade for Brassicaceae species differing in sensitivity to shade

In addition to Camelina lines—all grouped as 'moderately responsive' to shade in terms of their phenology and shoot morphology—selected lines of Arabidopsis and canola were tested for their response to disease under shade (Purnamasari *et al.*, unpublished, Chapter 4). We inoculated Arabidopsis genotype AtCol-0 (sensitive to shade, both in phenology and morphology), Arabidopsis genotype AtBla-6 and canola cv Mystic (both clustered as insensitive to shade showing stable phenology and leaf morphology across varying R:FR). The difference between the Arabidopsis lines in response to disease was striking (Figure 6.4). AtCol-0 is known to be susceptible to SR (Ge & Barbetti, 2019), which is enhanced under shade compared to full light ($DS_{0.39}$=64.9% vs DS_{10}=40%; Figure 6.4B). By 3 dpi, large necrotic lesions developed on AtCol-0 leaves under full light; under shade, plants had already collapsed and showed white cottony mycelial growth (Figure 6.4A). In contrast, shade-tolerant AtBla-6 was highly tolerant to SR under both full light and shade, with only a small necrotic lesion (<1 cm) developed by 3 dpi under either full light or shade (Figure 6.4A). The disease severity for AtBla-6 was 15.5% in high R:FR and 15.1% in low R:FR (Figure 6.4B). Similar to AtBla-6, the low R:FR had no adverse impact on the defence response of canola cv. Mystic at either the cotyledonary or flowering stage (Supplementary Figure 6.4). We note that the effects observed in Camelina and the other Brassicaceae lines were not due to differences in the pathogen's growth capacity under shade compared with full light, as they had similar growth rates on agar plates placed under shade and full light alongside the plants (data not shown).

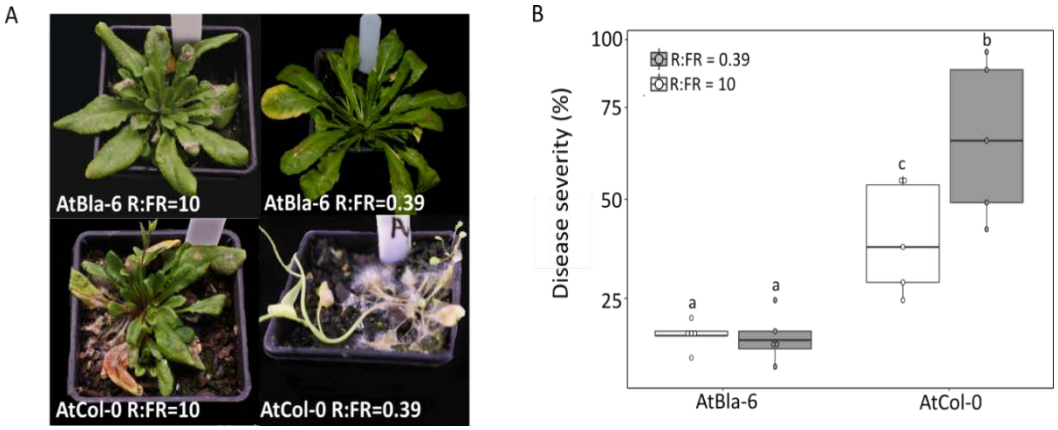

Figure 6.4: Shade effect on different shade-responsive *Arabidopsis thaliana* lines to *Sclerotinia sclerotiorum* infection. **A**, Arabidopsis lines (AtBla-6 and AtCol-0) under full light and shade-mimicking environments at 3 dpi (*n*=5 per genotype). **B**, Disease severity 3 dpi. Box plots represent disease severity of two Arabidopsis lines under contrasting light conditions. Different letter indicate significant differences between means (P<0.05).

6.5. Discussion

The genetic basis of plant response to disease and other stimuli is complex, but a scientific challenge of increasing relevance. Here, we target natural variation using a crop as the subject and QTL analysis as the approach to disrupting adapted genetic blocks and study plant response to disease under shade. We identified a locus associated with reduced defence in shade-avoiding species. This is the first attempt to uncover genetic determinants of defence under shade using QTL analysis. Identifying QTL that affect adaptation to a combination of environmental factors provides a foundation for developing plants tolerant to multiple challenges in the field.

Sclerotinia sclerotiorum, the necrotrophic fungus causing SR, was the pathogen of choice due to its recalcitrant, broad-host nature and significant adverse impact on natural and agroecosystems. Camelina was the plant choice due to its close relationship with important oilseed grain and vegetable crops and relatively neglected status in terms of literature. Our study demonstrated that shade downregulates the defence of Camelina to *S. sclerotiorum* at the cotyledonary and flowering stages, adding to accumulating evidence on the negative effects of shade on plant defence (Cerrudo *et al.*, 2012; de Wit *et al.*, 2013; Cargnel *et al.*, 2014; Ballaré, 2014; Schumacher, 2017). We identified two QTL affecting Camelina response to SR—RSR, with an additive effect on defence under full light but compromised under shade, and SIS, with a negative effect on defence under shade. To the best of our knowledge, these are the first QTL reported for Camelina resistance to SR and a good addition to the shortlist of genetic determinants of resistance against *Sclerotinia* in general.

Camelina resistance to *S. sclerotiorum* has a complex polygenic architecture, consistent with observations for other hosts in the Brassicaceae (*e.g.*, Rana *et al.*, 2017; Barbacci *et al.*, 2020; Qasim *et al.*, 2020), Fabaceae (*e.g.*, Vasconcellos *et al.*, 2017; Ashtari Mahini *et al.*, 2020; Liang *et al.*, 2020) and Asteraceae (*e.g.*, Fass *et al.*, 2020). Of the numerous QTL associated with resistance to SR, several genes have been identified, including IGMT5 (Wu *et al.*, 2013) and ARPC4 (Badet *et al.*, 2019). Taking advantage of the Camelina genome's paleo-allopolyploid structure, coupled with the availability of an annotated reference genome and high synteny between the Camelina and Arabidopsis genomes, we looked for candidate genes underlying RSR and SIS. For example, we identified a strong candidate gene underlying RSR, the MYB transcription factor (CsID 104710558), which has been under positive selection ($K_A/K_S > 1$). Recently, Chen *et al*. (2020) showed that Arabidopsis *myb28/myb29* mutant plants, deficient in aliphatic glucosinolate biosynthesis, were more susceptible to *S. sclerotiorum* than the wild type. Furthermore, Zhuang *et al*. (2012) detected seven MYB transcription factors upregulated in pea plants challenged with *S. sclerotiorum* infection. Another potential candidate underlying RSR, pectate lyase (CsID 104710564), has been reported as

a candidate gene affecting negative regulatory factors in soybean partial resistance to *S. sclerotiorum* (Sun *et al.,* 2020). SIS, the second QTL discovered in the current study, has a negative allelic effect (increased disease severity) on plant response to SR under shade. In the list of potential candidate genes behind SIS, only one gene has repression effects, annotated as JAZ13. Gommers *et al*. (2017) detected differential JAZ transcript accumulation between shade-avoidance and shade-tolerant *Geranium* species resulting in different immunity responses under low R:FR. JAZ proteins negatively regulate the JA-defence response by binding to the MYCs transcription factor (Guo *et al.,* 2018). Furthermore, the null mutation in JAZ genes can increase plant immunity to necrotrophic pathogens under low R:FR (*e.g.,* Cerrudo *et al.,* 2012, 2017). Interestingly, these studies were undertaken on leaves; the current study outcomes likely indicate that similar defence regulatory mechanisms are involved in other plant components, *viz.* stems. These findings will remain speculative until the genomic sequence of parental lines is available and causal genes are confirmed through map-based cloning or other approaches.

Shade weakens Camelina defence to SR by 1) compromising the RSR effect on defence and 2) further decreasing resistance through the effects of SIS. To further evaluate the utility of RSR and SIS in breeding programs for improving Camelina across agricultural production, field experiments are required to confirm that the host responses in the current study also occur under field conditions. If our findings are reproduced under field conditions, the approach of evaluating a crop's response to disease under shade within controlled-environment conditions is of considerable value for Camelina and other breeding programs. Our finding also contributes to understanding the epidemiology of a recalcitrant pathogen in the field and the selection pressure for the pathogen when it encounters a plant's molecular network adapted to shade. Studies on the evolutionary genetics of the plant–pathogen system under shade are scarce, with none on the plant–*S. sclerotiorum* pathosystem. Hence, strategies attempting to manage disease at full canopy closure or intercropping could be more challenging than appreciated. Further studies are needed to improve our understanding of this and other diseases that develop within the lower parts of plant canopies.

There was no correlation between defence-related traits under shade and shade-avoidance traits in Camelina. Studies on powdery mildew in cucurbits highlighted increased susceptibility under shade caused by morphology modifications under shade, such as higher specific leaf area and lower stem diameter (Shibuya *et al.,* 2011; Itagaki *et al.,* 2016). Since our resistant and susceptible lines do not differ in their response to shade, the RIL population is a great tool for studying defence without confounding effects of SAS. We discovered a distinct QTL profile responsible for disease response and flowering time (important SAS traits) that agrees with the lack of phenotypic correlation. We

detected SAS-TF, a QTL on chromosome 11 for flowering time response under different R:FR in Camelina. Our results support previous mutant analyses showing that growth and defence could be uncoupled in phytochrome B and JAZ10 mutants (Campos *et al.,* 2016; Cerrudo *et al.,* 2017). In Arabidopsis, a model species for many plant molecular studies, the response to disease and response to shade appear to be confounded in at least some lines, including the shade-stable and SR-resistant genotype, AtBla6. This genotype could maintain its immunity against *S. sclerotiorum* and normal morphology under shade, in contrast to the reduced defence observed in the shade-avoidance Arabidopsis genotype, AtCol-0. Other studies support this finding, revealing that strong competitors are likely to be more resistant to pathogens (Valladares & Niinemets, 2008; Viola *et al.,* 2010; Gommers *et al.,* 2017). The dissimilarity of response in AtBla-6 and AtCol-0 to *S. sclerotiorum* under shade provides a basis for future genetic analysis to understand the mechanism underlying such differences and should identify whether SAS traits and defence are cofounded. In addition, because there was a shade- and SR-tolerant genotype in Arabidopsis, we could confirm that the experimental conditions were ideal and conducive towards characterising defence responses under shade.

6.6. Conclusion

Overall, the current study supports the narrative that shade compromises the defence mechanism of shade-sensitive plants but has no impact to the defence response of shade-tolerant lines. In shade-avoiding species like Camelina, shade weakened the defence mechanism by compromising RSR and SIS development. Defence response under shade is a complex interplay among different hormonal signalling pathways so other mechanisms will be involved. However, the current study provides an initial step for breeding resilient crops under high planting density and/or where a dense crop canopy occurs. Additional future studies, including validation in other mapping populations, field studies and fine mapping should identify other potential candidate genes and validate their role in defence under shade. An exciting future challenge will be to identify the genetic architecture from shade-tolerant Arabidopsis lines, providing additional insight into developing effective management strategies that optimise the coordination of crop defence and crop growth under shade conditions.

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6.8. Supplementary tables and figures

Supplementary Table 6.1: Summary statistics for *Camelina sativa* parental lines and RILs for disease severity at two developmental stages (cotyledon and flowering stages) and two R:FR regimes. At the flowering stage, time to flower data is also shown.

Traits	Means		RILs							
	C370	C2305	Minimum	Maximum	Means	SE	Variance	Skewness	Kurtosis	W-test
Cotyledon stage										
C-$DS_{0.39}$	57.4	69.4	33.3	98.1	65.5	12.4	152.7	0.1	0.18	0.98
C-DS_{10}	42.1	67.1	30.6	83.8	59.3	10.7	115	-0.04	-0.12	0.98
C-DPI	-1.6	-0.2	-3.5	3.8	-0.6	1	1	-0.016	-0.03	0.98
Flowering stage										
F-$DS_{0.39}$	0.5	0.6	0.02	1	0.7	0.2	0.05	-0.8	0.7	0.91
F-DS_{10}	0.04	0.3	0.01	0.8	0.2	0.2	0.03	1.5	2.1	0.79
F-DPI	-2.8	-2.7	-6.1	0.7	-3.3	1.5	2.4	0.5	0.05	0.96
$TF_{0.39}$	47.4	48.6	36	55	47.8	5	25.3	-0.4	-1.1	0.88
TF_{10}	48	44	41.7	56	47.7	3	9.2	0.5	-0.04	0.96
TFPI	0.09	0.09	-1.2	1.6	0.1	0.07	0.004	0.6	-0.1	0.95

Supplementary Table 6.2: Cs370×Cs2305 RILs phenology and response to SR at two developmental stages and two light quality treatments. Key parameters for the two-way ANOVA analysis and broad-sense heritability (H^2). Number of RILs replicates for each trait are next to the trait name; no $DS_{0.39}$ or $FT_{0.39}$ are presented as RILs were not replicated.

Traits	Variation[a]	df	MS	F	P	H^2 (%)
C-$DS_{0.39}$ (*n*= 6)	G	105	914.1	2.6	0.0000	61.3
	B	5	3387.5	2.9	0.0000	
C-DS_{10} (*n*= 6)	G	105	73273	1.7	0.0004	41.4
	B	5	4387	2.2	0.0547	
F-DS_{10} (*n*= 3)	G	105	240.6	1.7	0.0033	42.8
	B	2	196.4	1.4	0.2423	
FT_{10} (*n*= 3)	G	105	27.7	2.2	0.0000	55
	B	2	14.9	1.2	0.3050	

a) G (genotype) is RIL genotype factor (*n*=106 RILs), B (block) is replication.

Supplementary Table 6.3: DArT markers distribution into linkage groups.

Chromosome	Number of markers	Total length (cM)	Average distance between markers (cM)
1	34	142.5	4.2
2	15	102.7	6.8
3	20	113.8	5.7
4	26	225.8	8.7
5	16	147.6	9.3
6	10	159.7	16
7	46	192.5	4.2
8	25	122.6	4.9
9	31	153.7	5.0
10	20	132.1	6.6
11	41	226	5.5
12	25	216.6	8.7
13	37	231.6	6.3
14	33	225.9	6.8
15	15	91.4	6.1
16	42	199.3	4.7
17	21	116.6	5.6
18	13	129.2	9.9
19	33	144.2	4.4
20	12	102	8.5
Total	515	3175.8	6.2

Supplementary Table 6.4: QTL pairs with significant epistatic interactions. Only pairs which have passed the significant threshold (logarithm of the odds ratio (LOD) >5) are presented.

Traits	Linkage Group 1	Position 1	Marker interval 1	Linkage Group 2	Position 2	Marker interval 2	LOD	R^2	Additive effect
$C\text{-}DS_{0.39}$	17	40	M443-M581	20	25	M954-M020	5	0.186	-0.16
$C\text{-}DS_{10}$	11	80	M844-M240	16	10	M674-M449	5.2	0.324	-0.1
$F\text{-}DS_{0.39}$	1	120	M098-M534	14	115	M113-M350	18.676	0.8212	-0.5534
	2	15	M177-M658	9	75	M231-M944	16.191	0.8233	-0.5718
	6	60	M610-M344	13	10	M146-M502	15.0528	0.8173	-0.5696
	8	100	M043-M192	11	215	M872-M187	19.8036	0.8234	-0.5944
	8	90	M280-M043	13	105	M191-M040	20.1601	0.8196	-0.551
	8	60	M010-M224	15	70	M850-M171	16.7435	0.8256	0.5527
	9	65	M810-M231	12	35	M964-M743	18.4692	0.8261	-0.5325
	9	130	M550-M601	18	85	M273-M444	17.9477	0.826	0.595
	10	95	M217-M982	13	145	M772-M471	14.5576	0.8167	0.561
	12	35	M964-M743	14	60	M226-M951	15.1119	0.8219	-0.5689
	16	5	M674-M449	18	110	M444-M937	17.7529	0.8174	0.564

Supplementary Table 6.5: Candidate genes underlying RSR (LL_{10}).

Gene ID (CsDH55-G3)[a]	Gene ID (CsDH55-G1)[a]	Gene ID (CsDH55-G2)[a]	K_S (G3-G1)	K_S (G3-G2)	K_A/K_S (G3-G1)	K_A/K_S (G3-G2)	Arabidopsis Homologue ID (At-Col1)[b]	Gene	Reference
104710550	104779956	-	0.06	-	0.33	-	AT3G27280	Prohibitin-4	Nadimpalli *et al.*, 2000
104710557	104779964	104790387	0.08	0.08	0.16	0.24	AT3G27330	Glycosyltransferase family 92 protein RCOM_0530710-like	Amos & Mohnen, 2019
104710564	104779971	104790395	0.09	0.06	0.06	0.07	AT3G27400	Putative pectate lyase 11	Sun *et al.*, 2020
104710570	104790424	104715061	0.02	0.04	0.41	0.37	AT3G27700	Zinc finger CCCH domain-containing protein 41-like	Zhao *et al.*, 2006
104710558	104790388	104779965	0.02	0.02	2.2	2.02	AT3G27810	Transcription factor MYB	Zhuang *et al.*, 2012

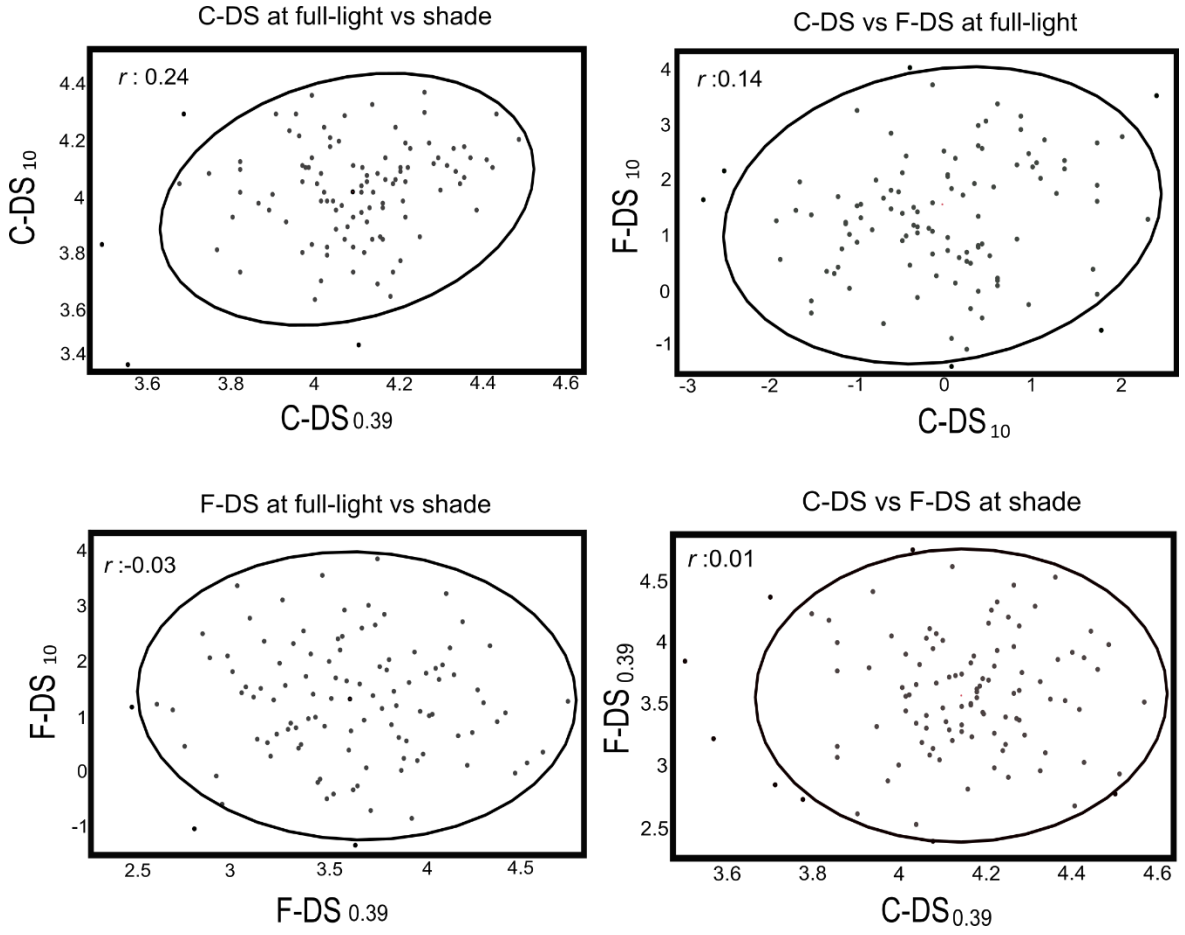
a /b references to the genome sequence or database.

Supplementary Table 6.6: Candidate genes underlying SIS ($LL_{0.39}$ and DPI at flowering).

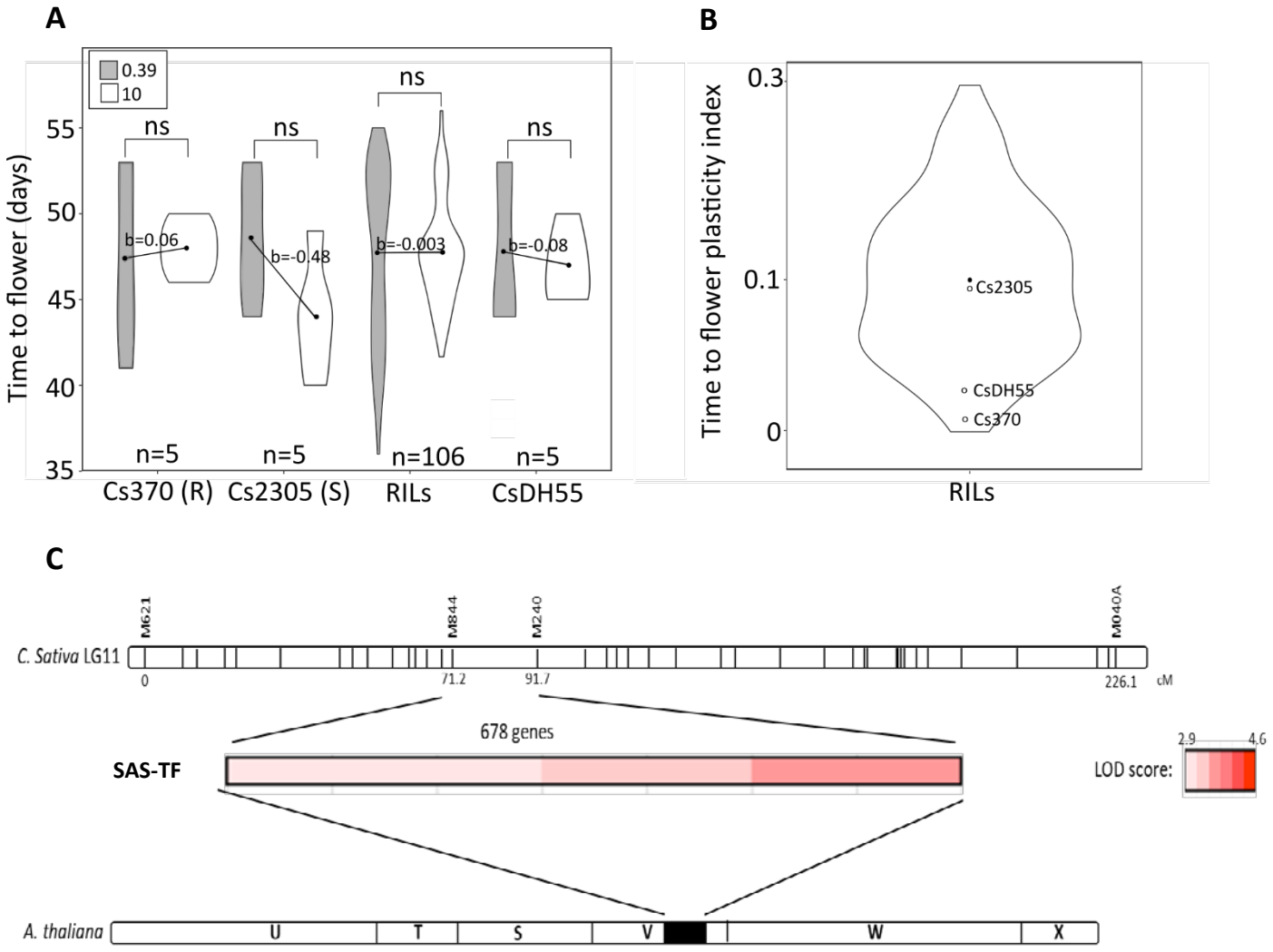
Gene ID (CsDH55-G3)[a]	Gene ID (CsDH55-G1)[a]	Gene ID (CsDH55-G2)[a]	K_S (G3-G1)	K_S (G3-G2)	K_A/K_S (G3-G1)	K_A/K_S (G3-G1)	Arabidopsis Homologue ID (At-Col1)[b]	GO term	Reference
104746572	104766041	104786497	0.09	0.09	0.07	0.04	AT3G21460	Monothiol glutaredoxin-S10-like	Li, 2014
104746591	104766063	104786678	0.05	0.03	0.62	1.01	AT3G21630	Chitin elicitor receptor kinase 1	Zhang *et al.*, 2013
104746624	104786894	104766087	0.05	0.07	0.39	0.4	AT3G21810	Zinc finger CCCH domain-containing protein 40-like	Gupta *et al.*, 2012
104746654	104766106	-	0.02	-	0.2	-	AT3G22104	BTB/POZ domain-containing protein At3g22104	Boyle *et al.*, 2009
104746661	-	104787176	-	0.01	-	0.17	AT3G22170	FAR-RED ELONGATED HYPOCOTYL 3 (FHY3)	Liu *et al.*, 2019
104746664	-	104787193	-	0.02	-	0.25	AT3G22190	IQ-DOMAIN 1-like	Bergey *et al.*, 2014
104746669	104787271	104766121	0.13	0.11	0.06	0.08	AT3G22231	Cysteine-rich and transmembrane domain-containing protein PCC1	Sauerbrunn & Schlaich, 2004
104746678	104766125	104787322	0.06	0.07	0.16	0.14	AT3G22275	JAZ13	Thireault *et al.*, 2015
104746715	-	104746036	-	0.2	-	1	AT3G22580	Defensin-like protein 46	Stotz *et al.*, 2009
104746744	104766176	104787772	0.03	0.06	0.04	0.02	AT3G22800	Leucine-rich repeat extensin-like protein 6	Wu *et al.*, 2016
104746776	104746791	104789209	0.04	0.07	0.17	0.04	AT3G23110	Receptor-like protein 12	Lv *et al.*, 2016
104746810	104788184	104766232	0.1	0.1	0.18	0.12	AT3G23240	Ethylene-responsive transcription factor 1B-like	Lorenzo *et al.*, 2003

a /b references to the genome sequence or database.

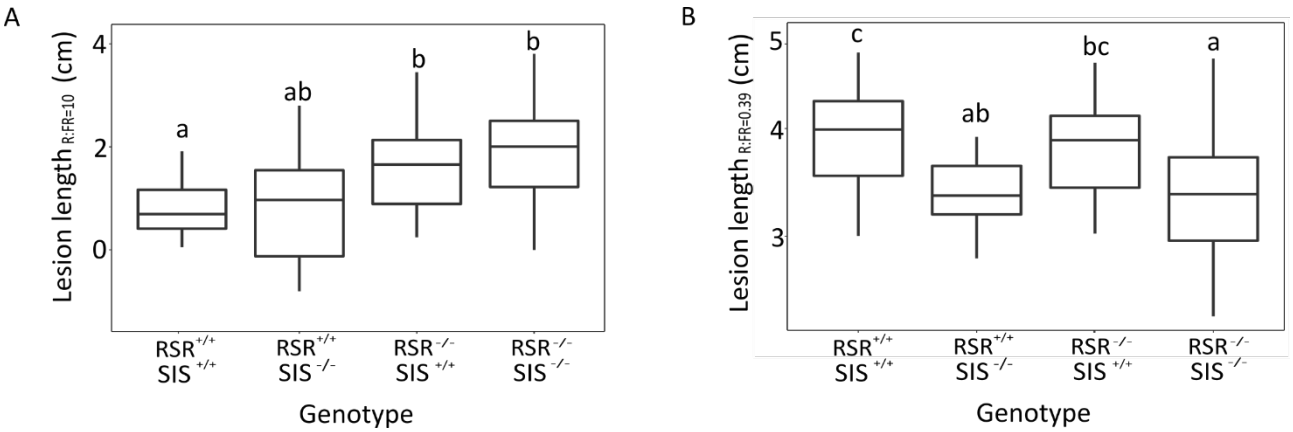
Supplementary Figure 6.1: Correlation between disease severity parameters in this study. *r* values represent Pearson correlation coefficients.



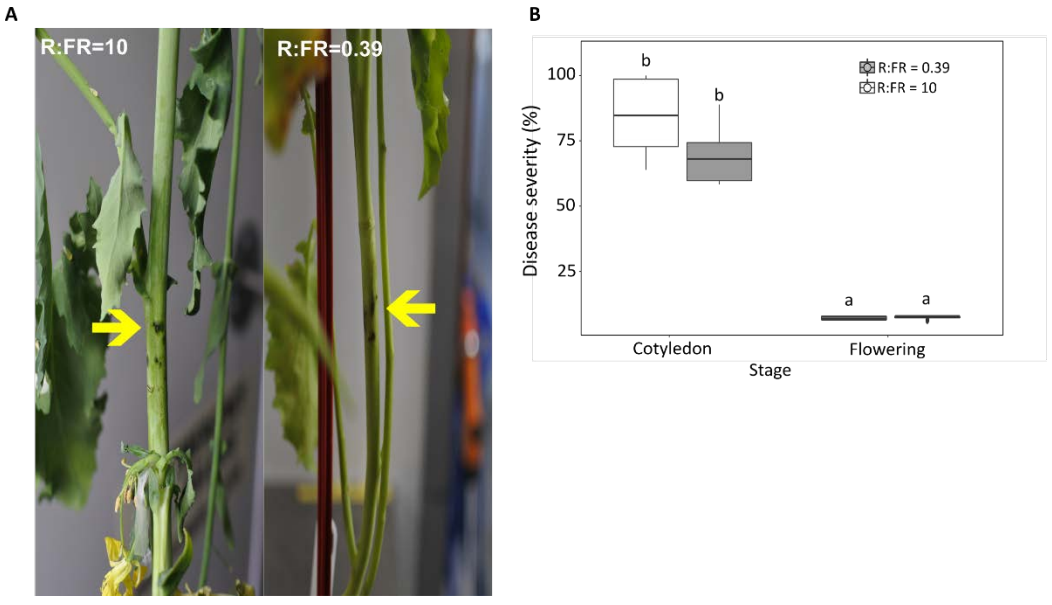
Supplementary Figure 6.2: *Camelina sativa* shade-avoidance syndrome-time to flower (SAS-TF) QTL. **A**, Violin plots for three lines of Camelina and recombinant inbred lines (RIL) F_5 derived from a cross between Cs370 (R) and Cs2305 (S). Violin plots represent the mean time to flower (n_i=5 per genotype and n_i=3 per RIL at cotyledon; n=106 RILs). **B**, Time to flower plasticity index (TFPI) calculated as the slope between the flowering response under low R:FR and high R:FR. The plasticity index value for R and S parental lines are indicated by open circles. **C**, LOD scores against the marker order of CsLG11 (equivalent to CsDH55 CHR11) obtained for TFPI on the Cs370×Cs2305 RIL population indicating the putative position of SAS-TF; genetic distances (cM) are provided above the CsLG11 bar, with the marker indicators below. The corresponding synteny blocks identified in Arabidopsis chromosome positions are labelled F–H.



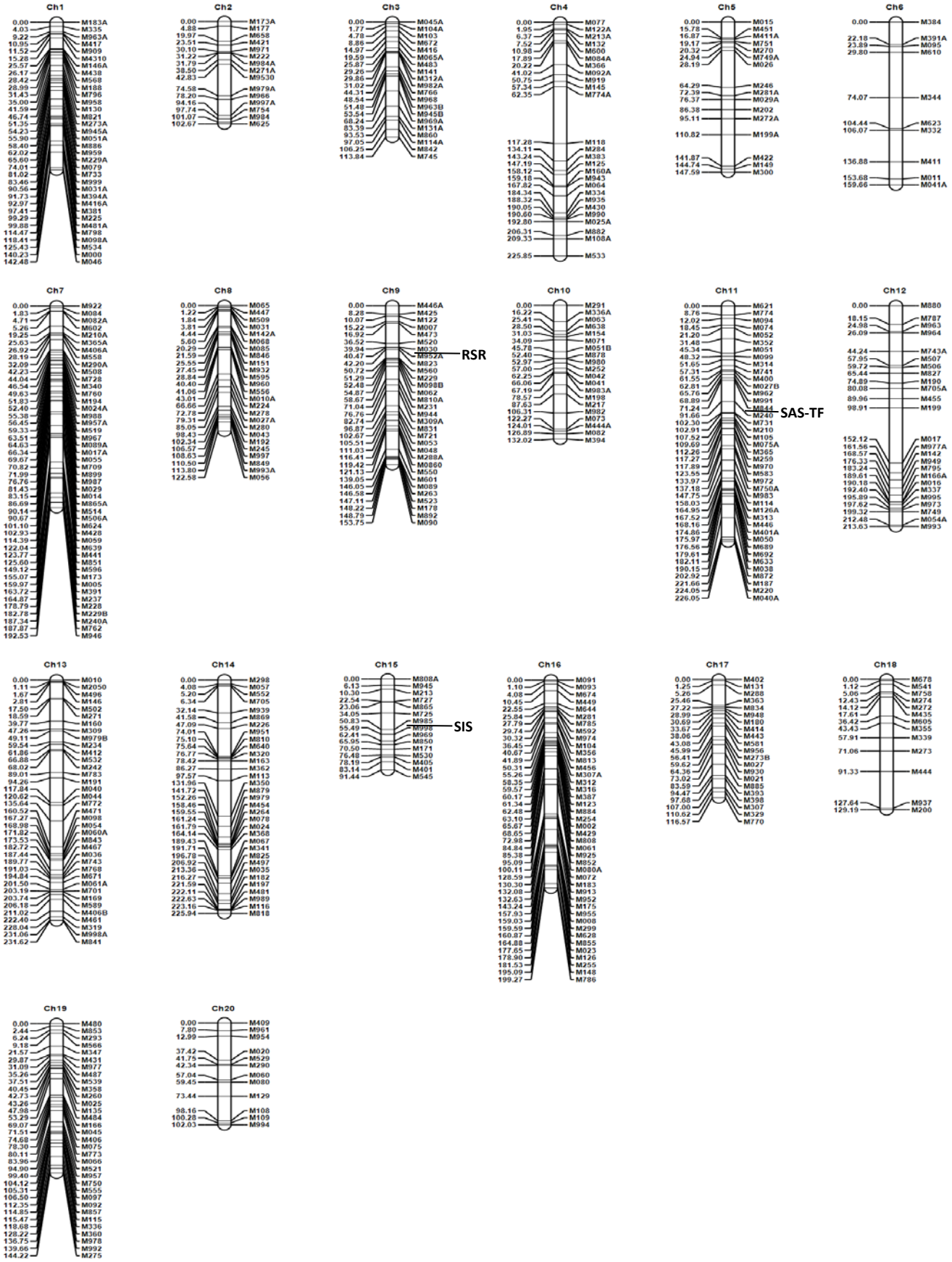
Supplementary Figure 6.3: Lesion length of different RSR and SIS allele genotypes under contrasting light conditions. **A**, Lesion length under full light. Box plots represent lesion length of different allele combinations. **B**, Lesion length under shade. Box plots represent lesion length of different allele combinations. Means with the same letter are not significantly different (P<0.05).



Supplementary Figure 6.4: Shade effect on *Brassica napus* cv. Mystic to *Sclerotinia sclerotiorum* infection. **A**, Canola under full light and shade-mimicking environments at 21 dpi (n=5). **B**, Disease severity at 21 dpi. Box plots represent disease severity of canola under different life stages and light conditions. Means with the same letter are not significantly different (P<0.05).



Supplementary Figure 6.5: QTL identified in this study mapped on the Cs370×Cs2305 genetic linkage map.



CHAPTER 7

General discussion

In this thesis, I constructed a model system using *Camelina sativa*, shade and *Sclerotinia sclerotiorum* to better understand how a plant responds to multiple environmental factors. How plants sense and adjust to various environmental stimuli through phenotypic plasticity is a fundamental biological question. A review of the literature in Chapter 2 demonstrated a knowledge gap on natural variation in plant defence under shade and Camelina phenotypic plasticity to shade, *S. sclerotiorum* and their interaction/s, which are essential factors for crop productivity. Expanding our knowledge in these areas is important from a fundamental and applied research perspective. In this final chapter, I discuss how the research presented in this thesis contributes to the current understanding of the genetic regulation of plant defence under shade. Moreover, I propose practical applications for Camelina improvement and potential research priorities.

7.1. Genetics of plant defence under shade

Implications for plant disease epidemiology

Shade alters both the microclimate in infection sites and the plant-signalling network(s) that regulate plant–pathogen interactions. Shade provides ecological niches for pathogens by changing other environmental factors. By controlling confounding factors in shady environments (*e.g.,* humidity and temperature), I characterise the genetic mechanism/s underlying reduced plant defence under shade using red to far-red ratio (R:FR) as parameter for shade (Chapter 6). Based on the Camelina genotype response to SR (Chapter 5), I used a RIL population comprising 106 F_5 lines of Camelina, derived from a cross between SR-resistant genotype C370 and SR-susceptible genotype C2305, to collect phenotypic and genotypic data. I developed the first genetic map for Camelina response to disease and found two novel QTL. This thesis collectively provides new insights into how shade affects defence mechanisms in shade-avoidance plants by compromising the plant resistance locus (Resistance to Sclerotinia Rot; RSR) and inducing the susceptibility locus (Shade-Induced Susceptibility; SIS). This information is crucial for developing control strategies in agricultural ecosystems, especially in high-density planting and/or intercropping systems.

Beyond a single genotype

In the last two decades, molecular and genetic studies have identified mechanisms underlying plant defence under shade, particularly the crosstalk between the phytohormones gibberellic acid and jasmonic acid (*e.g.,* Leone *et al.,* 2014; Campos *et al.,* 2016; Cerrudo *et al.,* 2017). Under shade, the defence mechanism is a highly tuned complex network that links light and defence signalling pathways. Consequently, a detailed explanation of the molecular mechanism of plant defence under shade is incomplete; in particular, little is known about the genetic basis of natural defence-under-shade variation in plants. Almost all characterised molecular pathways come from mutation studies

of model species and a few lines in these species. The natural variation in defence regulation under shade has considerable economic and scientific interest, as it provides some advantages over mutant studies. For example, the effect of mutations depends on genetic background, and phenotypes are not necessarily a direct result of mutations, leading to inaccurate interpretation (Borevitz & Nordborg, 2003; Zimmer *et al.*, 2019). Most importantly, natural variation occurs through natural selection, over long periods and numerous life cycles, through which species adapt to particular environments. Thus, natural variation provides a synthesis of an evolutionary process and its outcome. The literature review in Chapter 2 reveals that only two studies, by Gommers *et al*. (2017) and Ranade & Garcia-Gil (2020), have evaluated differences between shade-avoidance and shade-tolerant species in the context of their defence mechanisms under shade. These two studies highlight a possible connection of shade impact on pattern-triggered immunity-mediated defence and basal defence (cell wall regulation), suggesting how evolutionary change could significantly expand the scope of our understanding. My research in Chapter 6 marks a significant effort towards addressing this knowledge gap by evaluating natural variation using the Camelina–*S. sclerotiorum* pathosystem. To the best of my knowledge, this is the first genetic study using natural variation in a recombinant inbred line population to dissect the genetic mechanism of plant defence under shade.

Discovery of allelic variation controlling defence under shade

Arguably, the most important contribution of this thesis was finding a genomic region that contributes to plant susceptibility under shade—SIS—which affects both stem lesion length in shade-mimicking light (R:FR=0.39) and a differential response between disease severity at full light (R:FR=10) and shade-mimicking light (*i.e.*, slope). The SIS allele of resistant parent C307 leads to increased disease severity under shade compared to full light. Interestingly, the only repressor gene from the list of potential candidate genes in SIS is annotated as JASMONATE ZIM-domain 13 (JAZ13). JAZs have been reported as key players in regulating plant defence under shade by inhibiting MYCs transcription factor, thereby inactivating a downstream JA-defence mediated response (Hou *et al.*, 2010; Campos *et al.*, 2016; Cerrudo *et al.*, 2017; Gommers *et al.*, 2017). More research is required to identify and confirm the causal gene for SIS, as it represents a locus of keen interest for future research on defence response under shade.

A second significant achievement of this thesis was mapping RSR, the quantitative disease resistance (QDR) locus for Camelina resistance to *S. sclerotiorum*. There is limited knowledge of Camelina defence mechanisms to this recalcitrant broad-host pathogen, with only two studies in the literature (Chapter 2, Enyck *et al.*, 2012; Purnamasari *et al.*, 2015). This knowledge gap is due to a lack of substantive efforts to understand Camelina–pathogen interactions. For example, there was no genetic mapping study for disease resistance, with most Camelina QTL mapping undertaken for oil content,

seed yield, seed size and flowering time (*e.g.*, Gehringer *et al.*, 2006; King *et al.*, 2019; Chaudhary & Parkin, 2020). Therefore, RSR provides the opportunity to understand the genetic architecture of Camelina defence mechanisms, especially to a broad-host hemibiotrophic pathogen like *S. sclerotiorum*. RSR explained 14% of the phenotypic variation, and the presence of this QTL in genotypes carrying resistant parent alleles reduced disease severity more than the genotypes carrying susceptible parent alleles. Based on the Camelina DH55 gene annotation information, the chromosomal region of RSR spanned an average of 1.3 Mb on Linkage Group (LG) 9. This region contains candidate genes reported in plant resistance to SR, including MYB transcription factor (Zhuang *et al.*, 2012; Chen *et al.*, 2020) and pectate lyase (Sun *et al.*, 2020). Interestingly, RSR was not detected under shade-mimicking conditions, highlighting another reason why plant defence is compromised under shade. This information will undoubtedly enable researchers to alter crop genetics to develop better disease resistance under shade.

Shade-tolerant group as a resource for improving defence-under-shade tolerance

A question that arose when looking for natural variation in Camelina defence plasticity under shade was how do shade-tolerant lines respond to a pathogen? In Chapter 4, my results demonstrate Camelina lines exhibit no genotype by environment interaction (G×E) in their plasticity response to low R:FR; hence it was not possible to address this question using the Camelina lines used in my studies. Fortunately, using the multiple phenotype approach to concurrent factors, I identified three Brassicaceae lines, namely Camelina, Arabidopsis and canola that separated into three groups based on their plasticity to shade. Although seven Camelina lines clustered in Group 3, ten lines of Arabidopsis spread across three groups, representing different plasticity response to shade. Using this information, I chose to inoculate a shade-sensitive genotype AtCol-0 (Group 3) and shade-stable genotype AtBla-6 (Group 1) with *S. sclerotiorum* under full light and shade-mimicking light (Chapter 6). The shade-stable AtBla-6 maintained its immunity against *S. sclerotiorum* under shade-mimicking conditions. In contrast, the shade-sensitive AtCol-0 increased its disease severity under shade, a similar response to Camelina. This result agrees with a meta-analysis by Viola *et al*. (2010) and a study by Gommers *et al*. (2017) that found shade-tolerant species are likely to be more resistant to pathogens under shade. For the first time, this thesis demonstrates the natural variation in Arabidopsis lines for their defence response under shade. Natural diversity to this complex trait in plant model species has substantial implications for future genetic analysis to understand the mechanisms shade-tolerant plants use to optimise both growth and defence under shade.

Can growth and defence be uncoupled?

The natural variation in Camelina defence under shade agrees with results from mutant studies showing growth and defence under shade are not linked and can be uncoupled (Moreno *et al.,* 2009; Cerrudo *et al.,* 2012; Campos *et al.*, 2016). The phenotypic data showed no correlation between defence-related traits and SAS traits. Similarly, the SAS-TF (shade-avoidance syndrome-time to flower) QTL for time to flower plasticity under shade was found in chromosomal regions other than SIS. This finding highlights different pathways for regulating growth and defence under shade. The reason why plants reduced their defence under shade remains unknown. However, my study contributes evidence to the view that networks regulating SAS and defence under shade are not merely a factor of resource allocation constraints, in agreement with hypotheses postulated by Züst & Agrawal (2017) and Ballaré & Austin (2019). Nevertheless, the ability of AtBla-6 to maintain its growth and defence under shade highlights a possible relationship between SAS traits and defence under shade. It is important to note my study measured flowering time and stem diameters, while other mutant studies measured hypocotyl length. As such, SAS traits were analysed separately, which may have contributed to the lack of correlation observed in my study. Further elucidation of natural variation in Arabidopsis lines in multiple SAS traits (as in Chapter 4) and defence under shade would clarify this issue.

Future research opportunities

Given the importance of finding the cause and functionality of plant defence mechanism(s) to recalcitrant pathogens like *S. sclerotiorum*, further research to validate these QTL in different mapping populations—to narrow the interval and identify candidate genes in SIS and RSR—are essential. One potential limitation in my study was the inherent sample-size-restriction imposed by growth in controlled-environment shade conditions compared to what could be achieved in field studies. Therefore, it remains ecologically important to test the robustness of RSR and SIS in a natural environment with large population size. Increasing population size could also allow an increase in the number of recombination events between co-segregation markers in the QTL region. Availability of a complete genome sequence of parental lines would also aid the identification of candidate genes. Thus, further study to re-sequence the parental lines should reveal polymorphism for marker development and enable the construction of a saturated map. Integrating other molecular strategies would be beneficial for future functional candidate gene identification. For example, RNA sequencing of the two parents would reveal which specific genes are differentially expressed in the RSR or SIS interval.

7.2. Improvement of Camelina

Implications for Camelina improvement

A greater understanding of phenotypic plasticity to shade and disease in the understudied crop Camelina should facilitate improvement and broader adoption of this crop in suitable production regions. The low genetic diversity in available germplasm, coupled with small effective population sizes in the species, is a constraint to crop improvement and genetic studies (Brock *et al.*, 2018; Luo *et al.*, 2019). To overcome this constraint, I took a comparative analysis approach using the knowledge of well-established species related to Camelina: Arabidopsis and canola. Camelina and the model plant Arabidopsis are in lineage 1 of Brassicaceae with high similarity of genome sequence and transcriptome. This similarity allows the use of Arabidopsis as a reference for phenotypic and molecular studies. Canola, in lineage 2 of Brassicaceae, provides information on genetic alterations and phenotypic evolution of domesticated oilseed crops. Hence, the results outlined in this thesis have produced resources and novel information with practical implications for the improvement of Camelina, as discussed below.

Assessment of genetic diversity in Camelina

The characterisation of Camelina germplasm is important for the effective use of Camelina genetic resources in breeding. In Chapter 3, I revisited the assessment of 31 lines of Camelina held at UWA (Ghamkhar *et al.*, 2010), together with the genome reference line DH55, using new markers from genotyping-by-sequencing, flow cytometry and phenotypic evaluation. These lines were originally obtained from the N.I. Vavilov Research Institute of Plant Industry, Russia and pure-lined at The University of Western Australia by other researchers prior to my PhD. While I found that the Camelina germplasm has low genetic diversity as per other studies in other germplasm collections (*e.g.*, Singh *et al.*, 2015; Brock *et al.*, 2018; Luo *et al.*, 2019), I also found intraspecific diversity in genome size and phenotypic diversity. Using STRUCTURE and identity-by-state (IBS) analysis, I separated the lines into two population groups, offering a potential source of useful agronomic traits for breeding material and adoption in Australia. For example, two parental lines of the RIL population used in Chapters 5 and 6 (C370 and C2305) have low genetic relatedness (IBS value=0.47), which could explain their different response to SR. These findings are also relevant for breeding programs seeking to enhance diversity within Camelina spring types.

The phenotypic plasticity of Camelina in response to shade

A key agronomic finding from this thesis is the characterisation of phenotypic plasticity to shade (Chapter 4). Prior to this research, the response of Camelina to varying R:FR for morphology and phenology was unknown. Therefore, I explored different shade-avoidance responses in Camelina and its Brassicaceae relatives Arabidopsis and canola using multiple phenotypic traits to gain a deeper understanding of plant plasticity in response to light quality. I investigated seven lines of Camelina, ten lines of Arabidopsis and three canola lines, under five different R:FRs ranging from shade-mimicking (R:FR=0.2) to well above the normal sunlight ratio (R:FR=10). When I measured the reaction norm of 12 morphological traits at flowering stages, seven Camelina lines exhibited typical shade-avoidance syndrome under low R:FR, such as internode elongation, altered leaf shape and increased specific leaf area, all aimed to facilitate 'escape' from low R:FR and maximise light interception (Li & Kubota, 2009; Casal, 2013; Du *et al.*, 2017). This response to shade is useful when assessing the potential of planting Camelina into a dense monoculture system at a high seeding rate or growing it within a mixed-species intercropped stand. Furthermore, I identified that the capacity to reduce leaf area under shade is an essential determinant of increased biomass under shade. This finding offers selectable traits for Camelina breeding to develop shade-resilient cultivars. The introduction of a Camelina cultivar that could withstand pressures from high-density planting may prove key to improving yield in this crop, in the same way as this has been done for maize (Duvick, 2005). As shade is characterised not only by changes in the R:FR, a future study could collect empirical information of plastic responses in ecologically relevant conditions with as many replications as possible.

Camelina defence response to two major Brassicaceae pathogens

Another significant achievement of this thesis from a practical perspective was the characterisation of resistant lines for SR. In Chapter 5, I determined the response of 30 lines of Camelina to two pathotypes of *S. sclerotiorum* at cotyledon and adult stages. The study identified lines C370 and C253 that consistently exhibited *S. sclerotiorum* pathotype-independent cotyledon and stem resistance. Prior studies have noted the importance of using more than one type of inoculation test and using contrasting pathotypes when defining QDR (Ge *et al.*, 2012; Peltier *et al.*, 2012; Purnamasari *et al.*, 2015). Therefore, these lines are likely to be valuable for breeding SR-resistant Camelina varieties as very little is known about the level of pathogen variation in this oilseed crop. It would be beneficial to take these findings and validate them under field conditions, which was outside the time limitations of my study.

The research presented in Chapter 5 confirms the outstanding resistance in Camelina to six isolates of *Leptosphaeria maculans* (causal agent of blackleg) with diverse avirulence allele combinations. Several studies have reported very high levels of Camelina resistance to *L. maculans* (*e.g.*, Li *et al.*, 2005; Gregorich *et al.*, 2009; Séguin-Swartz *et al.*, 2009), but they involved relatively few lines and/or *L. maculans* races. One unanticipated result was a varied response for resistance to this pathogen, in which I showed that genotype C2305 was consistently more susceptible than the other lines to the four isolates of *L. maculans*. Importantly, C2305 was also consistently susceptible to two pathotypes of *S. sclerotiorum*. The similarity between resistant and susceptible Camelina lines to two contrasting pathogens of Brassicaceae may indicate a common mechanism that Camelina uses for defence and offers a potential opportunity to use the same mapping population to understand the genetic mechanism underlying Camelina response to different pathogens.

Application for sustainable intensification of food production

Using the Camelina–*S. sclerotiorum* pathosystem, my thesis provides significant insights and knowledge on plant–pathogen interactions, plant–plant interactions and plant–plant-pathogen interactions. The Camelina response to pathogen, shade and their interaction was identified to address current agricultural system challenges. The Green Revolution increased crop production in much of the developed world to meet global nutrition requirements. However, relying on this approach for the future seems questionable due to its negative environmental impacts. There is a need to consider alternative strategies focusing on more sustainable intensification practice, such as intercropping systems. Camelina produces high-quality oil and has more agronomic traits suited to growth in marginal climatic zones than other oilseed crops. The information relating to Camelina phenotypic plasticity to shade (Chapter 4) will enable agronomists and farmers to determine the optimum plant density for this crop and help breeders develop cultivars more tolerant of stresses occurring in crops under high plant density and/or intercropping. Selection for disease resistance (Chapter 5) is also critical for minimising yield reductions from pathogens without the dependence on fungicides. The plasticity to broad-host pathogens, shade and their interaction described in Chapter 6 would also better inform farmers using this crop under intercropping systems, particularly where both a broad-host pathogen and shade co-occur as limiting factors. Therefore, this thesis offers a framework for increasing yields and the more flexible use of Camelina in sustainable intensification required for future farming systems in the face of resource scarcity and broad-host pathogen challenges.

7.3. Summary

The findings summarised within this thesis contribute to understanding the regulation of plant defence in shade conditions, including the associated genomic location, links between SAS and defence and

the natural variation for this trait in a plant model. These findings provide key 'starting points' for future studies. A better understanding of plant–pathogen interactions under shade is required to identify strategies for managing these limiting factors, which are responsible for yield losses in sustainable agriculture intensification. In terms of plant breeding, this thesis extends previous literature regarding genetic diversity in this crop. It presents novel findings for broadacre crop species as regarding their plasticity to R:FR and their response to major Brassicaceae pathogens. For example, the finding of susceptible allele SIS now provides a new breeding target towards developing cultivars with reduced susceptibility to pathogens under shade. Furthermore, the exploitation of S-gene alleles in the breeding program, which are insensitive to manipulation by pathogen effectors, has been suggested to result in durable and broad-spectrum resistance that resembles nonhost resistance (Pavan *et al.,* 2010). Moreover, the improved characterisation of Camelina genomic resources—particularly the identification of two subpopulation groups—provides insight into the genetic structure of Camelina that will be valuable for genetic improvement efforts in this promising yet neglected species. More broadly, this study will assist future researchers by providing a framework for defining plasticity in related species and using multiple traits. The responses presented for plasticity to R:FR will assist in selecting morphological traits as markers for developing more shade-resilient cultivars. Finally, by identifying similar resistant and susceptible Camelina lines to the pathogens responsible for blackleg and *Sclerotinia*, I provide further options for understanding Camelina defence mechanisms to other pathogens (*i.e.,* using C370×C2305 RIL population). Together, these research findings provide a foundation for improving Camelina productivity while simultaneously minimising adverse impacts on the environment.

7.4. Looking ahead

Research on plant plasticity in response to critical environmental factors is essential for identifying genetic mechanisms underlying the responses and, ultimately, informing and enabling breeders to develop adapted varieties. Achieving robustness in measuring plasticity, especially across various factors, is challenging (Kissoudis *et al.,* 2014; Forsman, 2015). In this study, I used light-emitting diodes (LED) under controlled environments to provide different R:FR, from shade-mimicking light (R:FR<0.5) to full light (R:FR=10). The different responses of Arabidopsis lines to *S. sclerotiorum* inoculation in Chapter 6 confirmed the efficacy and efficiency of the experimental settings presented in this study. Furthermore, I found that the QTL for combination factors (SIS) had higher genetic variance than the QTL for a single factor (RSR). Studies have shown lower genetic variance under a combined stimulus than a single factor (*e.g.,* Rasmussen *et al.,* 2013; Kissoudis *et al.,* 2014). As low heritability and phenotypic variation are often identified as factors that may hinder QTL identification

in response to multiple environmental factors, this study should encourage further studies with different plants and pathogens. Moreover, my observation that shade-tolerant lines showed resistance to broad-host pathogens under shade raises an important question: Will disease phenotyping under shade be a strategy for detecting resistant lines? If the answer is 'yes', then implementing this strategy would provide an alternative, reliable and rapid means to identifying QDR genotypes valuable for developing resilient plants for challenging agricultural environments and their associated diseases.

7.5. References

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