Strigolactones positively regulate chilling tolerance in pea and in *Arabidopsis*

James W. Cooper[1], Yan Hu[1], Leila Beyyoudh[1], H. Yildiz Dasgan[1,2], Karl Kunert[1,3], Christine A. Beveridge[4], Christine H. Foyer[1*]

[1]Centre for Plant Sciences, School of Biology, Faculty of Biological Sciences, University of Leeds, Leeds, West Yorkshire, LS2 9JT, UK.

[2]Department of Horticulture, Agricultural Faculty, Cukurova University, Adana, Turkey.

[3]Forestry and Agricultural Biotechnology Institute, Department Plant and Soil Sciences, University of Pretoria, Hillcrest, Pretoria 0002, South Africa.

[4]School of Biological Sciences, University of Queensland, St Lucia, Brisbane, 4072, Australia

Running title: Strigolactone-dependent chilling tolerance

***Corresponding author:** Christine H. Foyer:c.foyer@leeds.ac.uk

[1]Centre for Plant Sciences, School of Biology, Faculty of Biological Sciences, University of Leeds, Leeds, West Yorkshire, LS2 9JT, UK.

ABSTRACT

Strigolactones (SL) fulfil important roles in plant development and stress tolerance. Here we characterised the role of SL in the dark chilling tolerance of pea and *Arabidopsis* by analysis of mutants that are defective in either SL synthesis or signalling. Pea mutants (*rms3, rms4, rms5*) had significantly greater shoot branching with higher leaf chlorophyll a/b ratios and carotenoid contents than the wild type. Exposure to dark chilling significantly decreased shoot fresh weights but increased leaf numbers in all lines. However, dark chilling treatments decreased biomass (dry weight) accumulation only in *rms3* and *rms5* shoots. Unlike the wild type plants, chilling-induced inhibition of photosynthetic carbon assimilation was observed in the *rms* lines and also in *max3-9, max4-1, max2-1* mutants that are defective in SL synthesis or signalling. When grown on agar plates the *max* mutant rosettes accumulated less biomass than the wild type. The synthetic SL, GR24 decreased leaf area in the wild type, *max3-9* and *max4-1* mutants but not in *max2-1* in the absence of stress. Moreover, a chilling-induced decrease in leaf area was observed in all the lines in the presence of GR24. We conclude that SL plays an important role in the control of dark chilling tolerance.

Summary statement:

Strigolactones (SL) are associated with drought tolerance but their functions in other stresses remain poorly characterised. Using a range of pea (*rms*) and *Arabidopsis thaliana* (*max*) mutants that are deficient in either strigolactone synthesis or signalling, we studied plant responses to dark chilling stress. In contrast to the pea and Arabidopsis wild types, which are insensitive to dark chilling, the *max* mutants and *rms* mutants showed chilling-induced inhibition of photosynthesis and a decrease in dry biomass accumulation. These findings demonstrate that SL regulate dark chilling tolerance in plants.

Key words: strigolactones, chilling tolerance, chilling stress, cystatins, legumes, photosynthesis

1. INTRODUCTION

Low temperatures negatively impact on agricultural productivity in many parts of the world causing yield losses (Thakur *et al*., 2010). Extreme freezing events (<-15 °C) are a particular concern for overwintering crops such as winter beans (Link *et al*., 2010), while chilling temperatures are a limiting factor for the growth and productivity of tropical legumes such as soybean (van Heerden *et al*., 2004). Enhancing the low temperature tolerance of chilling-sensitive crops is thus a major target for plant breeders (van Heerden *et al.,* 2004). Chilling tolerant plants are able to endure chilling (0 - 15 °C) temperatures and can even acclimate to freezing i.e. temperatures below 0 °C (Pearce, 1999). In contrast, tropical and subtropical species are generally sensitive to chilling and often show little capacity for chilling acclimation (Zhu *et al.,* 2007). The extensive reprogramming of gene expression and metabolism that underpins adaptation to chilling and freezing temperatures does not appear to be triggered in chilling-sensitive species such as maize (Sobkowiak *et al*., 2016). The ability of photosynthesis to withstand chilling-induced inhibition is an important mechanism underpinning chilling tolerance, alongside modifications in plant development and changes to cell wall and membrane properties.

Legumes are essential for future food security, because they are central components of sustainable agriculture and key protein sources in human and animal diets (Barton *et al*., 2014; Graham and Vance, 2003). However, the genetic improvement of legumes has lagged behind that of cereal crops (Foyer *et al*., 2016). Soybean (*Glycine max* Merr.) is the most economically important and widely grown legume globally (Ainsworth, *et al*., 2012). However, soybean is extremely sensitive to chilling (Cooper *et al*., 2015). Soybean yields are decreased when plants are exposed to temperatures below 15 °C (Gass *et al*., 1996). Chilling stress is hence an important constraint to soybean production in temperate regions such as Northern and Central Europe (Ray *et al*., 2012). Moreover, low temperatures can be experienced at night even in tropical countries, particularly when crops are grown at high altitude (van Heerden *et al*., 2003; Krüger *et al*., 2014). Chilling sensitivity is important at every stage of soybean development (Cheng *et al*., 2010; Gass*et al*., 1996). The chilling-dependent inhibition of photosynthesis is inherently linked to negative impacts on crop yield (Richards, 2000). In soybean the dark chilling-dependent inhibition of photosynthesis is underpinned by decreased enzyme activity (van Heerden *et al*., 2003) and photosystem II (PSII) efficiency (Krüger *et al*., 2014). Moreover, the chilling-induced loss of symbiotic nitrogen fixation in nodules can also contribute to the sensitivity of photosynthesis to low

temperatures (van Heerden *et al*., 2008). Exposure to chilling leads to an extensive loss of chlorophyll and premature senescence (van Heerden *et al*., 2003, 2008; Kunert *et al*., 2016).

The control of cellular protein composition and levels is essential for acclimation to changing environmental conditions (Pearce, 1999). Throughout the acclimation process, plant cells must maintain a strict balance between the production of new proteins and the degradation of less useful or undesirable proteins. Protein degradation is largely controlled by the ubiquitin-proteasome system in plants. Much research effort has recently focussed on the N-end rule pathway, which is part of the ubiquitin-proteasome system that links the stability of a protein to the identity of its N-terminal amino-acid residue (Gibbs *et al*., 2014). This pathway is regulated by the availability of oxygen and nitric oxide leading to the removal of undesired proteins by the ubiquitin-proteasome for the regulation of plant development and in response to pathogens (De Marchi *et al*., 2016). However, other proteases also contribute to the degradation of redundant proteins and also the production of new proteins. For example, cysteine proteases, particularly the papain type C1 cvsteine proteases are important enzymes in the regulation of plant development and stress tolerance (Beers *et al*., 2000; Bhalerao *et al*., 2003; Guo, 2013).

C1 cysteine proteases have been implicated in the regulated turnover of chloroplasts proteins such as ribulose bisphosphate carboxylase/oxygenase (RuBisCO) during leaf senescence (Otegui *et al*., 2005; Carrión *et al*., 2014; Martínez *et al*., 2008). RuBisCO degradation can occur both inside and outside of the chloroplast (Irving and Robinson, 2006). While metallo-proteases and aspartic proteases contribute to RuBisCO degradation inside the chloroplast stroma (Nair and Ramaswamy, 2004; Kato *et al*., 2004), C1 papain-like cysteine proteases also participate in RuBisCO turnover (Prins *et al*., 2008). In general, cysteine proteases are activated at acidic pH values and are regulated by redox and by chemical and protein-based inhibitors such as phytocystatins (Kunert *et al*., 2015). The most well characterised phytocystatin is oryzacystatin I (OCI) from rice (*Oryza sativa*, Masoud *et al*., 1993). Transgenic tobacco lines (*Nicotiana tabacum*) constitutively expressing OCI showed enhanced tolerance to dark chilling (Van der Vyver *et al*., 2003), as well as an increased accumulation of RuBisCo compared to the wild type (Prins *et al*., 2008). OCI expression in soybean increased nodule numbers and delayed leaf senescence, as well as leading to a significant increase in drought tolerance (Quain *et al*., 2015). Phytocystatin expression also prevented stress-induced programmed cell death in soybean (Solomon *et al*., 1999).

Our understanding of temperature-regulated signalling networks in plants has greatly increased in recent years. For example, chilling treatments activate the INDUCER OF CBF EXPRESSION-CBF/DREB1 (C-REPEAT BINDING FACTOR/DRE BINDING FACTOR1) or ICE pathway (Zhao *et al.*, 2011). The ICE1 protein, which is an HLH type (helix-loop-helix) transcription factor, fulfils a number of important functions in plant growth and development as well as chilling tolerance (Miura & Furumoto, 2013). The *BON1-ASSOCIATED PROTEIN1* (*BAP1*), which functions as a general inhibitor of programmed cell death, is directly regulated by ICE1 (Zhu *et al*., 2011). Moreover, ICE functions upstream of the CBF/DREB1 transcription factors, which bind to CRT/DRE *cis*-elements in the promoters of *COR* genes to regulate their expression. ICE and the CBF/DREBs are generally well conserved in higher plants (Miura & Furumoto, 2013).

A RING type E3 ubiquitin ligase called HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENE1 (HOS1) negative regulates the chilling response through the degradation of ICE1 by the 26S proteasome. The ubiquitination system plays important roles in the degradation of proteins particularly during stress responses, the E3 ubiquitin ligases catalyse the polyubiquitination of proteins, thereby targeting them for degradation by the 26S proteasome While many phytohormones, including abscisic acid, ethylene, gibberellins, jasmonic acid, salicylic acid and brassinosteroids, have been linked to plant adaptation to chilling stress (Eremina *et al*., 2016), there has been no evidence to date linking SL to chilling sensitivity/tolerance (Pandey *et al*., 2016). SLs have many important functions in plant growth, functioning in the control of seed dormancy, root and shoot branching, and in stress tolerance (Dun *et al*., 2009; Lopez-Obando *et al*., 2015). SL also plays a role in the control of plant lifespan and leaf senescence (Woo *et al*., 2001). *A. thaliana* mutants defective in either SL synthesis or signalling show increased sensitivity to drought and salt stresses (Ha *et al*., 2014).

The carotenoid cleavage dioxygenases CCD7 and CCD8 encoded by the *RMS5*/*MAX3*, *RMS1*/*MAX4* genes in garden pea (*Pisum sativum*) and *Arabidopsis* respectively (Supplemental Table 1) fulfil important roles in SL synthesis (Bennett *et al*., 2006). The *RMS4*/*MAX2* is an F-box signalling protein (Arite *et al*., 2009) interacts with the SKP1-CULLIN-F-BOX PROTEIN (SCF) complex and targets proteins for degradation. The closely related alpha/beta-hydrolases, DWARF14 (D14), RMS3 in pea, and KARRIKIN INSENSITIVE 2 (KAI2), perceive specific enantiomers of the synthetic SL analogue, GR24 and interact with the MAX2 protein (Nakamura *et al*., 2013, Waters and Smith, 2013, de

Saint Germain *et al*., 2016). Different associations of the SL receptor proteins with MAX2 in *Arabidopsis* likely facilitate ubiquitination, with subsequent 26S proteasomal degradation of specific members of the SMAX-Like (SMXL) family. D14/MAX2/SMXL 6, 7, 8 signalling complexes are involved in SL signaling to control processes such as shoot branching (Soundappan *et al*., 2015). Moreover, the KAI2/MAX2/SMAX1 signalling complex is involved in the perception of smoke-derived karrikins, and probably other molecules that control seed germination and hypocotyl growth (Waters and Smith, 2013, Soundappan *et al*., 2015). The RMS2 locus has been linked to SL feedback regulation via interactions with the PsBRC1 transcription factor (Braun *et al*., 2012) and has recently been reported as PsAFB4/5 which is required for perception of the auxin-related herbicide picloram (Ligerot *et al*., 2017).

The purpose of the study described below was to determine whether SL play a role in chilling tolerance in plants. We therefore compared the dark chilling sensitivity of different pea branching mutants that are called *ramosus* (*rms*), which are defective in either SL synthesis or signalling (Johnson *et al*., 2006; Supplemental Table 1). The *rms3* mutant is defective on the pea ortholog of the rice D14 SL receptor (de Saint Germain *et al*., 2016). We also determined the chilling sensitivity of the *Arabidopsis thaliana more axillary branches* (*max*) mutants, *max2-1*, *max3-9* and *max4-1*. We further characterised the chilling sensitivity of three independent OCI-expressing soybean lines (SOC1, SOC-2 and SOC-3) that had been previously characterized in terms plant growth and drought tolerance (Quain *et al*., 2014), as well as nodulation and growth under low nitrogen stress (Quain *et al*., 2015). The data presented here show that SL synthesis and signalling are important in the tolerance of soil-grown *A. thaliana* and pea plants to chilling stress, and they are also implicated in the decreased sensitivity of photosynthesis to chilling-induced photo inhibition in OCI-expressing soybean lines.

2. MATERIALS AND METHODS

2.1. Plant Material and Growth Conditions

Seeds of wild type peas (L107, Torsdag cultivar) and mutants deficient in SL biosynthesis or signaling (BL298, *rms5-3*; K164, *rms4-1*; K487, *rms3-1*), were allowed to germinate for 5 days under optimal temperatures (25 °C day/20 °C night) with a 12 h photoperiod at an irradiance of 250 µmol m^{-2} s^{-1}. Seedlings were then transferred to individual 10 x 10 cm pots containing compost and maintained under temperature conditions. Fourteen days later half of the plants from each genotype were either maintained under optimal temperature conditions or exposed to 7 consecutive nights of dark chilling at 4 °C.

Seeds of *A. thaliana* ecotype Colombia-0 (col-0/Wt) and mutant lines *max2-1*, *max3-9* and *max4-1* (Marquez-Garcia, *et al.*, 2013) were sown in 6x6 cm pots containing compost and grown under optimal temperatures (25 °C day/20 °C night, with a 12 h photoperiod) and an irradiance of 200 µmol m^{-2} s^{-1} for 7 days. Thereafter, seedlings were either allowed to grow for a further 7 days either in pots or transferred to individual 50 ml Falcon tubes containing compost for photosynthetic gas exchange measurements. Fourteen days later half of the plants from each genotype were either maintained under optimal temperature conditions or exposed to 7 consecutive nights of dark chilling at 4 °C.

A. thaliana seeds were also sown on 12x12 cm agar plates containing ½ strength MS media in the absence or presence of 2 µM GR24 (obtained from Professor Binne Zwanenburg, Utrecht University, Netherlands). Following incubation at 4 °C for 72 h, plates were transferred to controlled environment chambers and seedlings were grown under optimal temperatures (25 °C day/20 °C night, with a 12 h photoperiod) and an irradiance of 200 µmol m^{-2} s^{-1} for 4 days. Half of the plates for each genotype were either maintained under optimal temperature conditions or exposed to 11 consecutive nights of dark chilling at 4 °C.

Seeds of wild type soybean (*Glycine max* cv Williams 82) and 3 independent transformed lines expressing the rice cysteine protease inhibitor, oryzacystatin I (SOC-1, SOC-2 and SOC-3; Quain*et al.*, 2014) sown on vermiculite in 28x24cm trays and allowed to germinate for 7 days under optimal temperatures (25 °C day/20 °C night, with a 12 h photoperiod) and an irradiance of 250 µmol m^{-2} s^{-1}. Seedlings were transferred to individual 10 x 10 cm pots containing compost (Petersfield potting supreme, Petersfield Growing Mediums, 45 Cambridge Road, Cosby, Leicester, LE9 1SJ). 14 days after sowing, seedlings were either

maintained under optimal growth temperature conditions or were subjected to 9 consecutive nights of dark chilling (25 °C Day/4 °C night temperatures)

2.2. Photosynthetic gas exchange

Photosynthetic gas exchange measurements were performed using a LI-6400XT portable photosynthesis system (LI-COR Biotechnology UK Ltd, St. John's Innovation Centre, Cowley Road, Cambridge, CB4 0WS, UK)essentially as described by Soares*et al*. (2008).Unless otherwise stated, measurements were made 20 °Cat an irradiance of 800 µmol.$m^{-2}s^{-1}$ and a CO_2 concentration of 400 µmol mol^{-1}. For soybean and pea the youngest mature leaf per plant was measured in the 3 x 2 cm 6400-02B LED chamber. For *Arabidopsis* the whole plant was placed in the 6400-17L the *Arabidopsis* chamber. Therosette area was determined using ImageJ, Abramoff *et al*., 2004). Photosynthesis was allowed stabilise for 15 min prior to measurement. CO_2 response curves for photosynthesis were performed at 20 °C at an irradiance of 800 µmol.$m^{-2}s^{-1}$ with increasing CO_2 from 55-2000 µmol mol^{-1}. Light response curves for photosynthesis were performed at 20 °C and 400 µmol CO_2 with irradiance levels increasing from 0-2000 µmol.$m^{-2}s^{-1}$.

2.3. Quantitative real time PCR

RNA was extracted from soybean leaves using the Sigma-Aldrich Spectrum Plant Total RNA kit (Sigma Aldrich, Homefield Road, Haverhill CB9 8QP, UK). Extracted RNA was converted to cDNA using the QiagenQuantiTect Reverse Transcription Kit (Qiagen, Skelton House, Lloyd St N, Manchester M15 6SH, UK) and cDNA quantification was performed by nanodrop.Quantitative real time polymerase chain reaction (qPCR) was performed using a QuiagenQuantiTect SYBR Green PCR Kit and a BIO RAD CFX96 Real Time System with C1000 Thermal Cycler (www.bio-rad.com).

2.4. Germination of Striga seeds

Striga hermonthica seeds were germinated on agar plates in the absence or presence of GR24, or in the presence of pre-germinated wild type soybean seeds and pre-germinated OCI overexpressing lines.

2.5. Statistical analysis

Significant interactions were determined by two-way ANOVA ($p=<0.05$). A post-hoc LSD test was used to determine which pairwise interactions were significant in each data set.

3. RESULTS

3.1. Strigolactone deficiency induces chilling sensitivity in pea and *Arabidopsis*

In the first series of experiments, wild type and mutant pea or *Arabidopsis* lines were grown on compost for 14 days under optimal growth temperatures. Half of the plants were then maintained under optimal temperatures and half were subjected to seven consecutive nights of dark chilling. Unless otherwise stated, the following measurements were made during the first half of photoperiod on the 7th day.

The SL synthesis mutant *rms5-3* that is defective in the enzyme CCD7, and the SL signalling mutants, *rms3-1* that is defective in the pea orthologue of the D14 SL receptor, and the *rms4* mutant, which is defective in the *MAX2* F-box signalling protein, show an increased branching shoot phenotype compared to the wild type (L107 Torsdag cv) in the absence of chilling stress (Supplemental Figure 1). The enhanced branching phenotype was also present in the *rms* mutants following the dark chilling treatments (Figure 1 A, D). For simplicity, we show data only for *rms5-3* to illustrate the effect of dark chilling on the SL synthesis mutants.

The *rms4* mutant had lower shoot fresh and dry weights relative to the other lines in the absence of stress (Figure 1 B, C). The dark chilling treatment caused a significant decrease in shoot fresh weights of all lines (Figure 1). In contrast to the dry weights of the wild type (L107) and *rms4-1* shoots, which were unaffected by the chilling stress, the dry biomass of *rms5-3* and *rms3-1* mutants were significantly decreased in response to the chilling treatment (Figure 1 B, C).

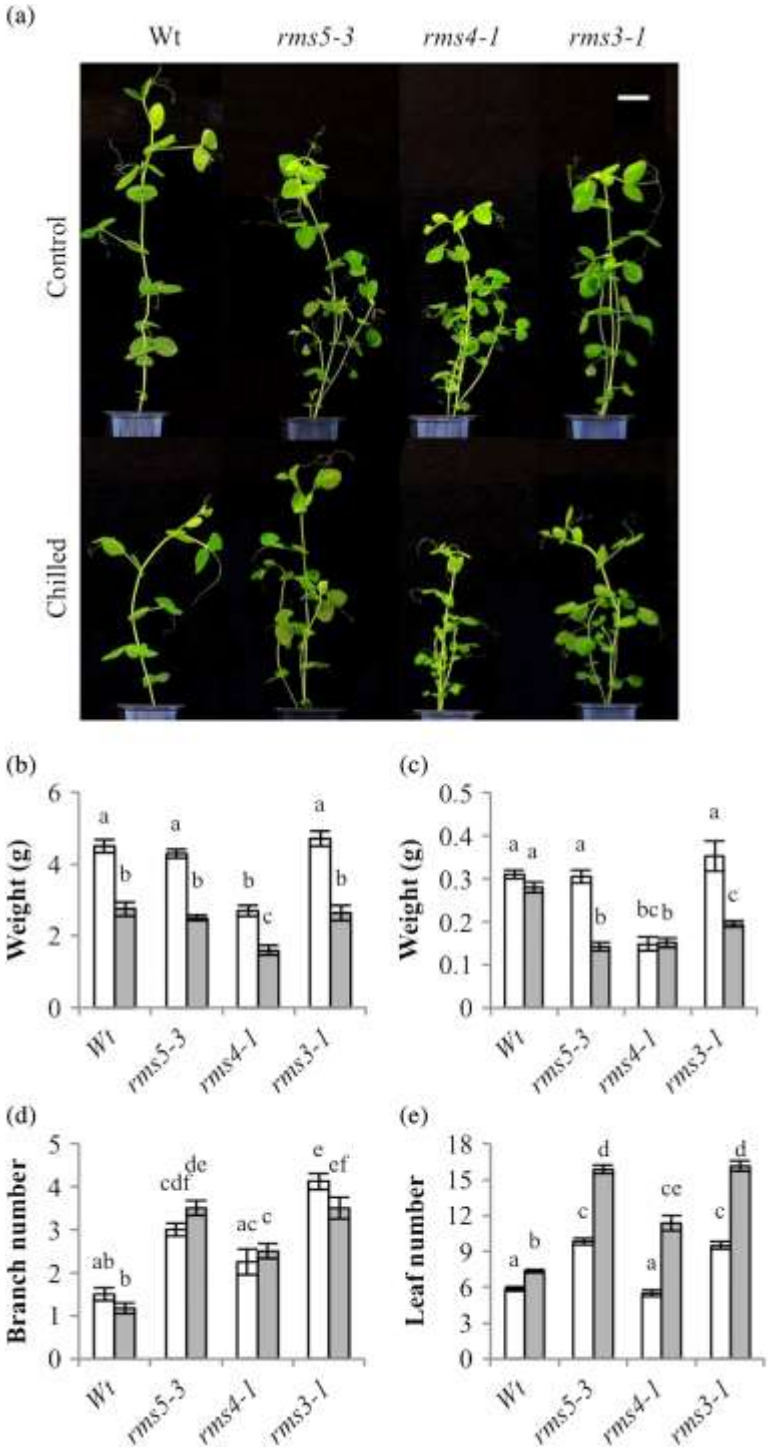


Figure 1. The effect of seven consecutive nights of dark chilling on the growth of wild type peas and mutants defective either in strigolactone synthesis (*rms5-3*) or in signalling (*rms3-1* and *rms4-1*). Plants were grown for either 21 days under optimal conditions or for 14 days under optimal conditions followed by seven consecutive nights of dark chilling (4 ± 1 °C). (a) Shoot phenotypes of plants grown either in the absence (control) of stress or following dark chilling (chilled). Scale bar shows 5 cm. (b) Shoot fresh weight. (c) Shoot dry weight. (d) Number of branches. (e) Number of leaves. Open bars indicate plants grown in the absence of chilling stress, whereas the grey bars indicate plants that had been subjected to the dark chilling treatment. Shared letters indicate no significant differences (mean ± SE) from three separate experiments. ($p > .05$)

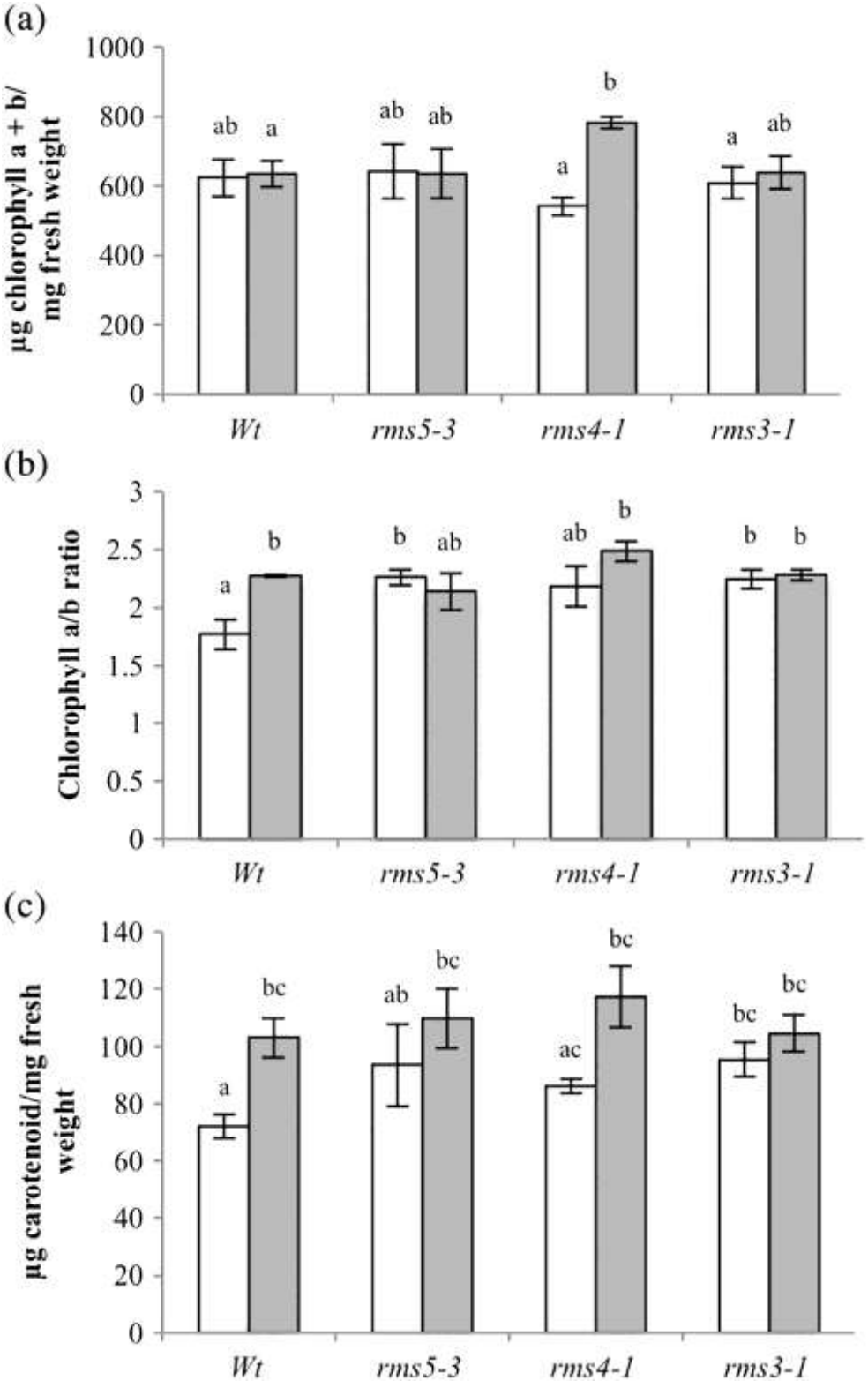


Figure 2. The effect of seven consecutive nights of dark chilling on the leaf pigment content and composition in wild type peas and mutants defective either in strigolactone synthesis (*rms5-3*) or signaling (*rms4-1*, *rms3-1*). Plants were grown for either 21 days under optimal conditions or for 14 days under optimal conditions followed by seven consecutive nights of dark chilling (4 ± 1 °C). (a) Total chlorophyll content, (b) chlorophyll a/b ratios, (c) total carotenoid content. Open bars indicate plants grown in the absence of chilling stress, whereas the grey bars indicate plants that had been subjected to the dark chilling treatment. Shared letters indicate no significant differences (mean ± SE) from three separate experiments. ($p > .05$)

The *rms5-3* and *rms3-1*shoots has significantly more leaves than the wild type or the *rms4-1* plants. The dark chilling treatment significantly increased the number of leaves in all lines. However, all the SL synthesis and signalling mutants had a significantly greater number of leaves than the wild type following the dark chilling treatments (Figure 1 E).

The leaves of all the lines had similar amounts of total chlorophyll in the absence of chilling (Figure 2 A). The chlorophyll content of the leaves of the wild type, *rms4-1* and rms3-*1* mutants was unchanged following the dark chilling treatments (Figure 2 A). In contrast, the leaves of the *rms4* mutant that is defective in the *RMS4*/*MAX2* F-box signalling protein contained significantly more chlorophyll following exposure to dark chilling compared to plants that had not been exposed to the stress (Figure 2 A). The *rms4-1*, *rms3-1* and *rms5-3* leaves had significantly higher chlorophyll a/b ratios (Figure 2 B) and carotenoid contents (Figure 2 C) than the wild type in the absence of stress. The chilling stress treatment resulted in significant increases in the leaf chlorophyll a/b ratios and the carotenoid contents (Figure 2 C) of all lines, such that there were no significant differences in these parameters between wild type and mutant lines following the chilling treatment (Figure 2 B, C).

Photosynthesis rates were similar in all pea lines grown under optimal conditions (Figure 3A). While photosynthesis was unaffected by the chilling treatment in the wild type (L107) pea leaves, the low temperature treatment led to a significant decrease in photosynthesis in the SL synthesis and signalling mutants (Figure 3 A). Stomatal conductance and transpiration rates were not significantly changed in the leaves of either the wild type (L107) or SL signalling mutants as a result of the dark chilling treatment (Figure 3 B, D). However, dark chilling caused a decrease in these parameters in the SL synthesis (*rms5-3*) mutants (Figure 3 B, D).

The *Arabidopsis* wild type, SL synthesis (*max3-9*, *max4-1*) and signalling (*max2-1*) mutants had a similar vegetative rosette phenotype when plants were grown in pots in the absence of chilling stress (Figure 4 A) with similar rosette biomass in terms of fresh weight (Figure 4 B). All the lines except for *max2-1* had a similar rosette biomass in terms of dry weight (Figure 4 C). Exposure to 7 consecutive nights of dark chilling caused a visible decrease in rosette biomass in all lines (Figure 4 A) with a trend towards lower shoot fresh weights (Figure 4 B). The rosette dry biomass also tended to decrease as a result of dark chilling in all the lines but this was only significant in the *max4-1* mutants (Figure 4 C).

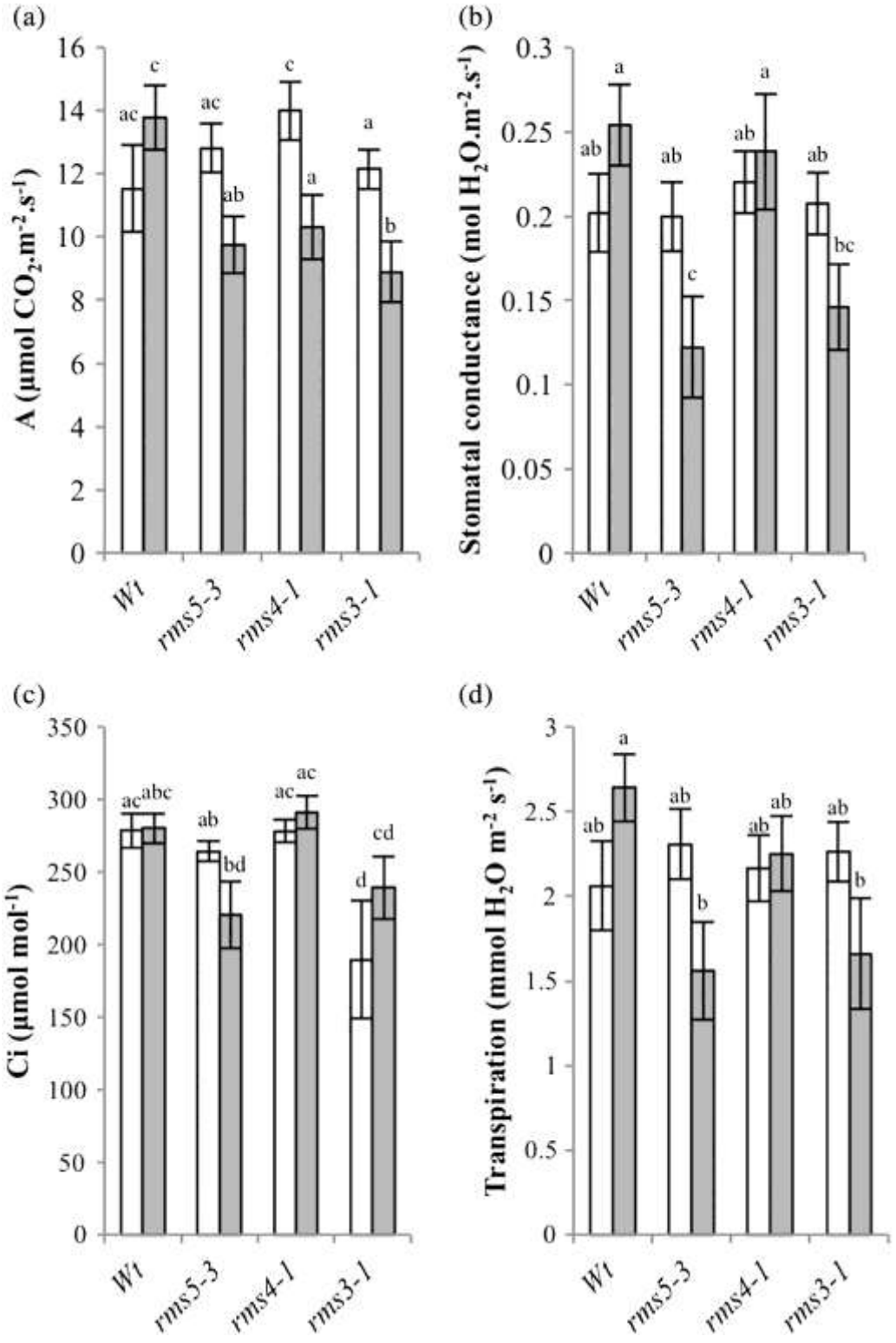


Figure 3. Chilling-induced inhibition of photosynthesis in the leaves of wild type pea plants and of pea mutants defective in SL synthesis (rms5-3) or signaling (*rms4-1, rms3-1*). Plants were grown for either 21 days under optimal conditions or for 14 days under optimal conditions followed by seven consecutive nights of dark chilling (4 ± 1 °C). Measurements were made at 20 °C, with an atmospheric CO_2 concentration of 400 ml L^{-1} and an irradiance of 800 µmol m^2 s^{-1}. (a) Carbon assimilation, (b) stomatal conductance, (c) intracellular CO_2 concentration, and (d) transpiration. Data are mean ± SE for plants grown at optimal temperatures (open bars) and after exposure to dark chilling (grey bars). Shared letters indicate no significant differences from three separate experiments. ($p > .05$)

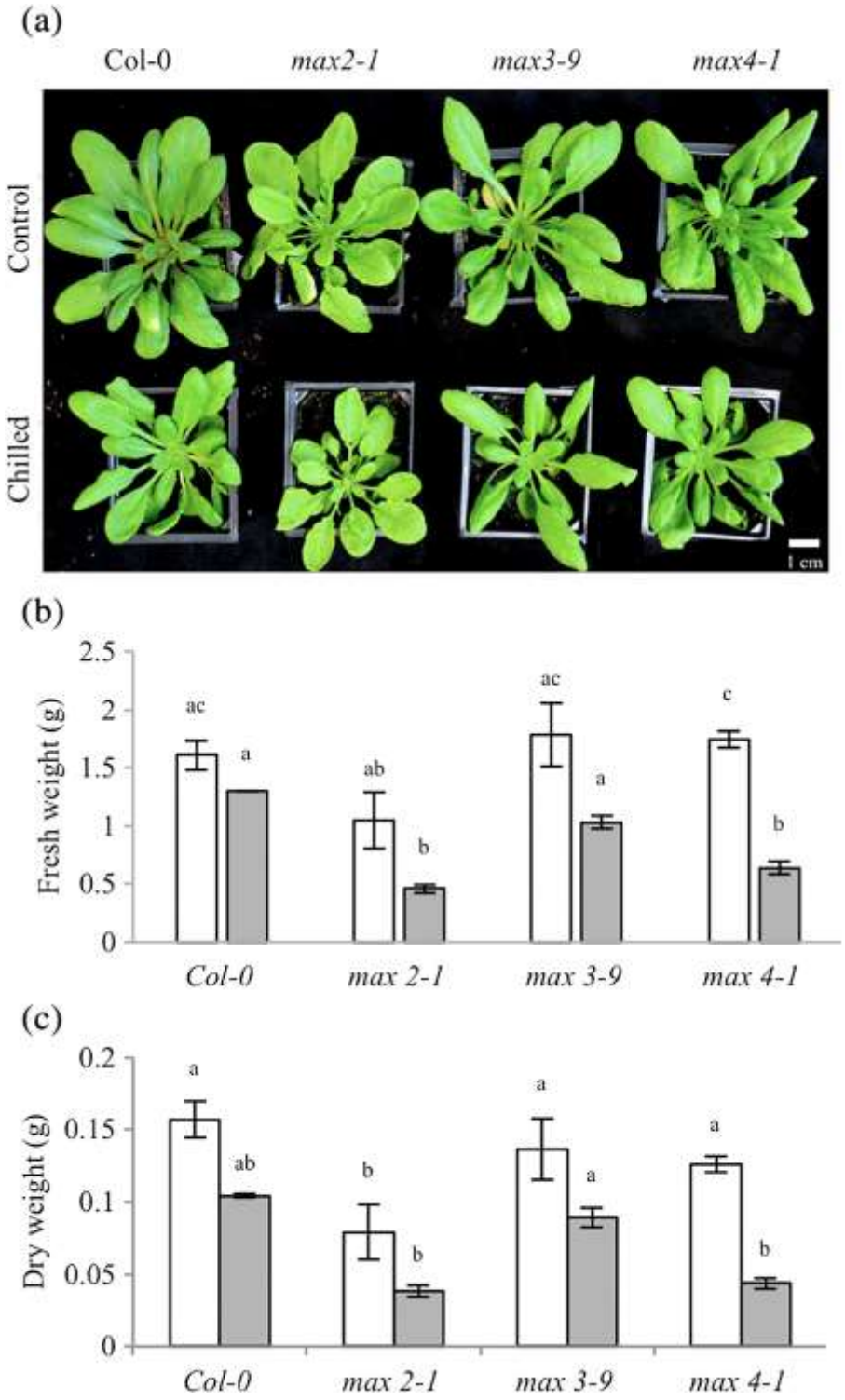


Figure 4. The effect of nine consecutive nights of dark chilling on the shoot phenotypes of soil-grown wild type (Wt) *Arabidopsis thaliana* and mutants defective in strigolactone synthesis (*max3-9, max4-1*) or signaling (*max2-1*). Plants were grown for 4 weeks on compost under optimal conditions before exposure to either seven consecutive nights of dark chilling (4 ± 1 °C). Controls were maintained under optimal temperatures. (a) Typical examples of *Arabidopsis* rosette phenotypes in the absence of chilling or after the dark chilling treatments. (b) Fresh biomass (weight) in the absence of chilling or after the dark chilling treatments. (c) Dry biomass (weight) in the absence of chilling or after the dark chilling treatments. Data are mean ± SE for plants grown at optimal temperatures (open bars) and after exposure to dark chilling (grey bars). Shared letters indicate no significant differences from three experiments. ($p > .05$)

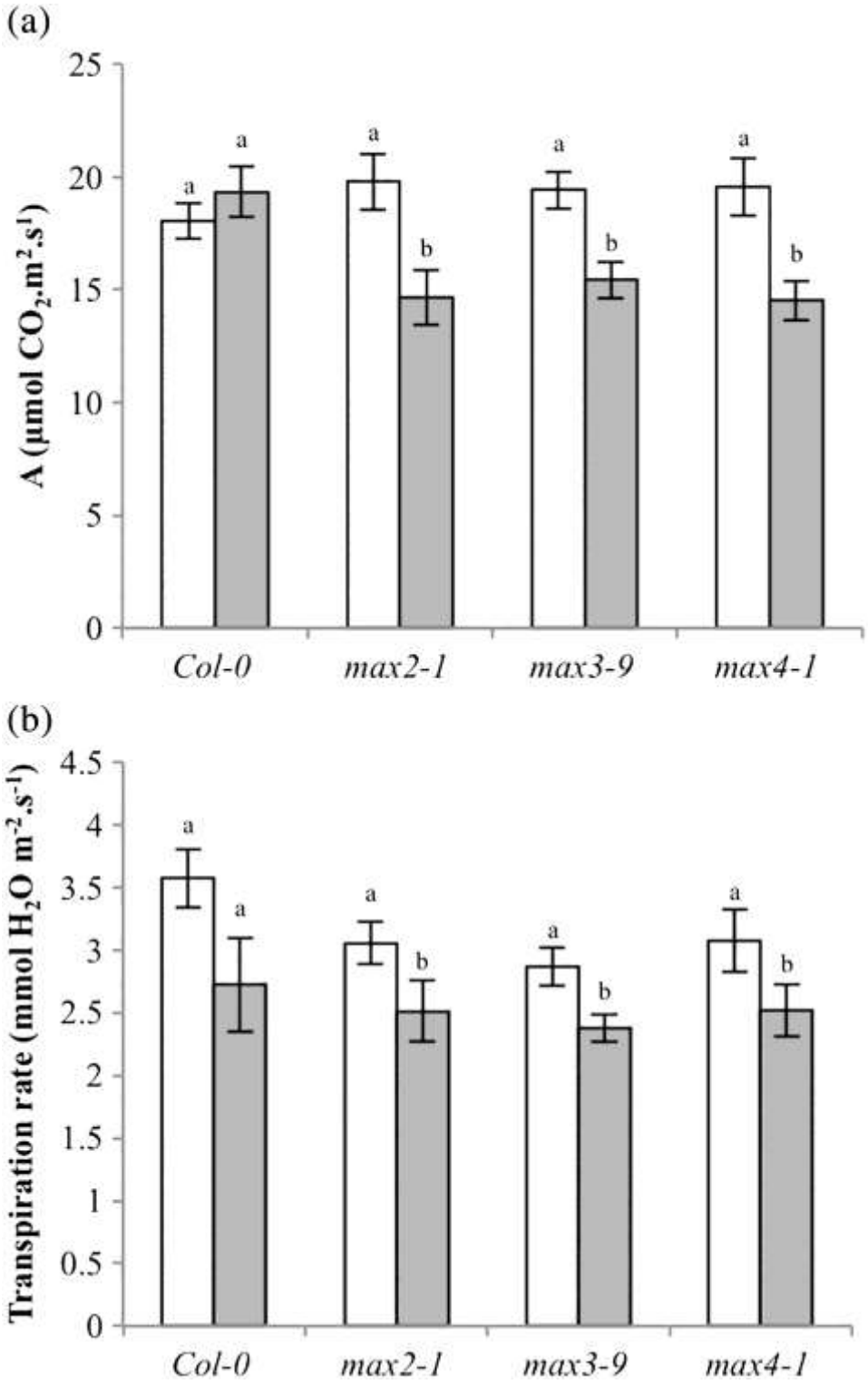


Figure 5. The effect of seven consecutive nights of dark chilling on photosynthesis in wild type *Arabidopsis thaliana* and mutants defective in strigolactone synthesis (*max3-9*, *max4-1*) or signaling (*max2-1*). Plants were grown on compost for either 35 days under optimal conditions or 28 days under optimal conditions followed by seven consecutive nights of dark chilling (4 ± 1 °C). Measurements were made at 20 °C, with an atmospheric CO_2 concentration of 400 ml L^{-1} and an irradiance of 800 µmol m^2 s^{-1}. (a) Carbon assimilation rate. (b) Transpiration rate. Data are mean ± SE for plants grown at optimal temperatures (open bars) and after exposure to dark chilling (grey bars). Shared letters indicate no significant differences from three independent experiments. ($p > .05$)

Photosynthesis rates were similar in the leaves of all the *Arabidopsis* lines when plants were grown in pots in the absence of chilling stress (Figure 5 A). While photosynthetic CO_2 assimilation rates were unaffected by dark chilling in the wild type, the stress treatment led to a significant decrease in photosynthesis in the *max2-1*, *max3-9* and *max4-1* mutants (Figure 5 A). Transpiration rates tended to be lower in all lines as a result of exposure to dark chilling but stress-induced inhibition was only significant in the SL synthesis and signalling mutants (Figure 5 B).

The growth phenotypes of the *Arabidopsis* lines were also compared on plants grown on agar plates (Figure 6 A). Under these conditions, the rosettes of all the mutant lines tended to accumulate less biomass with lower fresh weights than the wild type (Supplemental Figure 2). Under these growth conditions, the *max2-1* and *max4-1* mutants had significantly lower leaf areas but with the same numbers of leaves as the wild type (Figure 6 A). Leaf area was not significantly decreased in the wild type by the dark chilling stress (Figure 6 B).The dark chilling treatment caused a significant decrease in leaf area in the *max2-1* and *max4-1* mutants relative to the wild type (Figure 6 B).

We further assessed the role of SL on dark chilling tolerance by the addition of the synthetic SL, GR24. The presence of GR24 significantly decreased the rosette areas of the wild type, max3-*9* and *max4-1* mutants but not the *max2-1* line (Figure 6 C). A chilling-induced decrease in leaf area was observed in all the lines in the presence of GR24 (Figure 6 D).

3.2. Cysteine proteases are involved in the chilling sensitivity of photosynthesis in soybean

The shoot phenotype wild type soybean and three independent OCI-expressing soybean lines (SOC1, SOC-2 and SOC-3) grown in pots was similar in all lines in the absence of chilling stress (Figure 7). Following exposure to dark chilling, however, the shoots had fewer leaves than plants grown in the absence of stress (Figure 7). The chilling treatment led to a significant decrease in shoot biomass in all lines, determined as fresh (Figure 7 A) or dry (Figure 8 B) weights. Photosynthetic carbon assimilation rates were similar in the leaves of the wild type and different OCI-expressing lines when plants were grown under optimal conditions (Figure 8 C). While the dark chilling treatment led to a strong inhibition of photosynthesis in all the lines (Figure 8 C), photosynthetic rates were significantly higher the OCI-expressing lines than the wild type (Figure 8 C).

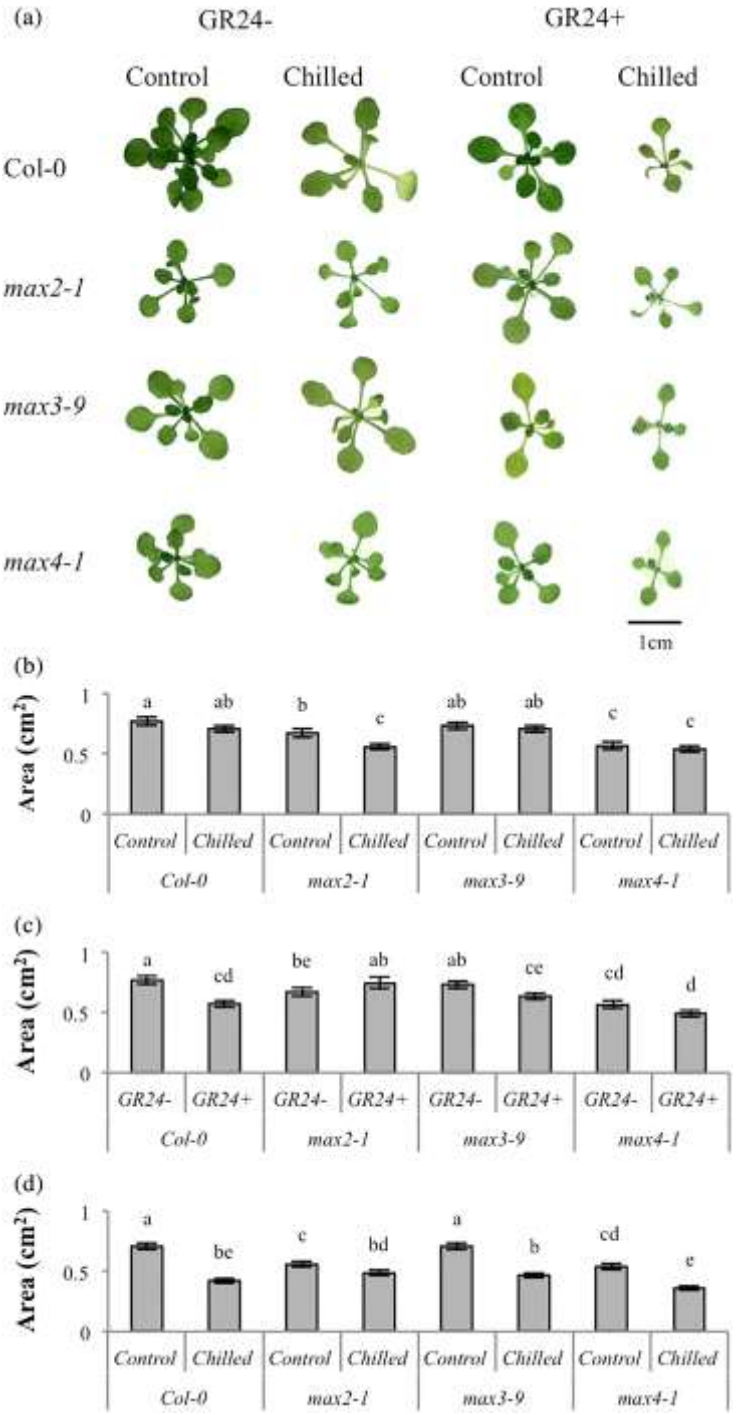


Figure 6. The effect of 11 consecutive nights of dark chilling on the shoot phenotypes of agar plate-grown wild type (Wt) *Arabidopsis thaliana* (Col-0) and mutants defective in strigolactone synthesis (*max3-9*, *max4-1*) or signaling (*max2-1*). Plants were grown on ½ strength Murashige and Skoog (MS) media in the absence or presence of 2-µM GR24, for either 25 days under optimal conditions or 4 days under optimal conditions followed by 11 consecutive nights of dark chilling (4 ± 1 °C). (a) Rosette phenotypes in the absence of chilling (control) or after 11 consecutive nights of dark chilling (chilled), in the presence or absence of 2-µM GR24 (scale bar = 1 cm). (b) Rosette area in the absence of chilling and after the dark chilling treatment with no application of GR24. (c) Rosette area (cm^2) in the presence or absence of GR24 with no application of chilling stress. (d) Rosette area (cm^2) in the presence of GR24 after growth at control temperatures or following 11 consecutive nights of dark chilling. Data are mean ± SE. Shared letters indicate no significant differences from three separate experiments. ($p > .05$)

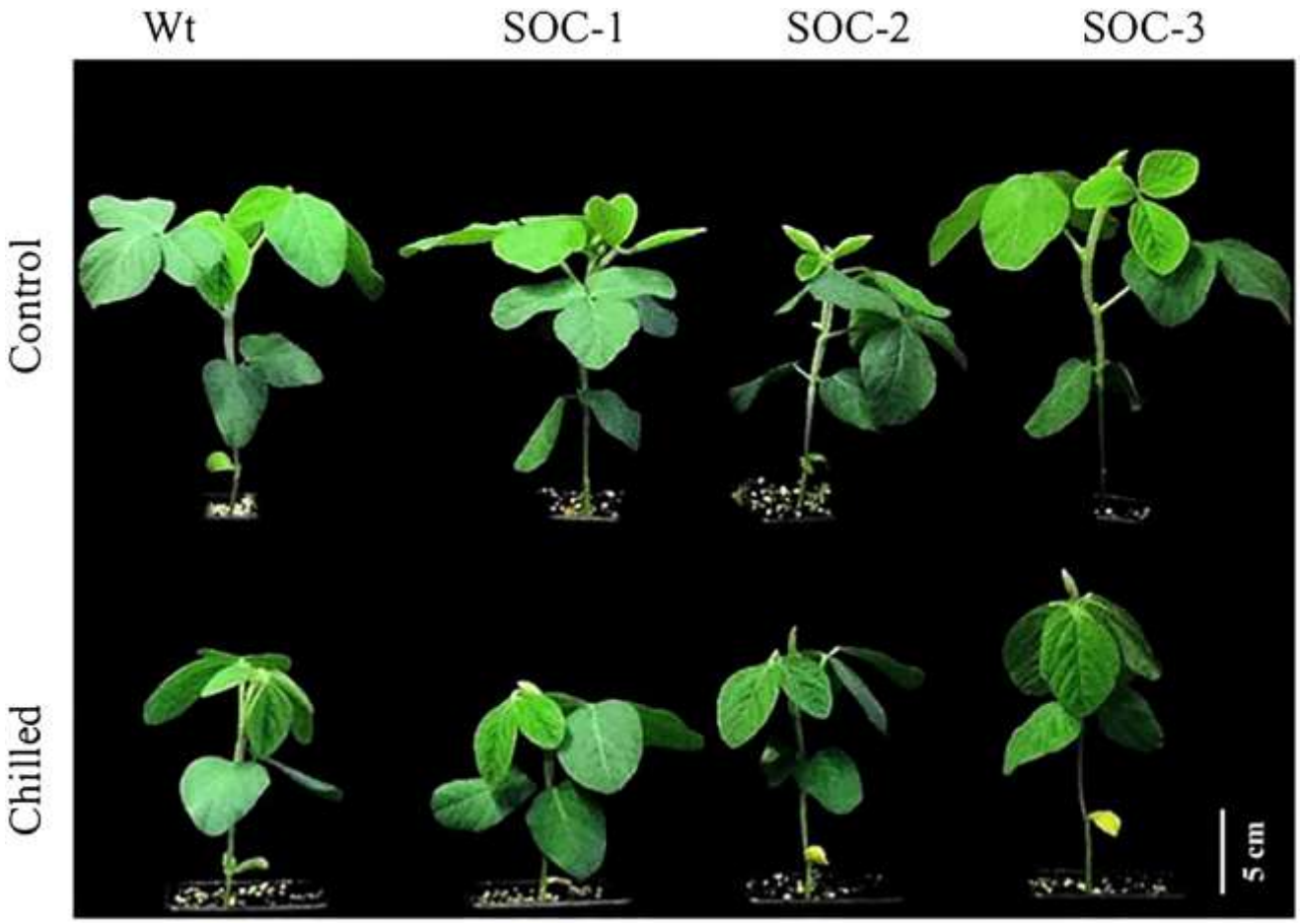


Figure 7. The effect of nine consecutive nights of dark chilling on the shoot phenotypes of the wild type (Wt) and three independent OCI-expressing soybean lines (SOC-1, SOC-2, and SOC-3). Plants were grown for 2 weeks under optimal growth conditions and then either maintained under optimal conditions (upper panel; control) or exposed to nine consecutive nights of dark chilling at 4 ± 1 °C (lower panel: chilled)

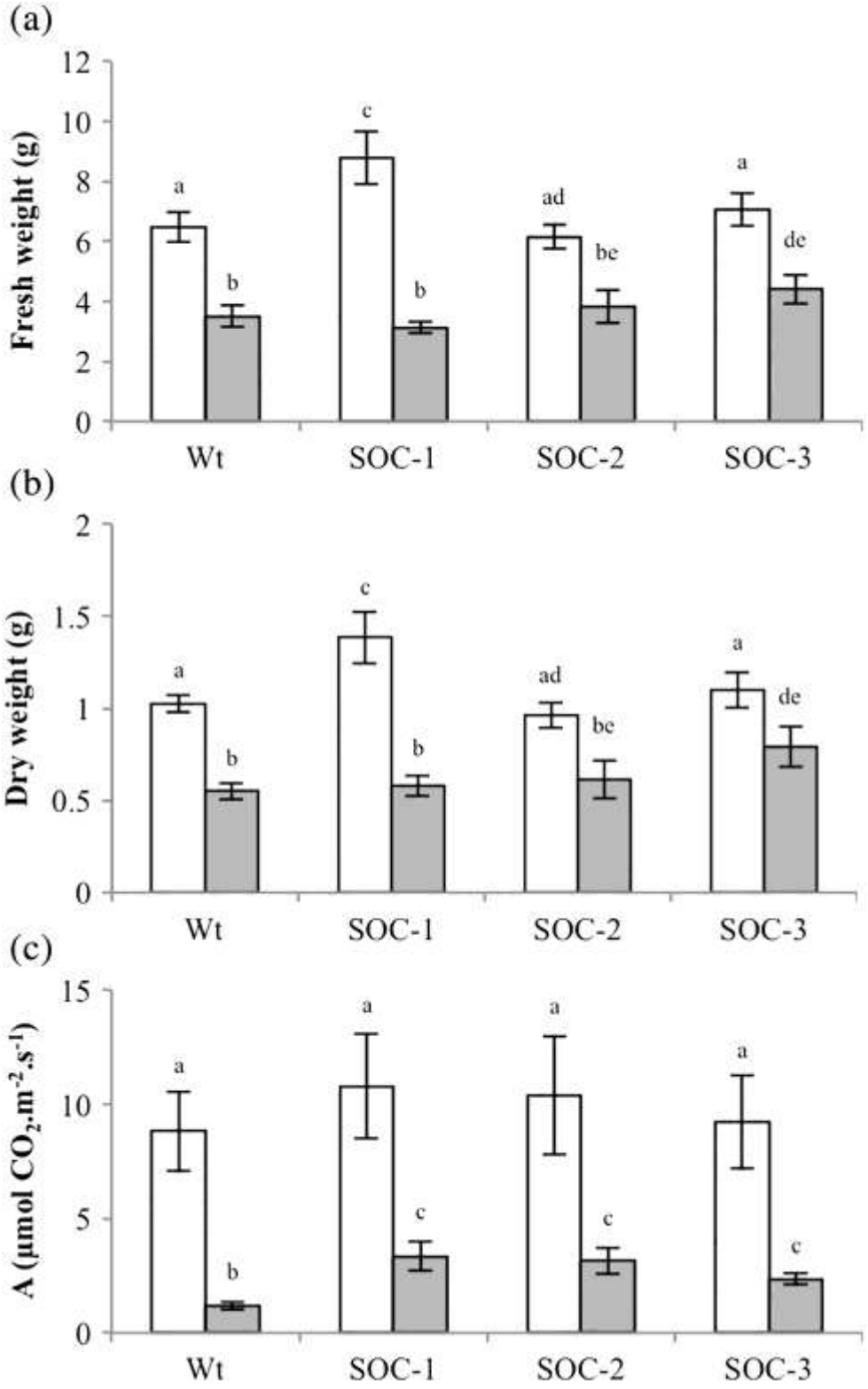


Figure 8. The effect of nine consecutive nights chilling on the shoot biomass and leaf photosynthesis in wild type (Wt) soybeans and three independent OCI-expressing lines (SOC-1, SOC-2, and SOC-3). (a) Shoot fresh weight. (b) Shoot dry weight. (c) Photosynthetic carbon assimilation (a) measured at 20 °C under 400 µmol/mol CO_2 and an irradiance of 800 µmol m^2 s^{-1}. Controls that had not been exposed to chilling stress (open bars); chilled plants (grey bars). Shared letters indicate no significant differences (mean ± SE) from three independent experiments. ($p > .05$)

3.3. OCI expression increases the levels of CCD7 and CCD8 transcripts

Wild type and OCI-expressing soybean lines (SOC1, SOC-2 and SOC-3) were grown under either optimal temperatures for 17 days or they were exposed to three consecutive nights of dark chilling following 14 days under optimal growth temperature conditions. The levels of transcripts encoding the SL biosynthesis enzymes CCD7 and CCD8 were measured in the roots, stems and leaves of wild type and SOC1, SOC-2 and SOC-3 lines, which were grouped for simplicity (Figure 8). The levels of *CCD7* mRNAs were broadly similar in the roots, stems and leaves of the wild type in the absence of stress (Figure 9 A, C, E). However, the leaves of the OCI-expressing lines had significantly higher levels of *CCD7* transcripts than the OCI-expressing stems or OCI-expressing roots (Figure 9). The abundance of *CCD7* transcripts in leaves was unaffected by dark chilling (Figure 9 A). In contrast, OCI-expressing stems had significantly lower levels of *CCD7* transcripts than the wild type in the absence of stress, but the abundance of *CCD7* transcripts was increased by dark chilling in the OCI-expressing stems (Figure 8 C). The roots of the wild type and OCI lines had similar levels of *CCD7* mRNAs in the absence of stress (Figure 8 C). However, dark chilling significantly increased root *CCD7* in the OCI lines but not the wild type (Figure 9 A, C).

The OCI-expressing lines had higher levels of *CCD8* transcripts was increased in the roots, stems and leaves than the wild type the absence of stress (Figure 9). The abundance of *CCD8 transcripts* was significantly decreased by dark chilling in wild type roots but not stems or leaves (Figure 9 B, D, F). However, the chilling treatment had no significant effect on the levels of *CCD8* transcripts in the roots, stems and leaves of the OCI-expressing lines (Figure 9 B, D, F).

We examined the effect of soybean seedlings on the germination of *Striga hermonthica* seeds to determine whether the level of SL exuded by the soybean plants was changed by OCI expression. However, while the *S. hermonthica* seeds germinated in the presence of GR24, there was no stimulation of germination in the presence of any of the soybean lines (Supplemental Figure 3).

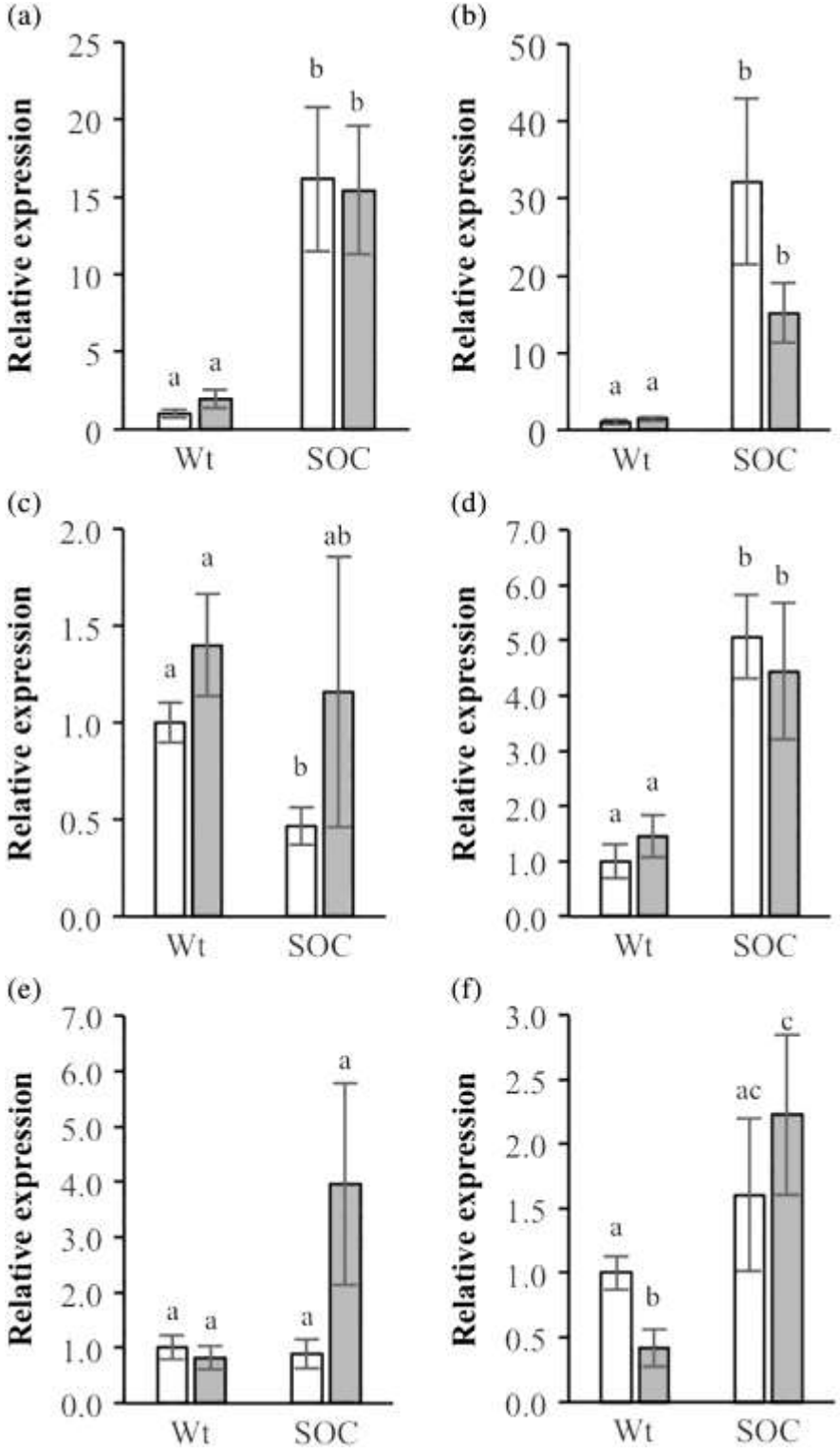


Figure 9. A comparison of the levels of transcripts encoding carotenoid cleavage dioxygenases (CCD) involved in strigolactone synthesis in the leaves (a, CCD7; b, CCD8), stems (c, CCD7; d, CCD8), and roots (e, CCD7; f, CCD8) of wild type soybean and three independent OCI-expressing lines (SOC-1, SOC-2, SOC-3). Measurements were made on plants that had been grown either under optimal growth conditions or after three consecutive nights of dark chilling. Data are expressed relative to mRNAs encoding elongation factor 1-β (with fold expression change relative to the wild type). Data obtained in the absence or presence of chilling stress were grouped for SOC-1, SOC-2, and SOC-3 lines. Shared letters indicate no significant differences (mean ± SE) from three separate experiments. ($p > .05$)

4. DISCUSSION

SLs fulfil multiple functions in plants participating in legume/rhizobium interactions, drought and nutrient deficiency responses and the stimulation of beneficial symbiosis with arbuscular mycorrhizal fungi (López-Ráez *et al.*, 2017). The data presented here demonstrate that SL also play a role in low temperature tolerance in pea and *A. thaliana.* In contrast to the wild types in during which photosynthesis is insensitive to dark chilling, low temperature stress significantly decreased photosynthesis in the *Arabidopsis max* and pea *rms4* mutants. These findings demonstrate that SL protect photosynthesis against the negative impacts of dark chilling. The chilling-dependent inhibition of photosynthesis may be related at least in part to the control of stomatal closure. However, since the chilling stress was applied in the dark period rather than during the photoperiod, any changes in transpiration and stomatal conductance are likely to be residual effects of processes occurring in the dark. Stomatal conductance and transpiration rates were lower following the dark chilling treatment in the *rms 3-1* and *rms 5-3* mutants but not in the pea wild type or *rms4-1* F-box protein mutants. The regulation of stomatal closure involves abscisic acid (ABA), PYR/RCAR and ABA receptors (Chater *et al*., 2015). Hence, the SL-dependent control of chilling tolerance is likely to involve crosstalk with the ABA-mediated signal transduction pathways that control stomatal movement, as has been reported for plant responses to drought (Liu *et al.*, 2013; Ha *et al.*, 2014; Visentin *et al.*, 2016). SLs positively regulate drought stress responses partly through ABA signal transduction pathways, which have also been shown to promote chilling and freezing tolerance (Shinkawa *et al.*, 2013). Moreover, the drought-sensitive phenotype of the *max* mutants has been attributed to effects on photosynthesis (Ha *et al.*, 2014). Crosstalk between MAX2 and ABA signalling has also been suggested to play a role in the resistance of *A. thaliana* to bacterial pathogens (Piisilä, *et al.*, 2015). The levels of transcripts encoding SL biosynthetic enzymes were increased following drought in *A. thaliana* leaves (Ha et al., 2014). In contrast, SL accumulation was decreased in drought-treated *L. japonicus* and tomato roots, and the expression of SL biosynthesis and transporter genes was repressed (Liu

et al., 2015; Ruiz-Lozano et al., 2016). Such data suggest that a complex interplay between SL and ABA signalling underpins plant responses to drought. Moreover, SL act in concert with other plant hormones such as cytokinin to protect plants against the negative impacts of environmental stresses by acting as systemic signals that regulate plant development and architecture (Zhuang *et al.*, 2017).

The data presented here showing that SL play a role in dark chilling tolerance of photosynthesis in pea and *A. thaliana* is convincing. However, SL functions in terms of the protection of biomass accumulation against the negative impacts of chilling is less well defined. Dark chilling caused a decrease in the shoot fresh weights of all the pea and *A. thaliana* lines. However, while shoot dry biomass accumulation was also significantly decreased in the *rms5-3* and *rms3-1* mutants and in the *max4-1* mutants (Figure 4 C). Taken together, these findings indicate that SL protects shoots against the negative impacts of chilling on dry biomass accumulation. However, the addition of exogenous SL (GR24) at a concentration, which we have previously shown to increases root glutathione (GSH) contents and block lateral root formation in a MAX2-dependent manner (Marquez-Garcia, *et al.*, 2013) had a negative impact on the chilling tolerance of all the *A. thaliana* lines grown on agar plates. One possible explanation of this rather contradictory finding is that the growth media used contained high levels of sucrose and is likely to have imposed a degree of osmotic stress. Osmotic stress acts together with sugar-dependent pathways to inhibit seedling development (Jossier *et al.,* 2009). However. SL are involved in sugar metabolism and sugar signalling pathways that modulate early seedling development (Li *et al.*, 2015). The *max* seedlings are hyposensitive to sugar-induced growth arrest (Li *et al.*, 2015). The results presented here may indicate that SL signalling interacts sugar-signalling in the expression of cold tolerance in *A. thaliana.*

One of the main effects of dark chilling tolerance on leaves is an interruption of the circadian control of starch breakdown (Graf *et al.,* 2011). Starch that was synthesized during the day is degraded at night to produce sucrose, which is transported throughout the plant to drive growth (Graf and Smith, 2011). Starch is rapidly mobilized at night by the synergistic action of b-AMYLASE1 (BAM1) and a-AMYLASE3 (AMY3). The regulation of starch degradation supports continued growth during the night while preventing carbon starvation or excessive carbon sequestration (Stitt and Zeeman, 2012). The circadian control of dark-

induced leaf starch degradation is perturbed by low night temperatures (Graf *et al.,* 2011), leading to a cessation of growth in plants exposed to dark chilling. The effect of dark chilling on starch degradation and hence growth will be much more important in soil/pot grown plants, which are dependent on photosynthesis to drive starch accumulation than in plants grown on plates where high levels of sucrose are always available. It is possible therefore that one mechanism by which SLs influence cold tolerance is through the control of sugar metabolism in the day and starch metabolism at night. Moreover, ABA controls the activities of BAM1 and AMY3 in leaves exposed to osmotic stress through the AREB/ABF-SnRK2 kinase-signalling pathway (Thalmann *et al.,* 2016). Drought stress-triggered ABA accumulation was found to impair responses to exogenous SL in roots (Liu *et al.,* 2015). Similarly, cold stress-induced increases in ABA levels my limit the effectiveness of GR24 in the present study and impair effective cold signalling responses, as reported in the case of drought (Liu *et al.,* 2015).

Other phytohormones in addition to ABA also exert a strong influence over chilling tolerance (Eremina *et al*., 2016). For example, the JASMONATE ZIM-DOMAIN (JAZ) proteins regulate the ICE-CBF/DREB1 pathway in *Arabidopsis* by repressing the transcriptional activity of ICE1 (Hu *et al.*, 2013). Moreover, the OPEN STOMATA 1 (OST1) kinase, which regulates stomatal closure mediated by reactive oxygen species (ROS), phosphorylates ICE1 and enhances chilling tolerance (Ding *et al.*, 2015). Jasmonates function as upstream regulators of *ICE-CBF/DREB1* pathway, which activates antioxidant systems and related pathways to counteract chilling-induced oxidation (Sharma and Laxmi, 2016). In addition to jasmonates, *ICE1* interacts with salicylic acid (SA)–dependent pathways, suggesting crosstalk between jasmonate and SA-mediated signal transduction pathways to avoid the negative impacts of chilling stress. GSH plays a crucial role in ROS-mediated regulation of gene expression mediated by the SA and jasmonate signalling pathways (Mhamdi *et al.,* 2010). Our earlier studies have also linked GSH to auxin and SLs signalling in the control of root architecture (Marquez-Garcia, *et al.*, 2013). SLs have been shown to interact directly with plant antioxidants and the redox signalling network (Woo *et al*., 2004). We have previously reported that the addition of GR24 to *Arabidopsis* seedlings exerted a greater

influence over the abundance of GSH and ascorbate than the activities of antioxidant enzymes such as catalase or superoxide dismutase (Bartoli *et al.*, 2013). Since GSH has long been associated with chilling tolerance (Foyer *et al.*, 2003; Gomez, *et al.*, 2004; Giauffret and Foyer, 2011), the SL-dependent control of chilling tolerance may also involve redox-mediated crosstalk with the jasmonate and SA-mediated signal transduction pathways.

The data presented here also implicate C1 papain-like cysteine proteases in the regulation of the chilling sensitivity of photosynthesis in soybean, as demonstrated previously in OCI-expressing tobacco lines (Van der Vyver *et al*., 2003). However, the observed decrease in chilling sensitivity of photosynthesis in soybean did not result in better shoot biomass accumulation in plants exposed to low night time temperatures. The data presented here links OCI expression to an increased abundance of *CCD7* and *CCD8* transcripts and therefore supports a role for SL in chilling tolerance.

ACKNOWLEDGEMENTS

This work was funded by BBSRC (BB/K501839/1). We thank Dr.Ottoline Leyser for providing the *max* mutants. We thank Dr Julie Scholes for the gift of *Striga hermonthica* seeds and her expert help and advice in setting up the protocols for determining soybean and GR24-mediated stimulation of seed germination.

DISCLOSURE DECLARATION

The authors declare no conflicts or competing interests.

REFERENCES

Abramoff M.D., Magalhaes P.J., Ram S.J. (2004) Image processing with Imagej. *Biophotonics International* **11**, 36 – 42.

Ainsworth E.A., Yendrek C.R., Skoneczka J.A., Long S.P. (2012) Accelerating yield potential in Soybean: Potential targets for biotechnological improvement. *Plant Cell and Environment* **35**, 38-52.

Arite T., Umehara M., Ishikawa S., Hanada A., Maekawa M., Yamaguchi S., Kyozuka J. (2009) D14, A Strigolactone-insensitive mutant of rice shows an accelerated outgrowth of tillers. *Plant and Cell Physiology* **50**, 1416–1424.

Bartoli C.G., Casalongue C., Simontacchi M., Marquez-Garcia B., Foyer C.H. (2013) Interactions between hormone and redox signalling pathways in the control of growth and cross tolerance to stress. *Environmental and Experimental Botany* **94**, 73-88.

Barton L., Thamo T., Engelbrecht D., Biswas W.K. (2014) Does growing grain legumes or applying lime cost effectively lower greenhouse gas emissions from wheat production in a semiarid climate? *Journal of Cleaner Production* **83**, 194–203.

Beers E.P., Woffenden B.J., Zhao C. (2000) Plant proteolytic enzymes: Possible roles during programmed cell death. *Plant Molecular Biology* **44**, 399–415.

Bennett T., Sieberer T., Willett B., Booker J., Luschnig C., Leyser O. (2006) The *Arabidopsis* MAX pathway controls shoot branching by regulating auxin transport. *Current Biology* **16**, 553–563.

Bhalerao, R., Keskitalo, J., Sterky, F., Erlandsson, R., Björkbacka, H., Birve, S.J., Karlsson, J., Gardeström, P., Gustafsson, P., Lundeberg, J., Jansson, S. (2003) Gene expression in autumn leaves. *Plant Physiology* **131**, 430–342.

Braun, N., de Saint Germain A., Pillot J.P., Boutet-Mercey S., Dalmais M., Antoniadi I., Li X., Maia-Grondard A., Le Signor C., Bouteiller N., Luo D., Bendahmane A., Turnbull

C., Rameau C. (2012). The pea TCP transcription factor Psbrc1 acts downstream of strigolactones to control shoot branching. *Plant Physiology* **158**, 225–38.

Carrión C., Martínez D., Lorenza Costa M., Guiamet J. (2014) Senescence-associated vacuoles, a specific lytic compartment for degradation of chloroplast proteins? *Plants* **3**, 498–512.

Chater, C., Peng, K., Movahedi, M., Dunn, J.A., Walker, H.J., Liang, Y.-K., McLachlan, D.H., Casson, S., Isner, J.C., Wilson, I., Neill, S.N., Hedrich, R., Gray, J.E. and Hetherington, A.M (2015) Elevated CO_2-induced responses in stomata require ABA and ABA signalling. *Current Biology* 25, 2709-2716.

Cheng L., Gao X., Li S., Shi M., Javeed H., Jing X., Yang G., He G. (2010). Proteomic analysis of soybean [*Glycine Max* (L.) Meer.] Seeds during imbibition at chilling temperature. *Molecular Breeding* **26**, 1–17.

Cooper J.W., Kunert K., Dasgan Y., Foyer C.H. (2015) Understanding chilling tolerance in soybean and faba bean *Aspects of Applied Biology* **124**, 117-121.

de Marchi, R., Sorel, M., Mooney, B., Fudal, I., Goslin, K., Kwaśniewska, K., Ryan, P. T., Pfalz, M., Kroymann, J., Pollmann, S., Feechan, A., Wellmer, F., Rivas, S., Graciet, E. (2016) The N-end rule pathway regulates pathogen responses in plants. *Scientific Reports* **6**, 26020.

de Saint Germain, A., Clavé, G., Badet-Denisot, M.–A., Pillot, J.–P., Cornu, D., Le Caer, J.–P., Burger, M., Pelissier, F., Retailleau, P., Turnbull, C., Bonhomme, S., Chory, J., Rameau, C., Boyer, F-D. (2016) An histidine covalent receptor and butenolide complex mediates strigolactone perception. *Nature Chemical Biology* **12**, 2147.

Ding Y., Li H., Zhang X., Xie Q., Gong Z., Yang S. (2015) OST1 kinase modulates freezing tolerance by enhancing ICE1 stability in Arabidopsis. In *Arabidopsis*. *Developmental Cell* **32**, 278–289.

Dun E.A., Brewer P.B., Beveridge C.A. (2009). Strigolactones: discovery of the elusive shoot branching hormone. *Trends in Plant Science* **14,** 364–372.

Eremina M., Rozhon W., Poppenberger B. (2016) Hormonal control of chilling stress responses in plants. *Cell and Molecular Life Sciences* 73, 797-810.

Foyer C.H., Lam, H.M., Nguyen, H.T., Siddique, K.H.M., Varshney, R.K., Colmer, T.D., Cowling, W., Bramley, H., Mori, T.A., Hodgson, J.M., Cooper, J.W., Miller, A.J., Kunert, K., Vorster, J., Cullis, C., Ozga, J.A., Wahlqvist, M.L., Liang, Y., Shou, H., Shi, K., Yu, J., Fodor, N., Kaiser, B.N., Wong, F-L., Valliyodan, B. Considine, M.J. (2016) Neglecting legumes has compromised Human Health And Sustainable Food Production. *Nature Plants* **2**, 1–10.

Foyer C.H., Vanacker H., Gomez L., Harbinson J. (2002). Regulation of photosynthesis and antioxidant metabolism in maize leaves at optimal and chilling temperatures. *Plant Physiology and Biochemistry* **40**, 659-668.

Gass T., Schori A., Fossati A., Soldati A., Stamp P. (1996). Chilling tolerance of soybean (*Glycine max* (L.) Merr.) during the reproductive phase. *European Journal of Agronomy* **5**, 71–88.

Giauffret, C., Foyer, C.H. (2011) Effects of chilling temperatures on maize growth and biomass production. In *Advances In Maize:* **3** *(Essential Reviews In Experimental Biology)* (eds J.L. Prioul, C. Thévenot and T. Molnar), pp. 347-372. The Society for Experimental Biology, UK.

Gibbs D.J., Bacardit J., Bachmair A., Holdsworth M.J. (2014) The eukaryotic N-end rule pathway: Conserved mechanisms and diverse functions. *Trends in Cell Biology* **24**, 603-611.

Gomez L., Vanacker H., Buchner P., Noctor G., Foyer C.H. (2004) Intercellular distribution of glutathione synthesis in maize leaves and its response to short term chilling. *Plant Physiology* **134**, 1662-1671.

Graf, A., Schlereth, A., Stitt, M., and Smith, A.M. (2010) Circadian control of carbohydrate availability for growth in Arabidopsis plants at night. *Proceedings of the National. Academy of Sciences of the United States of America* **107**, 9458–9463.

Graf, A., and Smith, A.M. (2011). Starch and the clock: the dark side of plant productivity. *Trends in Plant Sciences* **16**, 169–175.

Graham P.H., Vance, C.P. (2003). Legumes: Importance and constraints to greater use. *Plant Physiology* **131**, 872–877.

Guo Y. (2013) Towards systems biological understanding of leaf senescence. *Plant Molecular Biology* 82, 519–528

Ha CV, Leyva-González MA, Osakabe Y, Tran UT, Nishiyama R, Watanabe Y, Tanaka M, Seki M, Yamaguchi S Van Dong N, Yamaguchi-Shinozaki K, Shinozaki K, Herra-Estrella L, Tran L-S P. (2014) Positive regulatory role of strigolactone in plant responses to drought and salt stress. *Proceedings of the National. Academy of Sciences of the United States of America* **111**, 851–856.

Hu Y., Jiang L., Wang F., Yu D. (2013) Jasmonate Regulates The INDUCER OF CBF EXPRESSION−C-REPEAT BINDING FACTOR/DRE BINDING FACTOR1 Cascade And Freezing Tolerance In *Arabidopsis*. *Plant Cell* **25**, 2907–2924.

Hume D.J., Jackson A.K.H. (1981) Frost tolerance in soybeans. *Crop Science* **21**, 689–692.

Irving L.J., Robinson D. (2006) A dynamic model of rubisco turnover in cereal leaves. *New Phytologist* **169**, 493–504.

Johnson X., Brcich T., Dun E.A., Goussot M., Haurogné K., Beveridge C.A., Rameau C. (2006) Branching genes are conserved across species. Genes controlling a novel

signal in pea are coregulated by other long-distance signals. *Plant Physiology* **142**, 1014–26.

Jossier M, Bouly J-P, Meimoun P, Arjmand A, Lessard P, Hawley S, Grahame Hardie D, Thomas M (2009) SnRK1 (SNF1-related kinase 1) has a central role in sugar and ABA signalling in *Arabidopsis thaliana. Plant Journal* **59**, 316–328.

Kato Y., Murakami S., Yamamoto Y., Chatani H., Kondo Y., Nakano T., Yakota A, Sato F. (2004) The DNA-binding protease, CND41, and the degradation of ribulose-1, 5-bisphosphate carboxylase/oxygenase in senescent leaves of tobacco. *Planta* **220**, 97–104.

Krüger G.H.J., De Villiers M.F., Strauss A.J., de Beer M., van Heerden P.D.R., Maldonado R.., Strasser, R.J. (2014) Inhibition of photosystem II activities in soybean (*Glycine max*) genotypes differing in chilling sensitivity. *South African Journal of Botany* **95**, 85–96.

Kunert K.J., van Wyk S.G., Cullis C.A., Vorster B.J., Foyer CH (2015) Potential use of phytocystatins in crop improvement with a particular focus on legumes. *Journal of Experimental Botany* **66**, 3559-3570.

Kunert K.J., Vorster B.J., Fenta B.A., Kibido T., Dionisio G., Foyer C.H. (2016) Drought stress responses in soybean roots and nodules. *Frontiers in Plant Science* **7**, 1–7.

Li G.D., Pan L.N., Jiang K., Takahashi I., Nakamura H., Xu Y.W., Asami T., Shen R.F. (2016) Strigolactones are involved in sugar signaling to modulate early seedling development in *Arabidopsis. Plant Biotechnology* **33**, 1–11

Ligerot Y., de Saint Germain A., Waldie T., Troadec C., Citerne S., Kadakia N., Pillot J-P., Prigge M., Aubert G., Bendahmane A., Leyser O., Estelle M., Debelle F., Rameau C. (2017) The pea branching RMS2 gene encodes the PsAFB4/5 auxin receptor and is involved in an auxin-strigolactone regulation loop. PLoS Genetics 13, e1007089.

Link W., Balko C., Stoddard F.L. (2010) Winter hardiness in faba bean: Physiology and breeding. *Field Crops Research* **115**, 287–296.

Liu J., He H., Vitali M., Visentin I., Charnikhova T., Haider I., Schubert A., Ruyter-Spira C., Bouwmeester H.J., Lovisolo C. et al. (2015) Osmotic stress represses strigolactone biosynthesis in Lotus japonicus roots: exploring the interaction between strigolactones and ABA under abiotic stress. *Planta* **241**, 1435–1451.

Liu J, Novero M, Charnikhova T, Ferrandino A, Schubert A, Ruyter-Spira C, Bonfante P, Lovisolo C, Bouwmeester HJ, Cardinale F. (2013) CAROTENOID CLEAVAGE DIOXYGENASE 7 modulates plant growth, reproduction, senescence, and determinate nodulation in the model legume *Lotus japonicus*. *Journal of Experimental Botany* **64**, 1967–1981.

López-Ráez J.A., Shirasu K., Foo E. (2017) Strigolactones in plant interactions with beneficial and detrimental organisms: The Yin and Yang. *Trends in Plant Science* **22**, 527-537.

Lopez-Obando M., Ligerot Y., Bonhomme S., Boyer F.-D., Rameau C. (2015) Strigolactone biosynthesis and signaling in plant development. *Developmen**t*** **142**, 3615–3619.

Marquez-Garciá B., Njo M., Beeckman T., Goormachtig S., Foyer C.H. (2013) A new role for glutathione in the regulation of root architecture linked to strigolactones. *Plant Cell and Environment* **163**, 665-671.

Martínez D.E., Costa M.L., Gomez F.M., Otegui M.S., Guiamet J.J. (2008) 'Senescence-associated vacuoles are involved in the degradation of chloroplast proteins in tobacco leaves. *The Plant Journal* **56**, 196–206.

Masoud S.A., Johnson L.B., White F.F., Reeck G.R. (1993) Expression of a cysteine proteinase inhibitor (Oryzacystatin-I) in transgenic tobacco plants. *Plant Molecular Biology* **21**, 655–63.

Mhamdi A, Hager J, Chaouch S, Queval G, Han Y, Taconnat Y, Saindrenan P, Gouia H, Issakidis-Bourguet E, Renou J-P, Noctor, G. (2010) *Arabidopsis* GLUTATHIONE REDUCTASE 1 is essential for the metabolism of intracellular H_2O_2 and to enable appropriate gene expression through both salicylic acid and jasmonic acid signaling pathways. *Plant Physiology* **53**, 1144-1160.

Miura K., Furumoto T. (2013) Chilling signaling and cold response in plants. *International Journal of Molecular Sciences* **14**, 5312–5337.

Nair J.S., Ramaswamy N.K. (2004) Chloroplast proteases. *Biologia Plantarum* **48**, 321–326.

Nakamura H., Xue Y.L., Miyakawa T., Hou F., Qin H.M., Fukui K., Shi X, Ito E, Ito S, Park S-Hm Miyauchi Y, Asano A, Totsuki N, Ueda T, Tanokura M, Asami T. (2013) Molecular mechanisms of strigolactone perception by DWARF14. *Nature Communications* **4**, 2613.

Olesen J E., Trnka M., Kersebaum K.C., Skjelvag A.O., Seguin B., Peltonen-Sainio, P., Rossi F, Kozyra J, Micale, F. (2011) Impacts and adaptation of European crop production systems to climate change. *European Journal of Agronomy* **34**, 96–112.

Otegui, M.S., Noh, Y.S., Martínez, D.E., Vila Petroff, M.G., Staehelin, L.A., Amasino, R.M., and Guiamet, J.J. (2005) Senescence-associated vacuoles with intense proteolytic activity develop in leaves of *Arabidopsis* and soybean. *The Plant Journal* **41**, 831–844.

Pandey A., Sharma M., Pandey G.K. (2016). Emerging roles of strigolactones in plant responses to stress and development. *Frontiers in Plant Science* **7**, 1–17.

Pearce, R.S. (1999) Molecular analysis of acclimation to cold. *Plant Growth Regulation* **29**, 47–76.

Prins A., van Heerden P.D.R., Olmos E., Kunert K.J., Foyer C.H. (2008) Cysteine proteinases regulate chloroplast protein content and composition in tobacco leaves: A model for

dynamic interactions with ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) vesicular bodies. *Journal of Experimental Botany* **59**, 1935–1950.

Piisilä, M., Keceli MA, Brader G, Jakobson L, Jõesaar I, Sipari N, Kollist H, Palva ET, Kariola T. (2015) The F-box protein MAX2 contributes to resistance to bacterial phytopathogens in *Arabidopsis thaliana*. *BMC Plant Biology* **15**, 53.

Sorefan, K., Booker, J., Haurogne, K., Goussot, M., Bainbridge, K., Foo, E., Chatfield, S., Ward, S., Beveridge, C., Rameau, C., Leyser, O. (2003) *MAX4* and *RMS1* are orthologous dioxygenase-like genes that regulate shoot branching in *Arabidopsis* and pea *Genes & Development* **17,** 1469–1474.

Quain M.D., Makgopa M.E., Cooper J.W., Kunert K.J., Foyer C.H. (2015). Ectopic phytocystatin expression increases nodule numbers and influences the responses of soybean (*Glycine Max*) to nitrogen deficiency. *Phytochemistry* **112**, 179–187.

Quain M.D., Makgopa M.E., Márquez-Garciá B., Comadira, G., Fernandez-Garcia, N., Olmos, E., Schnaubelt, D., Kunert, K.J., Foyer, C.H. (2014). Ectopic phytocystatin expression leads to enhanced drought stress tolerance in soybean (*Glycine Max*) and *Arabidopsis thaliana* through effects on strigolactone pathways and can also result in improved seed traits. *Plant Biotechnology* **12**, 903–913.

R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Ray D.K., Ramankutty N., Mueller N.D., West P.C., Foley J.A. (2012) Recent patterns of crop yield growth and stagnation. *Nature Communications* 3, 1293.

Richards R.A. (2000) Selectable traits to increase crop photosynthesis and yield of grain crops. *Journal of Experimental Botany* **51**, 447–458.

Schmittgen T.D., Livak K.J. (2008) Analyzing real-time PCR data by the comparative CT method. *Nature Protocols* **3**, 1101–1108.

Shinkawa R., Morishita A., Amikura K., Machida R., Murakawa H., Kuchitsu K., Ishikawa M. (2013) Abscisic acid induced freezing tolerance in chilling-sensitive suspension cultures and seedlings of rice. *BMC Research Notes* **6,** 351.

Soares A.S., Driscoll S.P., Olmos E., Harbinson J., Arrabaça M.C., Foyer C.H. (2008). Adaxial/abaxial specification in the regulation of photosynthesis and stomatal opening with respect to light orientation and growth with CO_2 enrichment in the C4 species *Paspalum dilatatum. New Phytologist* **177**, 186–198.

Sobkowiak A., Jończyk M., Adamczyk J., Szczepanik J., Solecka D., Kuciara I., Hetmańczyk K., Trzcinska-Danielewicz J., Grzybowski M., Skoneczny M., Fronk J., Sowiński P. (2016) Molecular foundations of chilling-tolerance of modern maize. *BMC Genomics* **17**, 125.

Solomon M., Belenghi B., Delledonne M., Menachem E., Levine A. (1999) The involvement of cysteine proteases and protease inhibitor genes in the regulation of programmed cell death in plants. *Plant Cell* **11**, 431–44.

Soundappan I., Bennett T., Morffy N., Liang Y., Stanga J.P., Abbas A., Leyser O., Nelson D.C. (2015). SMAX1-LIKE/D53 family members enable distinct MAX2-dependent responses to strigolactones and karrikins in *Arabidopsis*. *Plant Cell* **27**, 3149 – 3159.

Stitt, M., and Zeeman, S.C. (2012). Starch turnover: pathways, regulation and role in growth. *Current Opinion in Plant Biology.* **15**, 282–292.

Thakur P., Sanjeev K., Malik J. A., Berger J. D., Nayyar H. (2010) Chilling stress effects on reproductive development in grain crops: An overview. *Environmental and Experimental Botany* **67**, 429 – 443.

Thalmann M, Pazmino D, Seung D, Horrer D, Nigro A, Meier T, Kölling K, Pfeifhofer HW, Zeeman,SC, Santeliaa D. (2016) Regulation of leaf starch degradation by abscisic acid is important for osmotic stress tolerance in plants. *Plant Cell* **28**, 1860–1878

Van Doorn W.G., Woltering E.J. (2004) Senescence and programmed cell death: Substance or semantics? *Journal of Experimental Botany* **55**, 2147–2153.

Van Heerden P.D., Strasser R.J., Krüger G.H. (2004) Reduction of dark chilling stress in N-fixing soybean by nitrate as indicated by chlorophyll a fluorescence kinetics. *Physiologia Plantarum* **121**, 239 – 249.

Van Heerden, P.D.R., Kiddle, G., Pellny, T.K., Mokwala, P.W., Jordaan, A., Strauss, A.J., de Beer, M., Schlüter, U., Kunert, K.J., Foyer, C.H. (2008) Regulation of respiration and the oxygen diffusion barrier in soybean protect symbiotic nitrogen fixation from chilling-induced inhibition and shoots from premature senescence. *Plant Physiology* **148**, 316–327.

Van Heerden P.D.R., Kruger G.H.J., Loveland J.E., Parry M.A.J., Foyer C.H. (2003) Dark chilling imposes metabolic restrictions on photosynthesis in soybean. *Plant Cell and Environment* **26**, 323–337.

Van der Vyver C., Schneidereit J., Driscoll S., Turner J., Kunert K., Foyer C.H. (2003) Oryzacystatin I expression in transformed tobacco produces a conditional growth phenotype and enhances chilling tolerance. *Plant Biotechnology Journal* **1**, 101–112.

Visentin, I, Vitali M, Ferrero M, Zhang Y, Ruyter-Spira C, Novak O, Strand M, Lovisolo C, Cardinale F. (2016) Low levels of strigolactones in roots as a component of the systemic signal of drought stress in tomato. *New Phytologist.* **212**, 954–963

Waters M.T., Smith S.M. (2013) KAI2- and MAX2-mediated responses to karrikins and strigolactones are largely independent of HY5 in *Arabidopsis* seedlings. *Molecular Plant* **6**, 63 – 75.

Woo H.R., Chung K.M., Park J.H., Oh S.A., Ahn T., Hong S.H., Jang S.K., Nam H.G. (2001) ORE9, An F-box protein that regulates leaf senescence in *Arabidopsis*. *Plant Cell* **13**, 1779–1790.

Zhang K., Halitschke R., Yin C., Liu C.-J., Gan S.-S. (2013) Salicylic acid 3-hydroxylase regulates *Arabidopsis* leaf longevity by mediating salicylic acid catabolism. *Proceedings of the. National Academy of Sciences of the United States of America* **110**, 14807–12.Zhuang L, Wang J, Huang B (2017) Drought inhibition of tillering in *Festuca arundinacea* associated with axillary bud development and strigolactone signaling. *Environmental and Experimental Botany* **142**, 15-23.

Zhou MQ, Shen C, Wu LH, Tang KX, Lin J (2011) CBF-dependent signaling pathway: A key responder to low temperature stress in plants. *Critical Reviews in Biotechnology* **31**, 186–192.

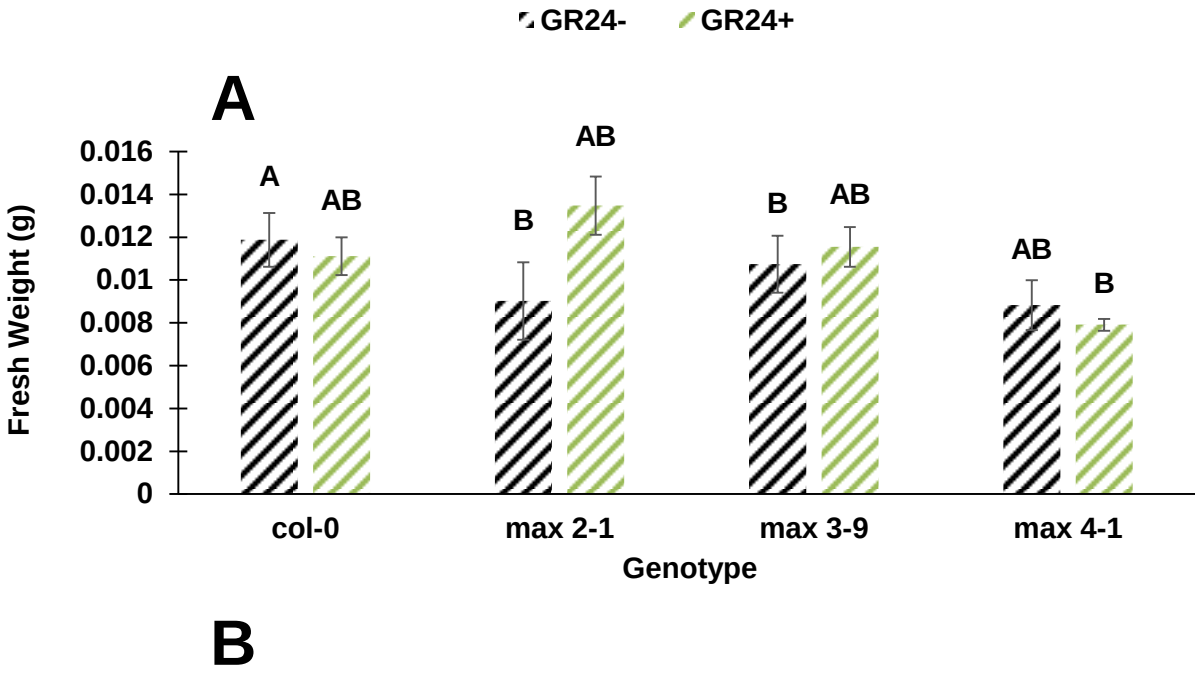
Zhu J., Dong C.H., Zhu J.K. (2007) Interplay between chilling-responsive gene regulation, metabolism and RNA processing during plant chilling acclimation. *Current Opinion in Plant Biology* **10**, 290–295.

Zhu Y., Yang H., Mang H.G., Hua J. (2011) Induction of BAP1 by a moderate decrease in temperature is mediated by ICE1 in *Arabidopsis*. *Plant Physiology* **155**, 580-588.

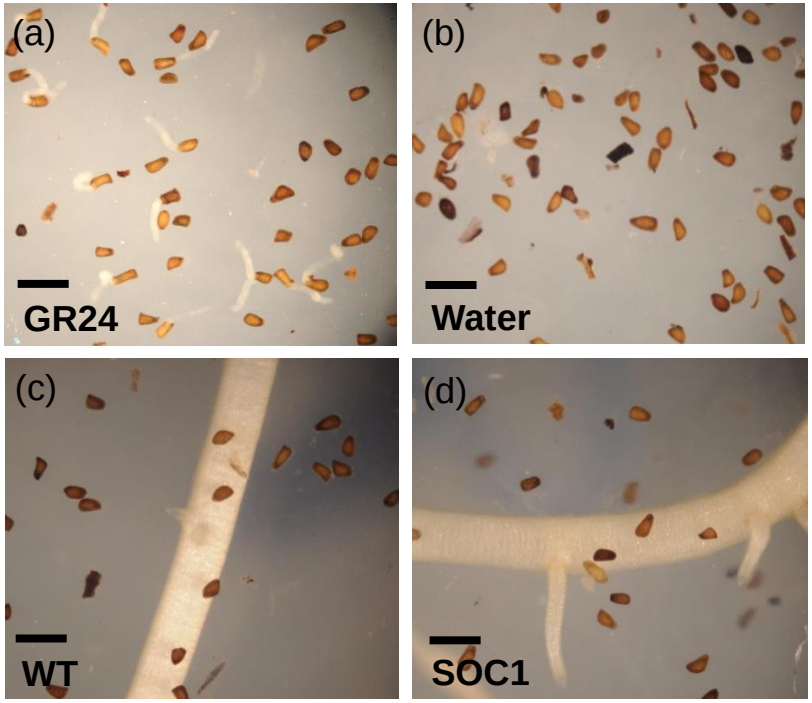
Supplementary Material

Supplemental Figure 1. A comparison of the shoot phenotypes of wild type peas and mutants defective in strigolactone synthesis either indirectly (*rms2-1*) or directly (*rms5-3*) or strigolactone signalling (*rms3-1* and *rms4-1*).

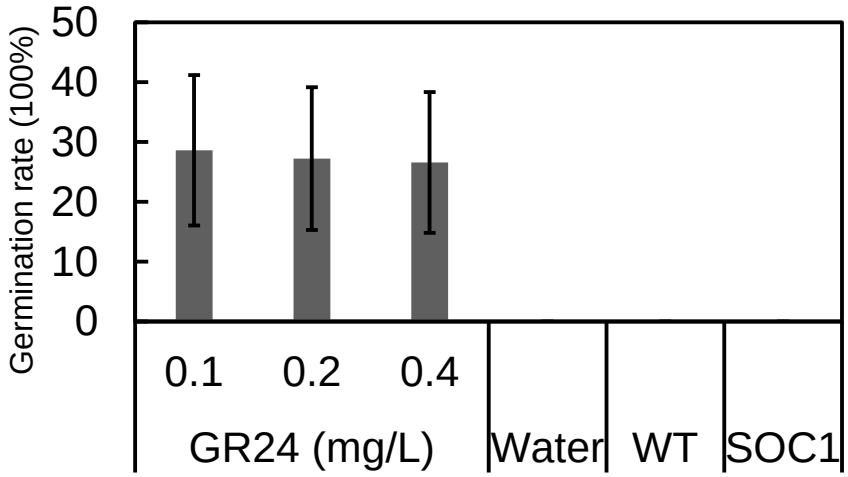
Supplemental Figure 2. The effect of GR24 on rosette biomass (fresh weight) of agar plate-grown wild type (Wt) *A. thaliana* (Col-0) and mutants defective in strigolactone synthesis (*max3-9*, *max4-1*) or signaling (*max2-1*). Plants were grown on ½ strength MS media in the absence or presence of 2 µM GR24, for 25 days under optimal growth temperatures.



Supplemental Figure 3. The effect of GR24 and soybean seedlings on the germination of *Striga hermonthica* seeds.



(e)



Supplemental Table 1: An overview of the wild type and mutant lines of pea and *A. thaliana* used in the present study. Proteins and their associated functions in the strigolactone pathway are listed.

Function	**Coded protein**	**Pea**	***A. thaliana***
-	-	L107 (Wt)	Col-0 (Wt)
Synthesis	CCD7	*rms5* (BL298)	*max3*
Synthesis	CCD8	-	*max4*
Signalling	F-box	*rms4* (K164)	*max2*
Signalling (probable)	unknown	*rms3* (K487)	-

Supplemental Table 2: Soybean gene accessions (phytozome), gene identities and primer sequences for carotenoid cleavage dioxygenases 7 and 8 and elongation factor 1 β.

Accession	**Gene ID**	**Forward sequence**	**Reverse sequence**
Glyma11g16370	CCD7	CACCAAACCCCTCCCTCTAT	CCTTCCACGGTGCTTAGAGT
-	CCD8	CTTGTTCCTGACATGCCTCA	CTAGTCCATGCAACGTGGT
Glyma02g44460	Elf 1 β	GTGGTACGATGCTGTCTCTC	CCACTGAATCTTACCCCTTGAG