Warsaw University of Life Sciences

Institute of Biology

M.Sc. Muhammad Kamran

CRK5 regulates senescence, photosynthesis, and dissipation of energy as heat in *Arabidopsis thaliana* by inhibitory effect on salicylic acid signaling

CRK5 reguluje starzenie, fotosyntezę, i rozpraszanie energii w postaci ciepła w liściach *Arabidopsis thaliana* poprzez inhibicję ścieżek sygnalnych kwasu salicylowego

Doctoral thesis

Rozprawa doktorska

Doctoral thesis prepared under the supervision of

Prof. dr hab. inż. Stanisław Karpiński

Department of Plant Genetics, Breeding and Biotechnology, Institute of Biology

Warsaw University of Life Sciences

Assistant supervisor:

Dr inż. Paweł Burdiak

Department of Plant Genetics, Breeding and Biotechnology, Institute of Biology

Warsaw University of Life Sciences

Warsaw 2026

Statement of the supervisor of the doctoral thesis

prepared by a student of the Doctoral School of the Warsaw University of Life Sciences

I represent that **the doctoral thesis by Muhammad Kamran has been prepared under my supervision based on research conducted during education at the Doctoral School of the Warsaw University of Life Sciences,** and I represent that it fulfils the requirements for submission thereof in the procedure for the award of the academic doctoral degree.

Date: 12/03/2026 Legible signature of the supervisor .....

Statement of the author of the doctoral thesis

being a student of the Doctoral School of the Warsaw University of Life Sciences

Aware of legal liability, including criminal liability for making a false statement, I hereby represent that **the present doctoral thesis has been prepared by myself based on research conducted during education at the Doctoral School of the Warsaw University of Life Sciences** and does not contain any content obtained in a manner inconsistent with the applicable laws, in particular the Act of 4 February 1994 on Copyright and Related Rights (consolidated text of 28 October 2022, Polish Journal of Laws of 2022, item 2509, as amended).

I represent that the thesis presented herein has not previously been the basis of any procedure related to the award of the doctoral degree.

I further represent that this version of the thesis is identical to the attached electronic version.

I acknowledge that the doctoral thesis will be subjected to the anti-plagiarism procedure.

Date: 12/03/2026 Legible signature of the author of the thesis .....

Acknowledgments

I would like to express my sincere gratitude to all those who contributed, directly or indirectly, to the completion of this doctoral thesis.

First and foremost, I am deeply grateful to my supervisor, ***Prof. dr hab. Stanisław Karpiński*** *and co-supervisor* ***Dr inż. Paweł Burdiak*** *for their invaluable scientific guidance, continuous support, and critical insight throughout this research.*

I sincerely thank the members of my ***doctoral committee*** *for their time, careful evaluation, and insightful comments, which significantly improved the quality and clarity of this thesis.*

To ***my colleagues*** *and* ***lab members****, thank you for creating a stimulating and supportive research atmosphere that makes daily work both productive and enjoyable.*

I sincerely thank ***all the co-authors*** *of the publications for the fruitful collaboration.*

I am thankful to the ***Warsaw University of Life Sciences****, which partially supported this research financially and provided the infrastructure necessary to conduct this work.*

Finally, I would like to express my heartfelt gratitude to ***God****, my parents –* ***Mr. Jamshaid Khan and Mrs. Husan Zarri,*** *my family members –* ***Mujeeb-ur-Rahman, Ashfaq Ahmad, Ayesha BiBi, Ismail Khan,*** *my nephews –* ***Affan, Sufyan, and Zahran****, and* ***close friends*** *throughout my doctoral journey. Thank you for being there when I needed you the most. Your encouragement and belief in me were a constant source of motivation.*

Financing

This research was performed as part of:

Project funded by the **National Science Centre (Opus 20 UMO-2020/39/B/NZ3/02103)**, project title: "**Unravelling molecular and physiological mechanisms of foliar temperature regulation during photoinhibition and its impact on retrograde cell death signalling and acclimatory responses in Arabidopsis**".

NARODOWE CENTRUM NAUKI

Table of contents

List of Figures

List of Tables

Summary

CRK5 regulates senescence, photosynthesis, and dissipation of energy as heat in *Arabidopsis thaliana* by inhibitory effect on salicylic acid signaling

Understanding how plants integrate stress perception, energy absorption management, and cell death (CD) regulation is important for understanding their survival strategies in variable, often extreme, environments. Plants are constantly exposed to complex combinations of abiotic and biotic stresses, which require tightly coordinated signaling networks to balance acclimation, defense, growth, and senescence. Although significant progress has been made in identifying individual stress response pathways, the mechanisms integrating hormonal and redox signaling and energy dissipation as heat into the cellular regulatory mechanisms, including photosynthesis, growth, development, acclimation, and CD regulation, remain largely unknown.

To address this knowledge gap, **the main hypothesis of this dissertation is that CYSTEINE-RICH RECEPTOR-LIKE KINASE 5 (CRK5) acts as a negative regulator of salicylic acid (SA)-dependent cellular signaling pathways, coordinating dark-induced senescence (DIS), reactive oxygen species (ROS) homeostasis, and CD in Arabidopsis leaves.** The thesis comprises three thematically related, peer-reviewed articles that collectively examine how CRK5- and SA-dependent cellular signaling influences the management of absorbed energy in excess (AEE) partitioning, thus, how it regulates the cellular homeostasis between the amount of heat produced from AEE dissipation, ROS production, and the level of CD induction in response to stress. **In particular, the work investigates: (i)** the role of transcription factors (TFs) and kinases in the regulation, management of AEE division in photosystem II, retroactive signaling from chloroplasts, transcriptional response of the cell nucleus and induction of CD in the integrated regulation of acclimation and defense mechanisms, i.e. in the so-called cross-tolerance phenomenon in plants; **(ii)** the function of CRK5 as a regulator integrating the inhibition of cellular SA signaling in the processes of cell senescence, photosynthesis and AEE dissipation in the form of heat; and; **(iii)** the role of CRK5 in the coordination of signaling of cellular homeostasis between SA, ROS and CD during DIS.

The first article is a comprehensive review discussing and presenting empirical evidence for cellular retrograde signaling from chloroplasts and the nuclear response in the integrated regulation of acclimation, defense, and CD mechanisms. It synthesizes the current knowledge on CD regulation in some leaf cells as a central and ultimate process underlying the induction of acclimation and defense, i.e., cross-tolerance in other leaf cells. The review highlights the importance of AEE, its dissipation as heat, and its role in inducing electrical and chemical signals, which serve as critical triggers for multiple retrograde signaling pathways from chloroplasts. Simultaneously, it highlights the role of non-photochemical energy quenching (NPQ) in photosystem II and its dissipation as heat, the redox status of the plastoquinone and ROS pools, and hormonal and electrical signals in local, systemic, and network retrograde signaling. Importantly, the review proposes the hypothesis that plants have evolved unified molecular mechanisms that jointly regulate CD and thus acclimation and defense mechanisms, i.e., cross-tolerance to abiotic and biotic stresses, rather than, as previously believed, having independent signaling pathways for these basic types of stresses. This conceptual framework provided the basis for formulating the central hypothesis that specific components of cellular and intercellular signaling coordinate SA-dependent senescence, AEE dissipation as heat, and cellular redox homeostasis, thereby enabling systemic and network-based acquired acclimation and immunity.

Based on this framework, the second article examines the role of CRK5 in integrating DIS signaling and photo-protective management of AEE, including inhibition of phytohormonal SA signaling. Using Arabidopsis mutants with loss of functional *CRK5* and *CPR1* (constitutive expression of *PR1*), mutant lines with blocked SA and ethylene phytohormone synthesis, such as *sid2* and *ein2*, transgenic lines with disturbed SA signaling (NahG), and double crosses between them, we analyzed physiological, biochemical, and molecular phenotypic differences. Based on these analyses, we identified deregulation of phenotypic traits, demonstrating that CRK5 acts as a negative regulator of SA signaling and a positive regulator of photosynthesis, AEE dissipation via heat, and plant growth. This was confirmed by the observation that loss of functional CRK5 resulted in increased SA levels, accelerated DIS, impaired photosynthetic efficiency, decreased NPQ, and lower leaf temperature during AEE, indicating impaired AEE dissipation as heat.

Transcriptomic analysis revealed extensive deregulation of genes, including transcription factors such as WRKY, involved in the regulation of senescence, SA, photosynthesis, and NPQ, under prolonged dark conditions. Crucially, crossing the *crk5* line with lines defective in SA synthesis or catabolism (*sid2* and NahG, respectively) completely reversed the *crk5* phenotype, whereas ethylene signaling dysfunction (*ein2*) did not, demonstrating that CRK5 specifically modulates SA-dependent pathways. The *cpr1* mutant, showing constitutive SA-accumulation, had a similar phenotype to *crk5*, and exogenous SA application further reduced NPQ and leaf temperature in all genotypes, confirming that SA is a negative regulator of photosynthetic efficiency and photo-protective dissipation of excess energy as heat. These results suggest a novel role for CRK5 as a key regulator that prevents DIS and maintains photosynthetic efficiency by inhibiting SA-driven reactions.

The third article further elucidates the role of CRK5 in integrating SA signaling with cellular ROS homeostasis and CD regulation during DIS. Using physiological, biochemical, and molecular analyses, this article demonstrates that *crk5* mutants exhibit distinct deregulations manifested by excessive ROS accumulation and increased electrolyte leakage from the cell (indicating increased and accelerated CD processes) under both control and dark conditions. These phenotypes were accompanied by a significant reduction in carotenoid and xanthophyll pools, increased phenolic compound accumulation, and enhanced ROS scavenging capacity, including increased activity of key antioxidant enzymes. Transcriptomic analysis revealed robust induction of genes in aging-related pathways, metacaspases, autophagy, and redox processes, positioning CRK5 loss-of-function as a precursor to SA-dependent transcriptional reprogramming. Importantly, these phenotypic features were reversed in the *crk5sid2* and *crk5NahG* double mutants, confirming that CRK5-negative SA signaling is a key driver of altered cellular redox homeostasis and CD induction. Comparisons of *crk5* and *cpr1* mutant phenotypes further highlighted CRK5's role as a regulator that modulates both antioxidant dynamics and transcriptional control of gene expression during CD.

In conclusion, these findings provide new insights into current knowledge on the manner in which plants coordinate phytohormonal, metabolic, and oxidative pathways to conditionally maintain optimal growth and development, and thus plant vigor, while

simultaneously adapting to unfavorable biotic and abiotic conditions. More broadly, this work presents a more holistic picture of plant responses to AEE stress, aimed at achieving conditionally optimal acclimation and defense during the ongoing climate change – the Earth's overheating.

Keywords: dark-induced senescence, development, absorbed energy in excess, receptor-like kinase, non-photochemical quenching, salicylic acid, reactive oxygen species, cell death, antioxidant system, acclimation

Streszczenie

CRK5 reguluje starzenie, fotosyntezę, i rozpraszanie energii w postaci ciepła w liściach *Arabidopsis thaliana* poprzez inhibicję ścieżek sygnalnych kwasu salicylowego

Zrozumienie, w jaki sposób rośliny integrują percepcję stresu, zarządzanie zaabsorbowaną energią i regulacją śmierci komórki (ang. CD), jest ważne dla zrozumienia ich strategii przetrwania w zmiennych, często ekstremalnych, środowiskach. Rośliny są stale narażone na złożone kombinacje stresów abiotycznych i biotycznych, które wymagają ściśle skoordynowanych sieci sygnalizacyjnych, aby zrównoważyć aklimatyzację, obronę, wzrost i starzenie się. Chociaż poczyniono znaczny postęp w identyfikacji indywidualnych szlaków reakcji na stres, mechanizmy integrujące sygnalizację hormonalną i redoks oraz rozpraszanie energii w postaci ciepła w ramach komórkowych mechanizmów regulacyjnych obejmujących fotosyntezę, wzrost, rozwój, aklimatyzację i regulację CD pozostają w dużej mierze nieznane.

Główną hipotezą niniejszej rozprawy doktorskiej jest to, że KINAZA RECEPTOROPODOBNA BOGATA W CYSTEINĘ 5 (CRK5) działa jako negatywny regulator komórkowych szlaków sygnałowych zależnych od kwasu salicylowego (ang. SA), koordynując indukowane ciemnością starzenie (ang. DIS), homeostazę reaktywnych form tlenu (ang. ROS) i CD w liściach Arabidopsis. Rozprawa składa się z trzech tematycznie powiązanych, recenzowanych artykułów, które łącznie badają, w jaki sposób komórkowa sygnalizacja zależna od CRK5 i SA wpływa na zarządzanie podziałem zaabsorbowanej energii w nadmiarze (ang. AEE) czyli jak reguluje komórkową homeostazę pomiędzy ilością wyprodukowanego ciepła z rozproszenia AEE, wyprodukowanych ROS i poziomu indukcji CD w odpowiedzi na stres. **W szczególności praca bada: (i)** rolę czynników transkrypcyjnych (ang. TF) i kinaz w zarządzaniu podziałem AEE w fotosystemie II, w retroaktywnej sygnalizacji z chloroplastów oraz w transkrypcyjnej odpowiedzi i indukcji CD w zintegrowanej regulacji aklimatyzacji i mechanizmów obronnych, czyli w tak zwanym zjawisku tolerancji krzyżowej u roślin; **(ii)** funkcję CRK5 jako regulatora integrującego inhibicję komórkowej sygnalizacji SA w procesach starzenia komórki, fotosyntezy i dyssypacji AEE pod postacią ciepła oraz; **(iii)**

rolę CRK5 w koordynacji sygnalnej homeostazy komórkowej pomiędzy SA, ROS i CD podczas DIS.

Pierwszy artykuł to kompleksowy przegląd omawiający i przedstawiający empiryczne dowody na komórkową sygnalizację wsteczną z chloroplastów i jądrową odpowiedź w zakresie zintegrowanej regulacji mechanizmów aklimatyzacji, obrony i CD. Syntetyzuje on aktualną wiedzę na temat regulacji CD w niektórych komórkach liści jako centralnego i ultymatywnego procesu leżącego u podstaw indukcji aklimatyzacji i obrony, czyli tolerancji krzyżowej w pozostałych komórkach liści. Artykuł podkreśla znaczenie AEE i jego rozpraszanie pod postacią ciepła oraz jego rolę w indukcji sygnałów elektrycznych i chemicznych jako krytyczny czynnik wyzwalający wieloraką sygnalizację wsteczną z chloroplastów. Jednocześnie podkreśla rolę niefotochemicznego wygaszania energii (NPQ) w fotosystemie II i jej rozpraszania pod postacią ciepła, statusu redoks puli plastochinonu i ROS, sygnałów hormonalnych i elektrycznych w lokalnej, systemowej i sieciowej sygnalizacji wstecznej. Co istotne, artykuł proponuje hipotezę, że u roślin wyewoluowały zunifikowane mechanizmy molekularne, które wspólnie regulują CD, a przez to mechanizmy aklimatyzacyjne i obronne, czyli tolerancję krzyżową na stresy abiotyczne i biotyczne. Jest to odmienny pogląd od przyjętego powszechnie, że rośliny mają niezależne ścieżki sygnalne dla tych podstawowych typów stresów. Te ramy koncepcyjne stanowiły podstawę do sformułowania centralnej hipotezy, że specyficzne komponenty komórkowej i między-komórkowej sygnalizacji koordynują zależne od SA starzenie się, rozpraszanie AEE, komórkową homeostazę redoks, a przez to systemową i scieciową nabytą aklimatyzację i odporność.

W oparciu o te ramy tematyczne, drugi artykuł bada rolę CRK5 w integracji sygnalizacji DIS i fotoprotekcyjnego zarządzania AEE, związanego z inhibicją sygnałów zależnych od SA. Wykorzystując mutanty Arabidopsis z dysfunkcją białek *CRK5* i *CPR1* (konstytutywna ekspresja *PR1*), linie mutantów z zaburzoną syntezą SA i etylenu takie jak: *sid2* i *ein2*, transgeniczne linie z zaburzonymi ścieżkami sygnalnymi SA (NahG) oraz podwójne krzyżówki pomiędzy nimi, podjęto analizę fizjologiczną, biochemiczną oraz molekularną tych linii. Na podstawie tych analiz, zidentyfikowano kluczowe szlaki regulacji tych cech fenotypowych, które wskazują, że CRK5 działa jako negatywny

regulator sygnalizacji SA i pozytywny regulator fotosyntezy, rozpraszania AEE pod postacią ciepła i wzrostu rośliny. Zostało to potwierdzone odkryciem, że utrata funkcjonalnego CRK5 skutkowała podwyższonym poziomem SA, przyspieszonym DIS, upośledzoną wydajnością fotosyntezy, obniżonym NPQ i niższą temperaturą liści podczas AEE, co wskazuje na zaburzone rozpraszanie AEE pod postacią ciepła. Analiza profilu transkryptomu ujawniła rozległą deregulację genów, w tym czynników transkrypcyjnych jak WRKY związanych z regulacją starzenia, SA, fotosyntezy i NPQ w warunkach długotrwałej ciemności. Istotną kwestią była obserwacja, że krzyżowanie linii *crk5* z liniami z dysfunkcją syntezy SA lub z zaburzonymi ścieżkami sygnalnymi SA (odpowiednio *sid2* i NahG) całkowicie odwróciły fenotyp *crk5*, podczas gdy dysfunkcja sygnalizacji etylenu (*ein2*) nie dała takiego efektu, co dowodzi, że CRK5 specyficznie moduluje szlaki zależne od SA. Linia mutanta *cpr1*, cechująca się akumulacją SA, miała podobny fenotyp do *crk5*, a egzogenne zastosowanie SA dodatkowo obniżyło NPQ i temperaturę liścia u wszystkich genotypów, potwierdzając, że SA jest negatywnym regulatorem fotoprotekcyjnego rozpraszania AEE pod postacią ciepła i wydajności fotosyntezy. Wyniki te wskazują na nową rolę CRK5 jako kluczowego regulatora, który zapobiega DIS i utrzymuje wydajność fotosyntezy poprzez hamowanie reakcji napędzanych przez SA.

Trzeci artykuł dodatkowo wyjaśnia rolę CRK5 w integracji sygnalizacji SA z komórkową homeostazą ROS i regulacją CD podczas DIS. Wykorzystując analizy fizjologiczne, biochemiczne i molekularne, niniejszy artykuł pokazuje, że mutant *crk5* wykazuje wyraźne deregulacje objawiające się nadmierną akumulacją ROS, zwiększonym wyciekiem elektrolitów z komórki (będącym wskaźnikiem zwiększonej i przyspieszonej CD) zarówno w warunkach kontrolnych, jak i w ciemności. Fenotypom tym towarzyszyło znaczne zmniejszenie puli karotenoidów i ksantofili, podwyższona akumulacja związków fenolowych i zwiększona zdolność do usuwania ROS, w tym zwiększona aktywność kluczowych enzymów antyoksydacyjnych. Analiza transkryptomiczna ujawniła silną indukcję genów związanych ze szlakami starzeniaautofagią i procesami utleniająco-redukcyjnymi, wskazując na istotną funkcję CRK5 w transkrypcyjnym przeprogramowaniu komórki zależnym od SA. Istotną kwestią jest to, że nastąpiło odwrócenie tych cech fenotypowych w podwójnych mutantach *crk5sid2* i *crk5NahG* i potwierdziło, że

sygnalizacja SA negatywnie zależna od CRK5 jest głównym czynnikiem powodującym zmianę komórkowej sygnalnej homeostazy utleniająco-redukcyjnej i indukcję CD. Porównania cech *crk5* z mutantem *cpr1* dodatkowo uwypukliły rolę CRK5 jako regulatora, który moduluje zarówno dynamikę przeciwutleniającą, jak i transkrypcyjną kontrolę ekspresji genów podczas DIS.

Podsumowując, odkrycia te dają nowe spojrzenie na to, jak rośliny koordynują szlaki fito-hormonalne, metaboliczne i utleniająco-redukcyjne, aby warunkowo utrzymać optymalny wzrost i rozwój, i jednocześnie przystosować się do niekorzystnych warunków biotycznych i abiotycznych. W szerszym ujęciu, praca ta przedstawia bardziej holistyczny obraz reakcji roślin na stres AEE, mający na celu osiągnięcie warunkowo optymalnej aklimatyzacji i obrony w czasie trwających zmian klimatu – przegrzania Ziemi.

Słowa kluczowe: indukowane ciemnością starzenie, rozwój, nadmiar zaabsorbowanej energii, kinaza receptoropodobna, wygaszanie niefotochemiczne, kwas salicylowy, reaktywne formy tlenu, śmierć komórki, układ antyoksydacyjny, aklimacja

List of articles comprising the thesis

Article 1: **Kamran, M**., Burdiak, P., & Karpiński, S. (2025). Crosstalk Between Abiotic and Biotic Stresses Responses and the Role of Chloroplast Retrograde Signaling in the Cross-Tolerance Phenomena in Plants. Cells, 14(3), 176. https://doi.org/10.3390/cells14030176. (Ministerial score: 140; IF: 5.2)

Article 2: **Kamran, M**., Burdiak, P., Zarrin Ghalami, R., Rusaczonek, A., Duszyn, M., Gołębiewska, K., Gawroński, P., Karpiński, SM (2026a). The kinase CRK5 regulates dark-induced senescence and dissipation of energy as heat by inhibiting salicylic acid signaling, *Plant Physiology*, Volume 200, Issue 2, February 2026, kiag046, https://doi.org/10.1093/plphys/kiag046. (Ministerial score: 140; IF: 6.9)

Article 3: **Kamran, M**., Burdiak, P., Rusaczonek A., Zarrin Ghalami R., & Karpiński, S. (2026b). CRK5 preserves antioxidant homeostasis and prevents cell death during dark-induced senescence through inhibiting the salicylic acid signaling pathway. Under peer review in BMC Plant Biology, Springer Nature. (Ministerial score: 140; IF: 4.8)

List of abbreviations used

Absorbed energy in excess	AEE
Ascorbate peroxidase	APX
Catalase	CAT
Cell death	CD
CYSTEINE-RICH RECEPTOR-LIKE KINASE 5	CRK5
Dark-induced senescence	DIS
Delta maximal foliar temperature	ΔT_{max}
Ethylene	ET
Hydrogen peroxide	H_2O_2
Maximum photosynthetic efficiency	*Fv/Fm*
Network acquired acclimation	NAA
Non-photochemical quenching	NPQ
Reactive oxygen species	ROS
Salicylic acid	SA
Superoxide dismutase	SOD
Systemic acquired acclimation	SAA
Total photosynthetic efficiency	ΦPSII

1. Introduction

Global climate change represents one of the most pressing challenges of the twenty-first century, threatening agricultural productivity, ecosystem stability, and global food security. Rising temperatures, increasing frequency of extreme weather events, prolonged droughts, and progressive desertification of fertile land have already been disrupting crop yields worldwide (Hatfield & Prueger, 2015; Peng et al., 2022). It is estimated that more than 800 million people lacked adequate access to food in 2021, with prediction to rise as climate extremes intensify (World Health Organization, 2022). Current projections suggest that desertified areas may expand from approximately 3% to nearly 10% of the Earth's land surface, further constraining agricultural production (Wilby et al., 2006). Under field conditions, major crop species typically achieve only ~30% of their genetic yield potential, with the remaining losses largely attributable to abiotic and biotic stresses (Boyer, 1982; Vij & Tyagi, 2007; Zhang et al., 2020; Zurbriggen et al., 2010). Addressing these challenges requires a deeper mechanistic insight into how plants perceive stress, integrate signaling pathways, and balance survival with productivity.

Plants are sessile organisms that must continuously adapt to fluctuating environmental conditions, often experiencing multiple stresses simultaneously. Among the most prevalent stressors intensified by climate change are absorbed energy in excess (AEE), heat waves, ultraviolet radiation, drought, salinity, and pathogen attacks (Fahad et al., 2017; Karpiński et al., 2013; Zhang et al., 2022). These stressors disrupt cellular homeostasis by altering energy balance, redox status, and metabolic fluxes, ultimately challenging plant survival. To cope with such conditions, plants rely on sophisticated signaling networks involving phytohormones, reactive oxygen species (ROS), calcium ions, electrical signals, and transcriptional reprogramming (Czarnocka & Karpiński, 2018; Mittler et al., 2022; Petrov et al., 2015; Szechyńska-Hebda et al., 2022). Central to these adaptive responses is cell death (CD), a genetically controlled process that eliminates damaged or unnecessary cells to protect the integrity of the organism and optimize resource allocation under stress (Górecka et al., 2020; Karpiński et al., 2013; Muhlenbock et al., 2008).

CD is not merely a terminal outcome of stress but a crucial regulatory mechanism that contributes to acclimation, defense, and development. Controlled activation of CD

enables plants to restrict pathogen spread, remodel tissues, and reallocate nutrients during senescence, thereby enhancing long-term fitness (Minhas et al., 2017; Petrov et al., 2015). Systemic- and network-acquired acclimation (SAA and NAA) processes further extend local stress perception into whole-plant responses, allowing plants to "memorize" stress exposure and mount faster or stronger responses upon subsequent challenges. Notably, exposure of low light acclimated plants to transient episodes of AEE can induce cross-tolerance, conferring enhanced resistance to pathogens and ultraviolet radiation through long-lasting molecular and physiological adjustments (Górecka et al., 2020; Karpiński et al., 2013; Szechyńska-Hebda et al., 2010, 2022). These phenomena underscore the integrative nature of plant stress responses, in which energy management, redox signaling, and CD are tightly interconnected.

Chloroplasts play a central role in coordinating these processes, acting as both energy converters and primary stress sensors. Under high light, excessive excitation of the photosynthetic apparatus necessitates rapid dissipation of AEE as heat to prevent photo-inhibition and oxidative damage. This dissipation occurs predominantly through non-photochemical quenching (NPQ), a photo-protective mechanism involving PSII antenna complexes and the xanthophyll cycle (Ruban, 2016; Zarrin Ghalami et al., 2025). NPQ converts excess excitation energy into heat, thereby limiting ROS production and safeguarding photosystem integrity. Conversely, under low light or extended darkness, photosynthetic activity declines, and chloroplasts undergo a controlled dismantling process during senescence, characterized by pigment degradation, nutrient remobilization, and eventual cell death (Liebsch & Keech, 2016; Paluch-Lubawa et al., 2021). Although high light and darkness represent opposite extremes, both conditions impose energetic imbalance and activate chloroplast-derived signaling pathways that regulate stress responses and cellular fate (Foyer & Hanke, 2022; Li & Kim, 2022).

A central regulator linking stress perception, energy management, and CD is the phytohormone salicylic acid (SA). SA is widely recognized for its role in plant immunity and systemic acquired resistance, but its functions extend far beyond pathogen defense (Devireddy et al., 2021; Hassan et al., 2022). SA modulates photosynthesis, respiration, thermogenesis, senescence, and multiple forms of cell death (Khan et al., 2013; Rivas-San

Vicente & Plasencia, 2011; Sangwan et al., 2022). During stress, SA signaling integrates with ROS, calcium, nitric oxide, and mitogen-activated protein kinase (MAPK) pathways to fine-tune cellular responses (Devireddy et al., 2021). Importantly, SA exhibits a dual role: at moderate levels, it enhances stress tolerance and acclimation, whereas excessive accumulation can promote oxidative stress, senescence, and CD (Bernacki et al., 2019; Miura et al., 2013; Morse et al., 2007).

The relationship between SA and redox homeostasis is particularly critical during senescence. ROS are unavoidable byproducts of aerobic metabolism and function as essential signaling molecules at low concentrations, but their uncontrolled accumulation leads to oxidative damage and cell death (Rogers & Munné-Bosch, 2016). Leaves undergoing senescence typically display increased ROS levels alongside a progressive decline in antioxidant capacity, tipping the balance from controlled signaling toward irreversible cellular damage (Ji et al., 2016). Antioxidant systems, including enzymatic components such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), as well as non-enzymatic antioxidants such as carotenoids, phenolic compounds, glutathione, and ascorbate, are therefore crucial determinants of cell fate (Hiltscher et al., 2014; Mordi et al., 2020; Rao et al., 2025). Genetic studies have demonstrated that enhanced antioxidant capacity can suppress ROS-induced CD, while compromised scavenging accelerates senescence and death (Hiltscher et al., 2014; Mateo et al., 2006; Rosenwasser et al., 2011).

SA is closely involved in regulating these antioxidant networks. Elevated endogenous SA levels often correlate with increased H_2O_2 accumulation, but this is frequently accompanied by upregulation of antioxidant buffers such as glutathione, suggesting that SA promotes a high-flux redox signaling state rather than uncontrolled oxidative stress (Mateo et al., 2006; Mishra et al., 2023). This delicate balance allows ROS to function as signals without immediately triggering catastrophic damage. However, prolonged or excessive SA accumulation can overwhelm antioxidant defenses, leading to redox collapse and activation of PCD, highlighting SA's role as a "double-edged sword" in plant stress responses (Radojičić et al., 2018).

The integration of SA signaling with upstream perception mechanisms is mediated partly by receptor-like kinases (RLKs), a large family of transmembrane proteins that transduce extracellular stimuli into intracellular responses. RLKs regulate diverse processes including growth, development, hormone signaling, and responses to abiotic and biotic stresses (Antolín-Llovera et al., 2014; Wrzaczek et al., 2010). Among these, cysteine-rich receptor-like kinases (CRKs) form a prominent subfamily characterized by extracellular DUF26 domains containing conserved cysteine motifs. CRKs have been implicated in immunity, redox signaling, stomatal regulation, and cell death, often acting as modulators of SA-dependent pathways (Burdiak et al., 2015; Wrzaczek et al., 2010).

CYSTEINE-RICH RECEPTOR-LIKE KINASE 5 (CRK5) has emerged as a particularly intriguing member of this family. Previous studies have identified CRK5 as a positive regulator of plant growth and a negative regulator of stomatal conductance, foliar senescence, and CD (Bourdais et al., 2015; Burdiak et al., 2022; Chen et al., 2003). Loss of CRK5 function leads to elevated SA, ROS levels, accelerated senescence, and altered stress responses. The *CRK5* promoter contains an unusually high number of W-box cis-elements, suggesting strong regulation by WRKY transcription factors, key mediators of SA signaling (Burdiak et al., 2022; Rushton et al., 2010). Recent evidence further indicates that CRK5 modulates SA signaling through MAPK cascades involving MPK3 and MPK6, positioning CRK5 upstream of major defense and senescence pathways (Zhao et al., 2022).

Despite these insights, critical gaps remain in understanding how CRK5 integrates SA signaling with senescence, AEE as heat dissipation, ROS homeostasis, and CD, particularly under conditions of dark-induced senescence. DIS provides an important experimental model for studying age-related senescence, as it induces coordinated chlorophyll degradation, photosystem remodeling, nutrient remobilization, and CD in a highly reproducible manner (Chaomurilege et al., 2023; Guo et al., 2021). The mechanisms by which SA and CRK5 modulate redox balance, antioxidant dynamics, and CD during DIS remain poorly resolved.

The primary objective of this doctoral thesis was to elucidate how CRK5 coordinates SA-dependent signaling with senescence, heat mechanism, photosynthetic performance, ROS homeostasis, and CD. By integrating conceptual advances in cross-

tolerance and systemic acclimation with detailed genetic, physiological, biochemical, and transcriptomic analyses, **this work aimed to define CRK5 as a central regulatory hub controlling the transition between acclimation and senescence.** Using Arabidopsis single and double mutant lines impaired in CRK5 function and SA synthesis or catabolism mutants (*sid2* and NahG), as well as SA-accumulating control plants (*cpr1*), this thesis dissects the molecular mechanisms through which CRK5 restrains SA-driven senescence, maintains photo-protective energy dissipation as heat, and preserves redox homeostasis.

Together, the studies presented in this thesis advance a unified framework in which CRK5 functions at the intersection of hormonal chloroplast retrograde signaling, absorbed energy management, and oxidative stress control, providing new insight into how plants balance survival, productivity, and longevity under adverse environmental conditions.

2. Research hypothesis and objectives

The main hypothesis of this doctoral thesis is that CYSTEINE-RICH RECEPTOR-LIKE KINASE 5 functions as a key upstream negative regulator of salicylic acid-dependent signaling pathways that coordinate dark-induced senescence, antioxidant homeostasis, and cell death in Arabidopsis. This hypothesis has been proven since the loss of CRK5 function disrupts this regulatory balance, leading to excessive SA accumulation, deregulated cellular redox homeostasis, impaired dissipation of AEE as heat, enhanced ROS accumulation, and accelerated senescence and CD. Furthermore, this regulatory role of CRK5 is hypothesized to be specific to SA-dependent pathways and largely independent of ET-signaling. This thesis further advances the understanding that CRK5 integrates hormonal, redox, and photo-protective signaling processes, thereby linking SA-mediated senescence and CD with antioxidant systems and the dissipation of AEE as heat, ultimately maintaining leaf functional stability under stress conditions such as extended darkness.

To test this principal hypothesis, **the study addresses the following objectives:**

Identification of CRK5 as an upstream regulator of SA-dependent DIS. To determine whether CRK5 negatively regulates DIS through SA signaling by comparing wild-type plants with CRK5 loss-of-function mutants and genetic lines with altered SA synthesis or catabolism (*sid2* and NahG). The contribution of ethylene signaling was additionally assessed using the ET-signaling mutant (*ein2*) to evaluate pathway specificity.

Assessment of CRK5 function in coordinating SA-mediated photo-protective and physiological responses. To investigate how CRK5 influences photosynthetic performance, NPQ, foliar temperature, and AEE as heat under control and stress conditions, and to determine whether these processes are modulated through SA signaling and accumulation.

Evaluation of CRK5-dependent regulation of antioxidant homeostasis and programmed cell death during dark-induced senescence. To examine how CRK5 modulates enzymatic and non-enzymatic antioxidant systems, ROS accumulation, pigment composition, and cellular integrity during senescence, and to establish whether enhanced CD and redox imbalance in *crk5* mutants are driven by SA-dependent mechanisms.

Transcriptomic dissection of SA-, senescence-, and redox-related pathways regulated by CRK5. To characterize global transcriptional reprogramming associated with loss of CRK5 function under control and dark conditions, with particular emphasis on senescence-associated genes, CD regulators, antioxidant and redox-related genes, and SA-responsive transcriptional networks.

This doctoral thesis, structured as a compilation of three thematically linked scientific articles, presents an integrated mechanistic framework that describes how a receptor-like kinase coordinates hormonal signaling, redox regulation, photo-protection, and cell-fate decisions. By positioning CRK5 as a central regulatory hub that links SA signaling with antioxidant homeostasis and senescence control, this work contributes to a deeper understanding of stress-induced leaf aging and CD, with broader implications for plant resilience and survival under adverse environmental conditions.

3. Materials and methods

3.1. Plant Material and growing conditions

Seeds of *Arabidopsis thaliana* wild-type Col-0 (ecotype Columbia), mutants in the same background: *crk5*, *cpr1*, *sid2*, *ein2*, the NahG line which is a transgenic line containing the bacterial *nahG* gene, and double mutants *crk5/sid2*, *crk5*/NahG, *crk5/ein2* were used for all the analyses. The generation of double mutant lines was achieved by crossing, while the generation of complementation lines was previously described (Burdiak et al., 2015). All primers used for genotyping are shown in Table 1. After stratification (3 days at 4 °C), plants were placed in the growing chamber and grown on Jiffy Pots for 4–5 weeks under a long-day photoperiod (16 h/8 h) with constant white light at 120 µmol photons m^{-2} sec^{-1} and a relative humidity of 70 ± 5% at 22 °C. To induce dark-associated senescence, four-week-old plants were transferred to continuous darkness for four days.

Table 1. Primers used in this study for genotyping.

Gene	AGI Code	Sequence LP Primer	Sequence RP Primer
CRK5	AT4G23130	AGGAGATCTCTCGCCAGAATC	CGATAGTCTCTTCACGGCAAC
NahG	Transgene line (M60055)	ACTCTGCCGCTACTCCCATA	CGAGCCCTAGGTACATCTGC
SID2	AT1G74710	AATTGGCAGGGAGACTTACGAA	TCCTCTATCGAATGATTCTATCTCCTT
EIN2	AT5G03280	TAAAATTGGTTTCAGCATCGG	CCTGAGCTTTGGGGAAAGTAC

3.2. Experimental design

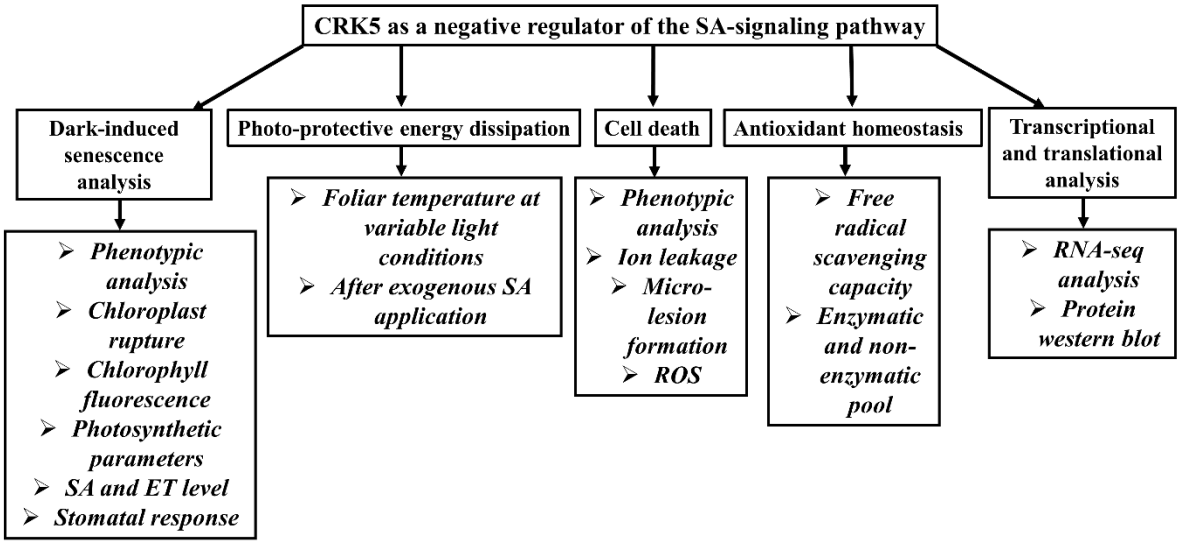


Figure 1. Conceptual framework for the present study.

3.3.Methods

In this thesis, a comprehensive, multi-level experimental framework combining genetic, physiological, biochemical, cellular, and transcriptomic approaches was employed to explain the role of CRK5 in SA-dependent regulation of senescence, energy dissipation as heat, redox balance, and cell death in Arabidopsis. Cellular integrity and cell death progression were assessed through relative electrolyte leakage measurements and trypan blue staining, allowing for quantitative and spatial evaluation of membrane damage and micro-lesions formation during dark-induced senescence. Chloroplast stability was further examined using confocal laser scanning microscopy in transgenic lines expressing stromal GFP markers.

Photosynthetic performance and energy management were analyzed using chlorophyll *a* fluorescence imaging and PAM fluorometry to determine PSII efficiency, NPQ, and electron transport rates, complemented by new technique developed in our lab to measure the delta maximal foliar temperature under variable light conditions. In this newly developed technique the thermal imaging analysis was conducted on intact Arabidopsis rosettes grown in soil using a FLIR A600-Series infrared camera. Measurements utilized a custom LabVIEW program under variable light conditions: 30 s ambient light (150 μmol

m^{-2} s^{-1}), followed by 60 s high-intensity blue light (4000 µmol m^{-2} s^{-1}), then 60 s ambient light recovery (150 µmol m^{-2} s^{-1}). Background temperature rose approximately 1 to 2°C during measurements. Gas-exchange measurements provided insight into stomatal conductance, transpiration, and CO_2 assimilation. Pigment composition, including chlorophylls, carotenoids, and xanthophylls, was quantified by high-performance liquid chromatography (HPLC).

Hormonal and redox-related parameters were evaluated by quantifying salicylic acid *via* HPLC, ethylene production by gas chromatography, and hydrogen peroxide levels using both spectrophotometric assays and histochemical 3,3'diaminobenzidine (DAB) staining. Antioxidant capacity was characterized using 2,2-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), and Ferric Reducing Antioxidant Power (FRAP) assays, along with detailed profiling of phenolic compounds, flavonols, anthocyanins, and phenylpropanoids by spectrophotometric methods. Enzymatic antioxidant activities, including APX, CAT, and SOD, were determined from protein extracts using established spectrophotometric assays, with protein abundance of selected senescence- and defense-related markers analyzed by Western blotting.

At the molecular level, transcriptomic profiling was conducted using RNA sequencing of control and dark-treated plants, followed by differential expression and gene ontology enrichment analyses to identify CRK5- and SA-dependent regulatory networks. Statistical analyses were performed across all physiological, biochemical, and molecular datasets using appropriate software to assess genotype- and treatment-specific effects.

Overall, this integrated methodological approach enabled us for a robust, systemic characterization of CRK5 function in coordinating hormonal signaling, energy dissipation as heat, antioxidant homeostasis, and cell death during dark-induced senescence.

All methods are described in detail in the attached publications (Kamran et al., 2026a (article 2), Kamran et al., 2026b (article 3).

4. Main results and general comments

Article 1: Kamran, M., Burdiak, P., & Karpiński, S. (2025). Crosstalk Between Abiotic and Biotic Stresses Responses and the Role of Chloroplast Retrograde Signaling in the Cross-Tolerance Phenomena in Plants. Cells, 14(3), 176. https://doi.org/10.3390/cells14030176

This review article provides a comprehensive conceptual and mechanistic framework from the last decade, incorporating new information on how plants integrate abiotic and biotic stress responses through chloroplast-centered signaling networks, thereby establishing the theoretical foundation for the subsequent experimental studies presented in this thesis. The main result of the review is the synthesis of dispersed evidence into a unified model in which chloroplast retrograde signaling, driven primarily by AEE, functions as a central hub coordinating acclimation, defense, CD, and cross-tolerance.

A key contribution of the article is a clear demonstration that AEE as heat is not merely a consequence of stress but a primary trigger of multiple, simultaneous stress signals, including heat, photo-oxidative, photo-inhibitory, osmotic, and photo-respiratory stress. The dissipation of AEE as heat through NPQ, electrical signals, and ROS initiate rapid retrograde signaling from chloroplasts to nucleus. These signals operate across multiple temporal and spatial scales, from sub-second photo-physical events within photosystem II (PSII) to long-distance systemic- and network-acquired acclimation (SAA and NAA) within plants and even between neighboring plants.

The review consolidates evidence that ROS, redox changes in the plastoquinone pool, phytohormones (notably SA, JA, ET, and ABA), calcium fluxes, and electrical signaling waves do not act independently. Instead, they form tightly interconnected signaling networks that jointly determine cellular fate decisions, acclimation versus CD under fluctuating environmental conditions. Importantly, CD is presented not as a purely detrimental outcome, but as a conditionally beneficial, developmentally and environmentally regulated process that can optimize whole-plant survival and stress resilience (Figure 2).

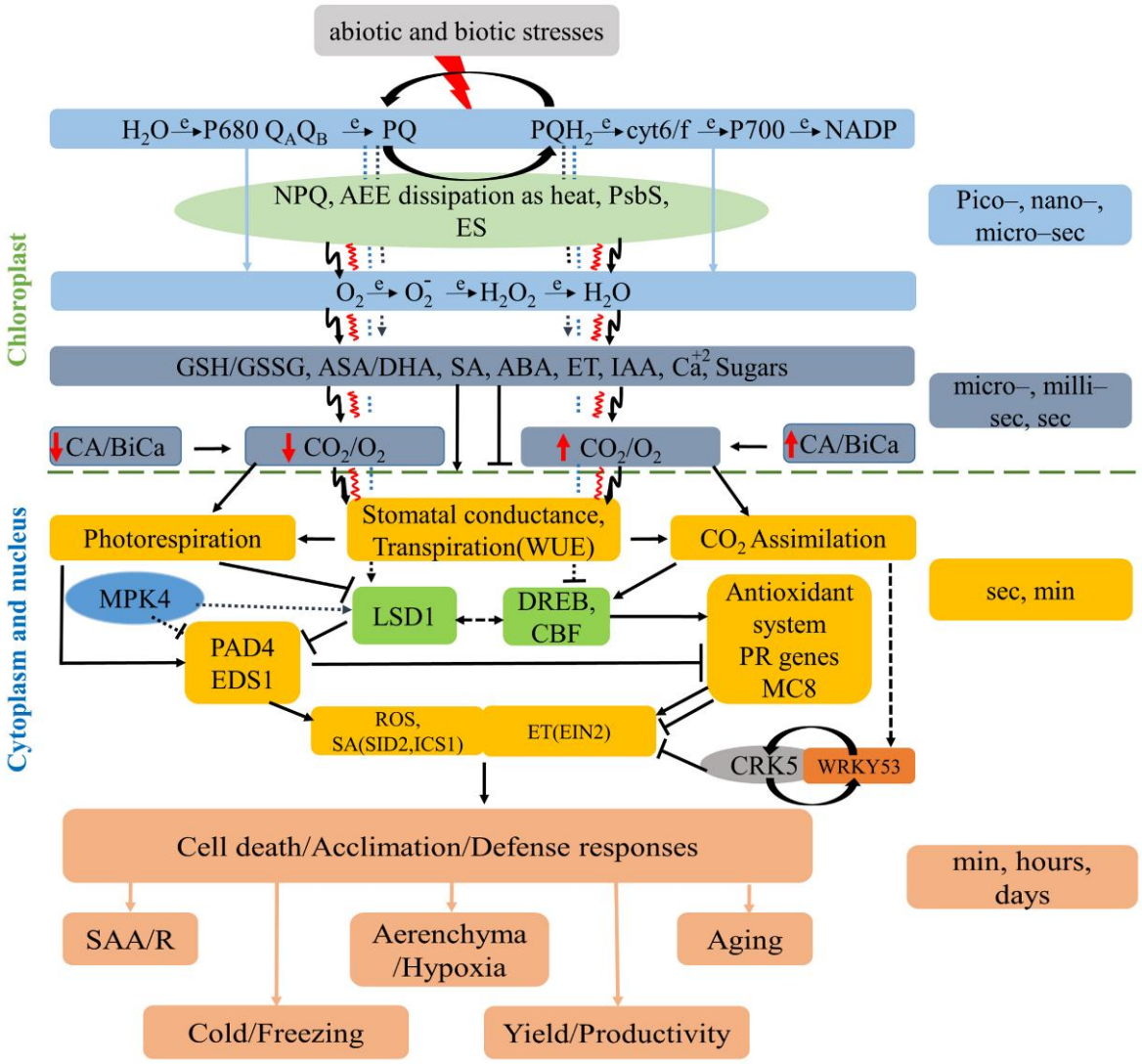


Figure 2. Model of the chloroplast retrograde signaling pathways that regulate cell death (CD) and integrate immune defenses and plant acclimation responses in cross-tolerance phenomenon to biotic and abiotic stresses. Figure from article 1 (Kamran et al., 2025).

Another central outcome of the article is the integration of cross-tolerance phenomena into this signaling framework. The review highlights how prior exposure to one stress (e.g., excess light) can induce a "stress memory" that enhances tolerance to subsequent, mechanistically distinct stresses (e.g., UV radiation or pathogen attack). This memory is shown to rely on NPQ-dependent processes, redox regulation, and transcriptional reprogramming, supporting the concept that plants employ a shared

molecular system to manage diverse stress combinations rather than deploying entirely stress-specific pathways.

The article further emphasizes the role of key regulatory proteins and transcription factors such as LSD1, EDS1, PAD4, WRKYs, RRTF1, and cysteine-rich receptor-like kinases (CRKs) as conditional regulators, modulators of stress signaling, and CD. Among these, CRK5 emerges as an important regulator positioned at the interface of extracellular stress perception, SA signaling, cellular redox homeostasis, and CD control. Although the review itself is not experimental, it identifies knowledge gaps and hypotheses for novel CRK5 functions in cross-tolerance mechanisms, conditionally optimizing SA signaling and CD with photosynthesis efficiency, and photoprotection against chloroplast-derived stress responses, which remained unresolved at the time (Figure 2).

Ultimately, the article advocates a broader conceptual shift, framing plants as highly dynamic, information-processing systems capable of integrating photo-physical, biochemical, and electrical signals across cells, organs, and entire plant communities. The proposed models of SAA and NAA highlight that stress responses and cross-tolerance are not limited to individual cells or plants, but rather operate at the level of interacting networks, enabling coordinated acclimation and defense within plant populations.

In the context of this doctoral thesis, this review provides the theoretical and conceptual foundation for the subsequent research articles. It establishes the importance of chloroplast retrograde signaling, AEE as heat, NPQ, PSII performance, SA, ROS, and conditional CD regulation in cross-tolerance phenomena. These insights directly motivate and justify the experimental focus of the following studies on CRK5 as a central regulatory hub linking SA signaling, photo-protective energy dissipation, antioxidant homeostasis, senescence, and cell death under stress-induced conditions.

Article 2: **<u>Kamran, M</u>**., Burdiak, P., Zarrin Ghalami, R., Rusaczonek, A., Duszyn, M., Gołębiewska, K., Gawroński, P., Karpiński, SM (2026a). The kinase CRK5 regulates dark-induced senescence and dissipation of energy as heat by inhibiting salicylic acid signaling, *Plant Physiology*, Volume 200, Issue 2, February 2026, kiag046, https://doi.org/10.1093/plphys/kiag046.

This study demonstrates that CRK5 functions as a central regulator that integrates SA signaling with DIS and photo-protective energy dissipation. Loss of CRK5 activity leads to excessive SA-accumulation, which in turn accelerates leaf senescence under prolonged darkness, as evidenced by enhanced chlorophyll degradation, chloroplast destabilization, and premature induction of senescence-associated genes and proteins. Genetic analyses using single and double mutants clearly demonstrate that this phenotype is strictly SA-dependent: disruption of SA synthesis or catabolism mutants (*sid2*, NahG) fully restores wild-type (Col-0) senescence progression in the *crk5* background, whereas disruption of ethylene signaling (*ein2*) does not. These findings firmly position CRK5 as a negative regulator of SA-mediated DIS (Figure 3).

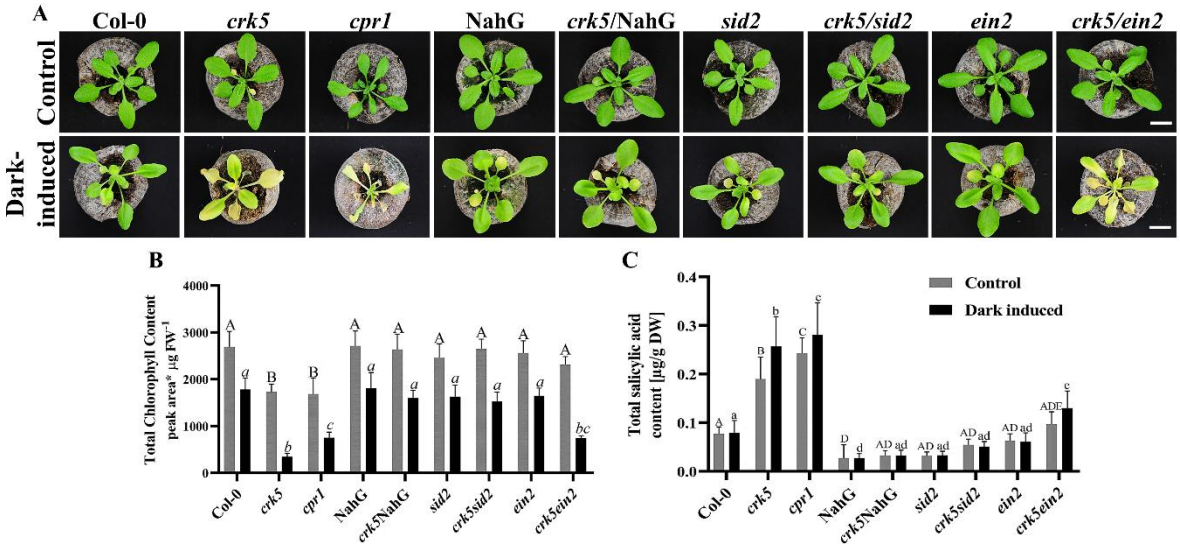


Figure 3. Morphology, total foliar chlorophyll, and salicylic acid (SA) content of the Arabidopsis salicylic acid-synthesis or -catabolism dysfunctional mutants and ethylene-signaling mutants in control and after exposure for 4 days to permanent darkness. The picture shows a phenotype of 4-week-old plants growing under ambient light (120 µmol m^{-2} s^{-1}) conditions and exposed to four days of permanent darkness (**A**), Total chlorophyll content **(B)**, and Total SA content (**C**). White scale bars indicate 1 cm. Mean values (±SD) were

derived from 9 plants (n = 9). Statistical analysis was performed according to a t-test at a level of $p < 0.05$. Letters A, B, C, D, E, and a, b, c, d, e above the bars indicate homogenous groups, and values sharing common labels (letters) are not significantly different from each other. Figure from article 2 (Kamran et al., 2026a).

Beyond its role in aging, CRK5 also has a decisive influence on leaf energy management under conditions of excess light. SA-accumulating lines, including *crk5* and the constitutively active SA mutant *cpr1*, display reduced NPQ, lower foliar temperatures (ΔT_{max}), and impaired photosynthetic efficiency (*Fv/Fm* and ΦPSII). These physiological alterations are fully reversed in SA-deficient *crk5* double mutants, demonstrating that CRK5 positively regulates NPQ, thermal dissipation AEE as heat, and photosynthetic performance through suppression of SA signaling. Correlation analyses further support this relationship, revealing strong negative associations between endogenous SA levels and both NPQ and ΔT_{max} amplitude (Figure 4).

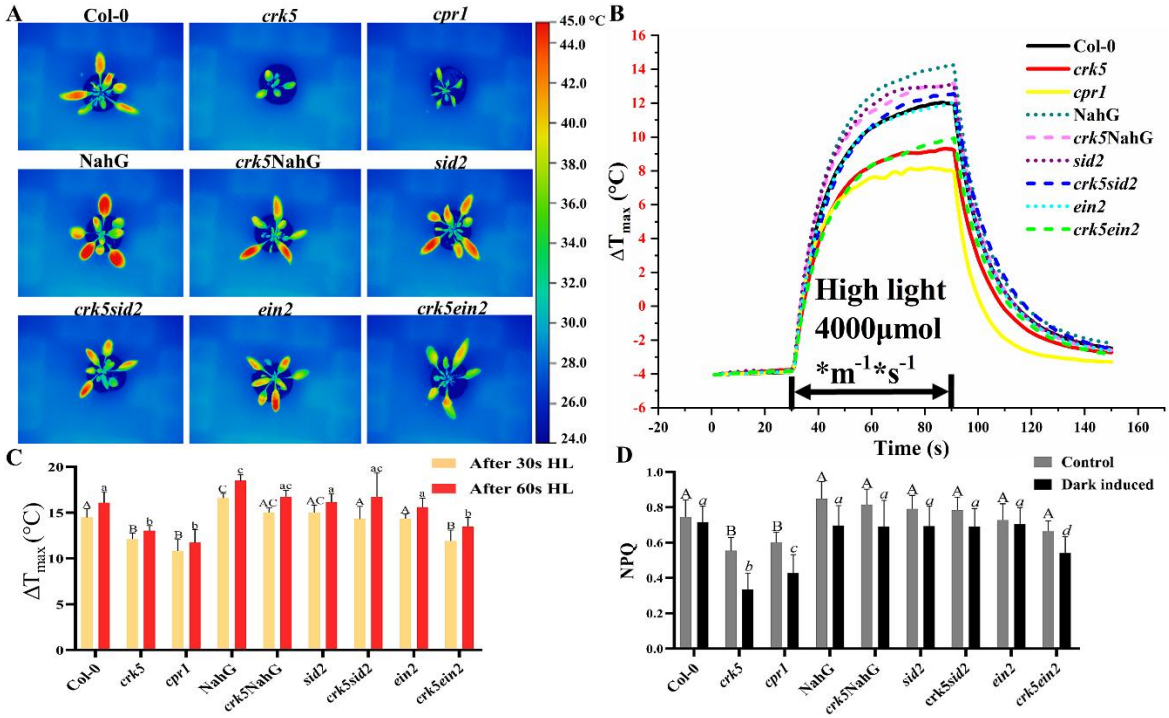


Figure 4. Analysis of foliar temperature under variable light conditions and non-photochemical quenching (NPQ) in Arabidopsis salicylic acid-synthesis or -catabolism dysfunctional mutants and ethylene-signaling mutants. The thermograms displaying whole rosettes of various Arabidopsis genotypes after 30 seconds of high light (4000 µmol photons m^{-2} s^{-1}) (**A**). The plot displays dynamic changes in delta foliar maximal temperature (ΔT_{max}) under varying light levels relative to the white background on which the plants were

placed for the measurement. The white surface reflected light, and exposure to intense light only caused a 1.5 °C rise in temperature. The following was the program's setup: 30 s of ambient light (150 μmol photons m^{-2} s^{-1}), followed by 60 s of blue LED light (4000 μmol photons m^{-2} s^{-1}), and lastly by 60 s of ambient light (150 μmol photons m^{-2} s^{-1}) (**B**). The average leaf temperature changes after 30 s and 60 s of high light exposure (**C**). NPQ (**D**). For temperature analysis, data represent mean values of 3 different leaves from 9 plants (n = 27), and for NPQ, mean values were derived from 9 plants ± SD (n = 9). Statistical analysis was performed according to a t-test at a level of $p < 0.05$. Letters A, B, C, and a, b, c above the bars indicate homogenous groups, and values sharing common labels (letters) are not significantly different from each other. Figure from article 2 (Kamran et al., 2026a.)

Transcriptomic analyses provide molecular support for these physiological observations and identify CRK5 as an upstream modulator of SA-dependent transcriptional networks during DIS. The *crk5* mutant exhibits extensive transcriptional reprogramming under darkness, with strong induction of WRKY transcription factors and genes associated with chlorophyll catabolism, autophagy, and SA signaling. Importantly, these transcriptomic changes are largely abolished when SA accumulation is genetically suppressed in the *crk5* background, supporting that CRK5 controls senescence-related gene expression primarily through SA signaling. In contrast, the *cpr1* mutant, despite showing a similar physiological phenotype, exhibits more limited transcriptional changes, suggesting that CRK5 acts upstream of CPR1 to coordinate SA-mediated responses. Protein level analyses of NPR1, PR1, and SAG12 further corroborate the activation of SA-dependent defense and senescence pathways in *crk5* and their reversion in SA-deficient backgrounds.

Overall, this work reveals a previously unrecognized dual role of CRK5 in simultaneously restraining SA-driven senescence and promoting photo-protective energy dissipation and photosynthesis. By fine-tuning SA signaling, CRK5 conditionally maintains leaf longevity and functional stability under conditions of energy imbalance caused by either prolonged darkness or excess light. These findings place CRK5 at the center of a regulatory hub that coordinates hormonal, transcriptional, and physiological processes, thereby providing important mechanistic insight into how plants balance stress-induced senescence with photo-protective energy management (Figure 5). The article presents further elaboration, a more in-depth interpretation, and a discussion of these findings.

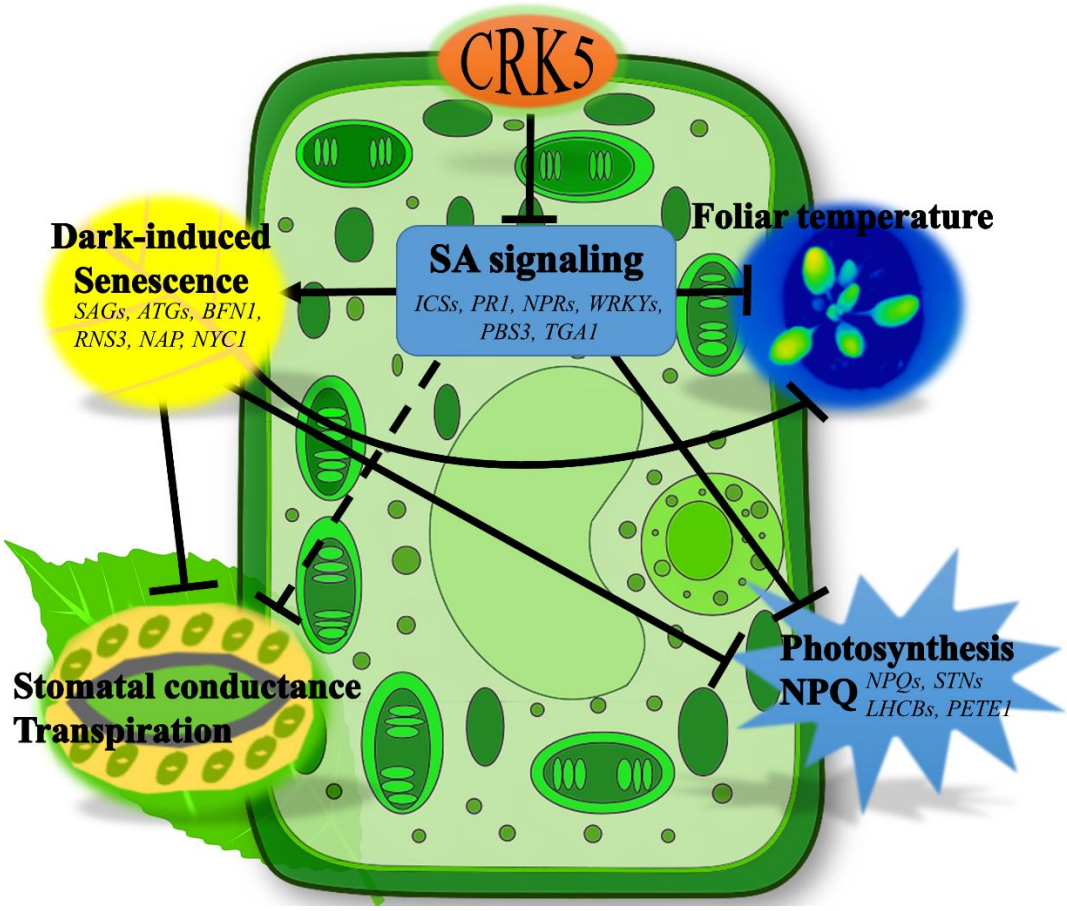


Figure 5. A model summarizing the negative regulatory role of CRK5 in the salicylic acid signaling. The CRK5-driven inhibition of SA signaling leads to the induction in the foliar temperature, photosynthesis, and non-photochemical quenching and delays the onset of aging processes. Figure from article 2 (Kamran et al., 2026a).

Article 3: Kamran, M., Burdiak, P., Rusaczonek A., Zarrin Ghalami R., & Karpiński, S. (2026b). CRK5 preserves antioxidant homeostasis and prevents cell death during dark-induced senescence through inhibiting the salicylic acid signaling pathway. Under revision in BMC Plant Biology.

The present article demonstrates that CRK5 functions as a key upstream regulator that restrains SA-dependent DIS and CD by maintaining the ROS and redox homeostasis. Loss of CRK5 activity resulted in constitutively elevated SA levels leading to accelerated senescence, increased electrolyte leakage, and enhanced micro-lesion formation under both control and dark conditions, indicating premature activation of CD processes. These phenotypes were consistently observed in the SA-accumulating *crk5* and *cpr1* mutants, whereas SA synthesis or catabolic dysfunction lines (*sid2* and NahG) and their corresponding double mutants in *crk5* background exhibited a wild-type (Col-0) like phenotype. The complete phenotypic reversion in *crk5sid2* and *crk5*NahG plants clearly suggests that accelerated CD and antioxidant homeostasis response in *crk5* is driven by SA signaling (Figure 6).

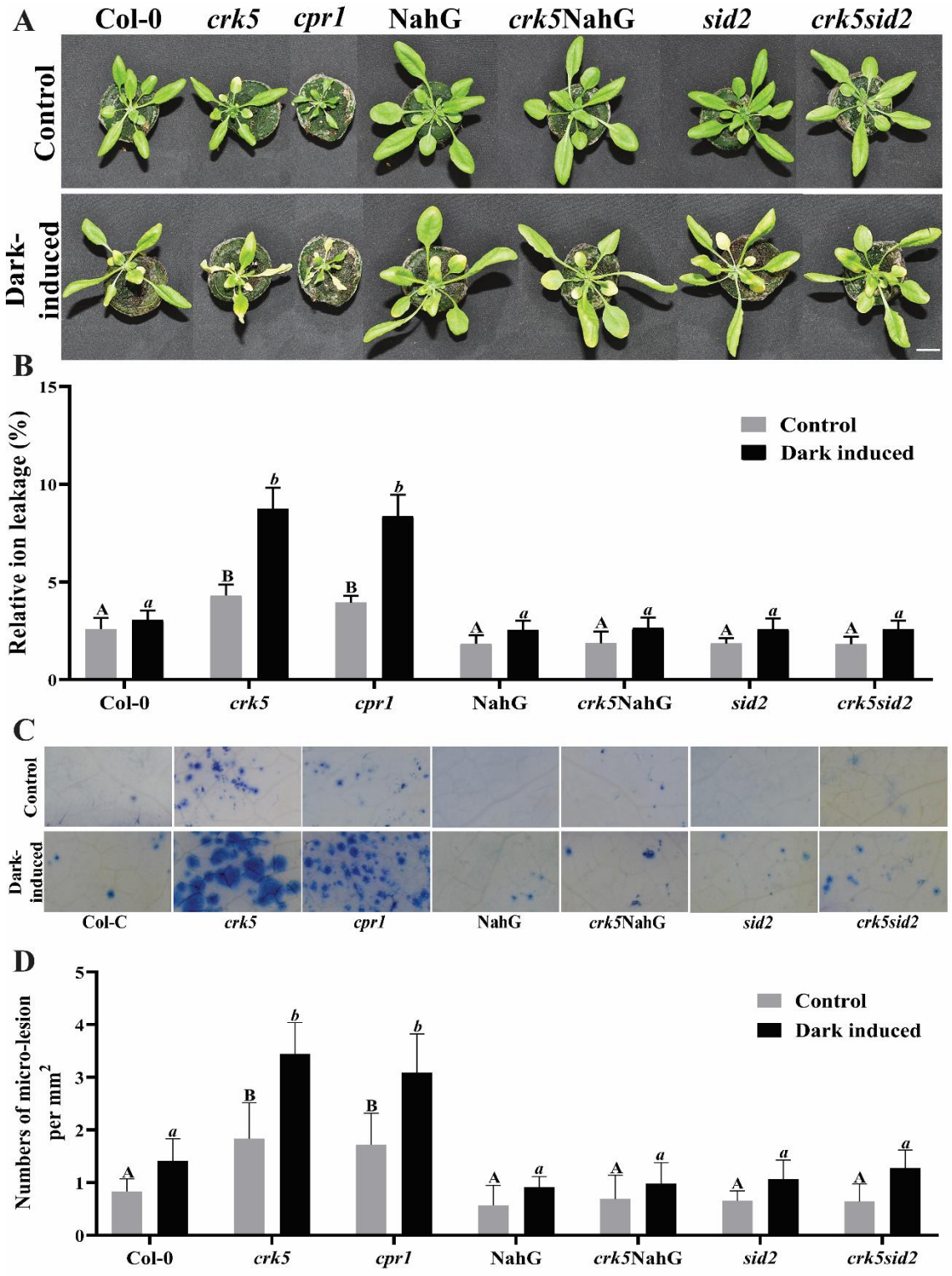


Figure 6. CRK5-dependent blockade of salicylic acid accumulation and signaling during dark-induced senescence and cell death. Morphology (**A**), relative ion leakage (**B**), trypan blue staining for the detection of cell death (**C**), and cell death quantified as micro-lesion number per mm^2 (**D**) of wild-type, *crk5*, c*pr1*, and

salicylic acid-synthesis dysfunction *sid2* mutant or salicylic acid-catabolism transgenic NahG line after exposure for 4 days to permanent darkness. The picture shows a phenotype of 4-week-old plants growing under ambient light (120 μmol m^{-2} s^{-1}) conditions and exposed to four days of permanent darkness. White scale bars indicate 1 cm. Mean values (±SD) were derived from 9 plants (n = 9). Statistical analysis was performed using a t-test at a significance level of $p < 0.05$. Letters A, B, and a, b above the bars indicate homogenous groups, and values sharing common labels (letters) are not significantly different from each other. Figure from article 3 (Kamran et al., 2026b).

At the metabolic level, CRK5 was shown to protect chloroplast integrity during senescence by preventing excessive SA-dependent degradation of carotenoids and xanthophylls. SA-accumulating lines displayed markedly reduced pools of photo-protective pigments under both light and prolonged darkness, whereas suppression of SA synthesis or catabolism in the *crk5* background restored pigment levels to those of Col-0 plants. In parallel, elevated SA was associated with pronounced oxidative stress, as evidenced by increased H_2O_2 accumulation and a strong induction of both enzymatic and non-enzymatic antioxidant systems. Enhanced activities of SOD, CAT, and APX, together with increased phenolic compounds and total radical-scavenging capacity (ABTS and DPPH), reflect a compensatory response to excessive ROS production rather than an effective suppression of oxidative damage. Importantly, these redox imbalances were fully reverted when SA signaling was genetically suppressed in *crk5* (Figure 7).

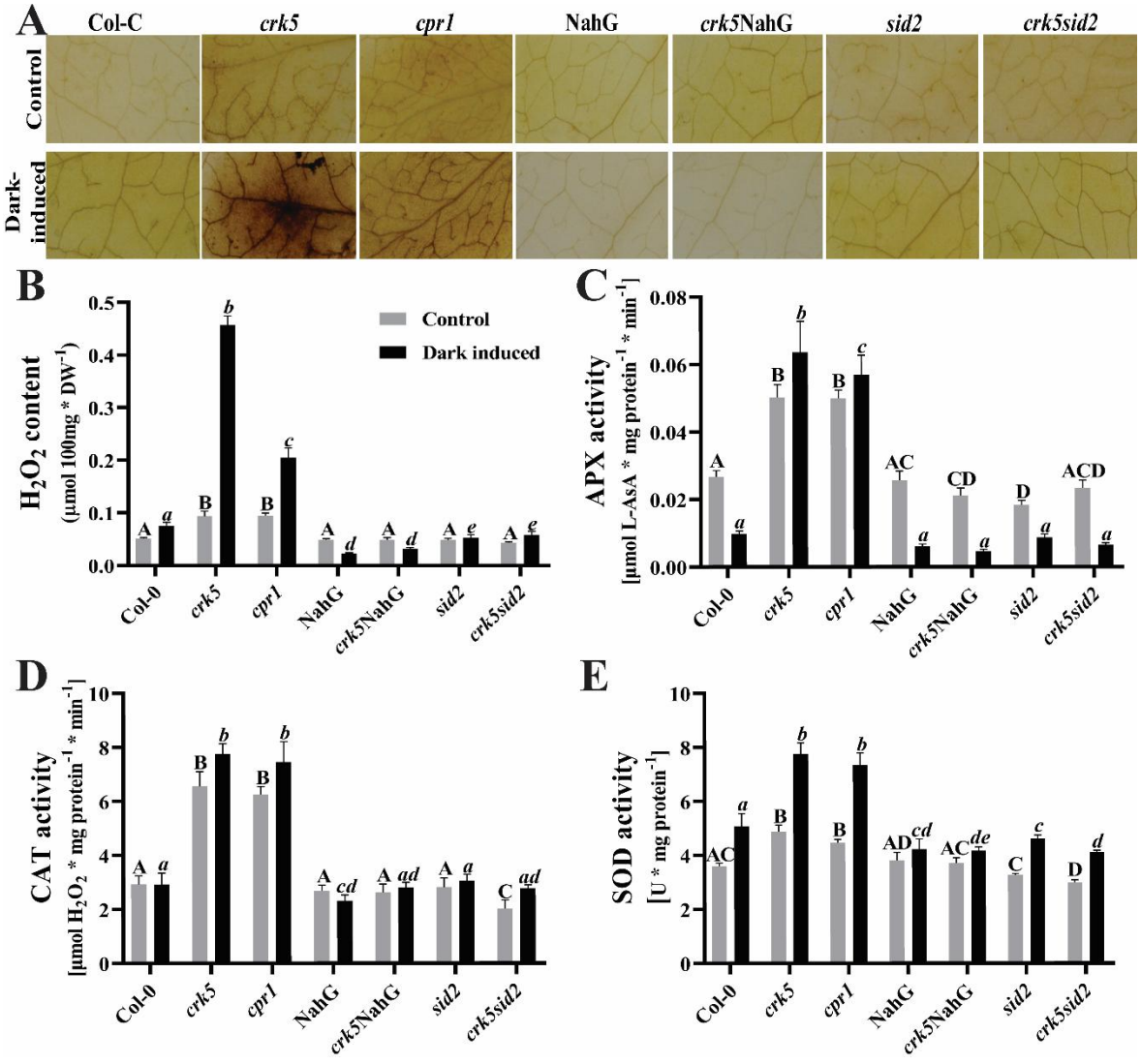


Figure 7. Foliar reactive oxygen species (ROS) levels and enzymatic ROS scavenging activity of wild-type, *crk5*, *cpr1*, and salicylic acid-synthesis dysfunction *sid2* mutant or salicylic acid-catabolism transgenic NahG line in control and after exposure for 4 days to darkness. 3,3'diaminobenzidine (DAB) staining showing H_2O_2 foliar levels (**A**), hydrogen peroxide (H_2O_2) foliar content (**B**), ascorbate peroxidase (APX) (**C**), catalase (CAT) (**D**), and superoxide dismutase (SOD) (**E**) foliar enzymatic activity. Mean values (±SD) were derived from 9 plants (n = 9). Statistical analysis was performed using a t-test at a significance level of $p < 0.05$. Letters A, B, C, D, and a, b, c, d, e above the bars indicate homogenous groups, and values sharing common labels (letters) are not significantly different from each other. Figure from article 3 (Kamran et al., 2026b).

Transcriptomic analysis further revealed that CRK5 controls senescence-, cell death-, and redox-related gene networks during DIS. Among all analyzed genotypes, *crk5* exhibited the most extensive transcriptional reprogramming under darkness, including

strong induction of senescence-associated genes, autophagy components, metacaspases, and redox-regulatory pathways. In contrast, the *cpr1* mutant, despite showing a visible similarity in phenotype to *crk5*, displayed comparatively limited transcriptional changes, highlighting CRK5 as a critical upstream modulator of SA-dependent gene expression. The near-complete restoration of Col-0 transcriptional patterns in *crk5sid2* and *crk5*NahG plants confirms that CRK5 controls these processes primarily through inhibition of SA signaling.

Overall, this study positions CRK5 as a central regulatory hub that integrates phytohormonal signaling with ROS homeostasis to delay senescence and prevent CD during DIS. By restraining SA-accumulation and its downstream oxidative burst, CRK5 preserves pigment stability, limits ROS overload, and prevents excessive activation of senescence and CD processes. These findings provide a mechanistic framework for understanding how CRK5 coordinates redox balance and phytohormonal cues during leaf aging and further explore SA-dependent regulation of senescence and stress-induced cell death. The article presents further elaboration, a more in-depth interpretation, and a discussion of these findings (Figure 8).

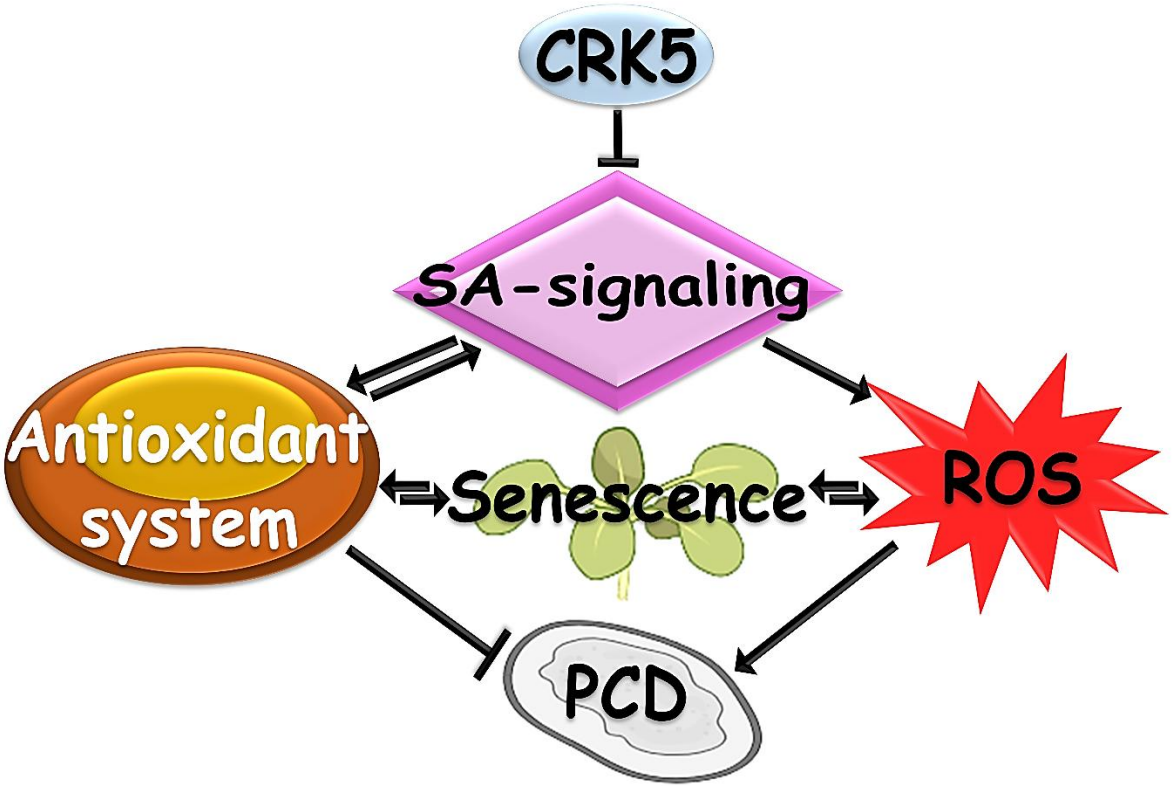


Figure 8. Schematic model illustrating the negative regulatory role of CRK5 in the salicylic acid signaling. The CRK5-driven inhibition of SA signaling coordinates hormonal, metabolic, and oxidative pathways to maintain leaf viability, providing mechanistic insight into the control of stress-induced senescence and cell death in Arabidopsis. Figure from article 3 (Kamran et al., 2026b).

5. Conclusions

Based on the research presented, **the following conclusions may be drawn:**

CRK5 negatively regulates senescence by inhibiting SA signaling. Loss of CRK5 leads to higher SA levels and premature leaf aging, whereas blocking SA synthesis or catabolism (*sid2* or NahG) restores normal senescence timing.

CRK5 promotes photo-protective heat dissipation and photosynthetic efficiency. CRK5 mutants (and high-SA line *cpr1*) exhibit reduced NPQ, lower foliar temperature (ΔT_{max}) under AEE stress, and impaired photosynthesis, indicating CRK5 is needed for effective dissipation of AEE as heat.

CRK5 acts specifically through the inhibition of SA but not ethylene signaling. The *crk5* mutant phenotypes are rescued by disrupting SA signaling but not by blocking ethylene signaling, demonstrating that CRK5's regulatory role in senescence and AEE dissipation as heat is mediated through inhibition of SA signaling.

CRK5 conditionally regulates leaf aging (CD) and AEE management. By preventing SA signaling, it enhances photosynthesis and photo-protection, thus conditionally optimizing cellular ROS homeostasis, growth, and development.

CRK5 preserves cellular antioxidant homeostasis and pigments content during stress. It conditionally optimizes carotenoid and xanthophyll pigment content and maintains balanced cellular ROS production.

CRK5 regulates gene expression by inhibiting SA and ROS signaling. This regulation involves WRKYs, metacaspases, senescence, and, photosynthetic-related genes and other metabolic stress-responsive factors.

CRK5 functions as important chloroplast retrograde signaling and nuclear anterograde responses regulator in acclimatory and defense responses.

6. References

Antolín-Llovera, M., Petutsching, E. K., Ried, M. K., Lipka, V., Nürnberger, T., Robatzek, S., & Parniske, M. (2014). Knowing your friends and foes–plant receptor-like kinases as initiators of symbiosis or defence. *New Phytologist*, *204*(4), 791–802.

Bernacki, M. J., Czarnocka, W., Rusaczonek, A., Witoń, D., Kęska, S., Czyż, J., Szechyńska-Hebda, M., & Karpiński, S. (2019). LSD1-, EDS1-and PAD4-dependent conditional correlation among salicylic acid, hydrogen peroxide, water use efficiency and seed yield in *Arabidopsis thaliana*. *Physiologia Plantarum*, *165*(2), 369–382.

Bourdais, G., Burdiak, P., Gauthier, A., Nitsch, L., Salojärvi, J., Rayapuram, C., Idänheimo, N., Hunter, K., Kimura, S., & Merilo, E. (2015). Large-scale phenomics identifies primary and fine-tuning roles for CRKs in responses related to oxidative stress. *PLoS Genetics*, *11*(7), e1005373.

Boyer, J. S. (1982). Plant productivity and environment. *Science*, *218*(4571), 443–448.

Burdiak, P., Mielecki, J., Gawroński, P., & Karpiński, S. (2022). The CRK5 and WRKY53 are conditional regulators of senescence and stomatal conductance in Arabidopsis. *Cells*, *11*(22), 3558.

Burdiak, P., Rusaczonek, A., Witoń, D., Głów, D., & Karpiński, S. (2015). Cysteine-rich receptor-like kinase CRK5 as a regulator of growth, development, and ultraviolet radiation responses in *Arabidopsis thaliana*. *Journal of Experimental Botany*, *66*(11), 3325–3337.

Chaomurilege, Miyagi, A., Ishikawa, T., Yamaguchi, M., Murayama, H., & Kawai-Yamada, M. (2023). Metabolic changes associated with dark-induced leaf senescence in Arabidopsis *nadk2* mutants. *Plant Signaling & Behavior*, *18*(1), 2215618.

Chen, K., Du, L., & Chen, Z. (2003). Sensitization of defense responses and activation of programmed cell death by a pathogen-induced receptor-like protein kinase in Arabidopsis. *Plant Molecular Biology*, *53*, 61–74.

Czarnocka, W., & Karpiński, S. (2018). Friend or foe? Reactive oxygen species production, scavenging and signaling in plant response to environmental stresses. *Free Radical Biology and Medicine*, *122*, 4–20.

Devireddy, A. R., Tschaplinski, T. J., Tuskan, G. A., Muchero, W., & Chen, J.-G. (2021). Role of reactive oxygen species and hormones in plant responses to temperature changes. *International Journal of Molecular Sciences*, *22*(16), 8843.

Fahad, S., Bajwa, A. A., Nazir, U., Anjum, S. A., Farooq, A., Zohaib, A., Sadia, S., Nasim, W., Adkins, S., & Saud, S. (2017). Crop production under drought and heat stress: Plant responses and management options. *Frontiers in Plant Science*, *8*, 1147.

Foyer, C. H., & Hanke, G. (2022). ROS production and signalling in chloroplasts: Cornerstones and evolving concepts. *The Plant Journal*, *111*(3), 642–661.

Górecka, M., Lewandowska, M., Dąbrowska-Bronk, J., Białasek, M., Barczak-Brzyżek, A., Kulasek, M., Mielecki, J., Kozłowska-Makulska, A., Gawroński, P., & Karpiński, S. (2020). Photosystem II 22kDa protein level-a prerequisite for excess light-inducible memory, cross-tolerance to UV-C and regulation of electrical signalling. *Plant, Cell & Environment*, *43*(3), 649–661.

Guo, Y., Ren, G., Zhang, K., Li, Z., Miao, Y., & Guo, H. (2021). Leaf senescence: Progression, regulation, and application. *Molecular Horticulture*, *1*(1), 5.

Hassan, M. U., Aamer, M., Chattha, M. U., Afzal, A., Batool, M., Ahmed, H. A. I., Guoqin, H., & Shahzad, B. (2022). Salicylic Acid-Mediated Physiological and Molecular Mechanisms in Plants Under Heat Stress. *Managing Plant Stress Using Salicylic Acid: Physiological and Molecular Aspects*, 163–182.

Hatfield, J. L., & Prueger, J. H. (2015). Temperature extremes: Effect on plant growth and development. *Weather and Climate Extremes*, *10*, 4–10.

Hiltscher, H., Rudnik, R., Shaikhali, J., Heiber, I., Mellenthin, M., Meirelles Duarte, I., Schuster, G., Kahmann, U., & Baier, M. (2014). The radical induced cell death protein 1

(RCD1) supports transcriptional activation of genes for chloroplast antioxidant enzymes. *Frontiers in Plant Science*, *5*, 475.

Ji, Y., Liu, J., & Xing, D. (2016). Low concentrations of salicylic acid delay methyl jasmonate-induced leaf senescence by up-regulating nitric oxide synthase activity. *Journal of Experimental Botany*, *67*(17), 5233–5245.

Karpiński, S., SZECHYŃSKA-HEBDA, M., Wituszyńska, W., & Burdiak, P. (2013). Light acclimation, retrograde signalling, cell death and immune defences in plants. *Plant, Cell & Environment*, *36*(4), 736–744.

Khan, M. I. R., Iqbal, N., Masood, A., Per, T. S., & Khan, N. A. (2013). Salicylic acid alleviates adverse effects of heat stress on photosynthesis through changes in proline production and ethylene formation. *Plant Signaling & Behavior*, *8*(11), e26374.

Li, M., & Kim, C. (2022). Chloroplast ROS and stress signaling. *Plant Communications*, *3*(1).

Liebsch, D., & Keech, O. (2016). Dark-induced leaf senescence: New insights into a complex light-dependent regulatory pathway. *New Phytologist*, *212*(3), 563–570.

Mateo, A., Funck, D., Mühlenbock, P., Kular, B., Mullineaux, P. M., & Karpinski, S. (2006). Controlled levels of salicylic acid are required for optimal photosynthesis and redox homeostasis. *Journal of Experimental Botany*, *57*(8), 1795–1807.

Minhas, P. S., Rane, J., & Pasala, R. K. (2017). Abiotic stresses in agriculture: An overview. *Abiotic Stress Management for Resilient Agriculture*, 3–8.

Mishra, N., Jiang, C., Chen, L., Paul, A., Chatterjee, A., & Shen, G. (2023). Achieving abiotic stress tolerance in plants through antioxidative defense mechanisms. *Frontiers in Plant Science*, *14*, 1110622.

Mittler, R., Zandalinas, S. I., Fichman, Y., & Van Breusegem, F. (2022). Reactive oxygen species signalling in plant stress responses. *Nature Reviews Molecular Cell Biology*, *23*(10), 663–679.

Miura, K., Okamoto, H., Okuma, E., Shiba, H., Kamada, H., Hasegawa, P. M., & Murata, Y. (2013). SIZ1 deficiency causes reduced stomatal aperture and enhanced drought tolerance via controlling salicylic acid-induced accumulation of reactive oxygen species in A rabidopsis. *The Plant Journal*, *73*(1), 91–104.

Mordi, R. C., Ademosun, O. T., Ajanaku, C. O., Olanrewaju, I. O., & Walton, J. C. (2020). Free radical mediated oxidative degradation of carotenes and xanthophylls. *Molecules*, *25*(5), 1038.

Morse, A. M., Tschaplinski, T. J., Dervinis, C., Pijut, P. M., Schmelz, E. A., Day, W., & Davis, J. M. (2007). Salicylate and catechol levels are maintained in nahG transgenic poplar. *Phytochemistry*, *68*(15), 2043–2052.

Muhlenbock, P., Szechynska-Hebda, M., Płaszczyca, M., Baudo, M., Mateo, A., Mullineaux, P. M., Parker, J. E., Karpinska, B., & Karpiński, S. (2008). Chloroplast signaling and LESION SIMULATING DISEASE1 regulate crosstalk between light acclimation and immunity in Arabidopsis. *The Plant Cell*, *20*(9), 2339–2356.

Paluch-Lubawa, E., Stolarska, E., & Sobieszczuk-Nowicka, E. (2021). Dark-induced barley leaf senescence–a crop system for studying senescence and autophagy mechanisms. *Frontiers in Plant Science*, *12*, 635619.

Peng, P., Li, R., Chen, Z.-H., & Wang, Y. (2022). Stomata at the crossroad of molecular interaction between biotic and abiotic stress responses in plants. *Frontiers in Plant Science*, *13*, 1031891.

Petrov, V., Hille, J., Mueller-Roeber, B., & Gechev, T. S. (2015). ROS-mediated abiotic stress-induced programmed cell death in plants. *Frontiers in Plant Science*, *6*, 69.

Radojičić, A., Li, X., & Zhang, Y. (2018). Salicylic acid: A double-edged sword for programed cell death in plants. *Frontiers in Plant Science*, *9*, 1133.

Rao, M., Duan, M., Zhou, C., Jiao, J., Cheng, P., Yang, L., & Zheng, B. (2025). *Antioxidant defense system in plants: Reactive oxygen species production, signaling, and scavenging during abiotic stress-induced oxidative damage. Horticulturae 11 (5): 477*.

Rivas-San Vicente, M., & Plasencia, J. (2011). Salicylic acid beyond defence: Its role in plant growth and development. *Journal of Experimental Botany*, *62*(10), 3321–3338.

Rogers, H., & Munné-Bosch, S. (2016). Production and scavenging of reactive oxygen species and redox signaling during leaf and flower senescence: Similar but different. *Plant Physiology*, *171*(3), 1560–1568.

Rosenwasser, S., Rot, I., Sollner, E., Meyer, A. J., Smith, Y., Leviatan, N., Fluhr, R., & Friedman, H. (2011). Organelles contribute differentially to reactive oxygen species-related events during extended darkness. *Plant Physiology*, *156*(1), 185–201.

Ruban, A. V. (2016). Nonphotochemical chlorophyll fluorescence quenching: Mechanism and effectiveness in protecting plants from photodamage. *Plant Physiology*, *170*(4), 1903–1916.

Rushton, P. J., Somssich, I. E., Ringler, P., & Shen, Q. J. (2010). WRKY transcription factors. *Trends in Plant Science*, *15*(5), 247–258.

Sangwan, S., Shameem, N., Yashveer, S., Tanwar, H., Parray, J. A., Jatav, H. S., Sharma, S., Punia, H., Sayyed, R., & Almalki, W. H. (2022). Role of salicylic acid in combating heat stress in plants: Insights into modulation of vital processes. *Frontiers in Bioscience-Landmark*, *27*(11), 310.

Szechyńska-Hebda, M., Kruk, J., Górecka, M., Karpińska, B., & Karpiński, S. (2010). Evidence for light wavelength-specific photoelectrophysiological signaling and memory of excess light episodes in Arabidopsis. *The Plant Cell*, *22*(7), 2201–2218.

Szechyńska-Hebda, M., Lewandowska, M., Witoń, D., Fichman, Y., Mittler, R., & Karpiński, S. M. (2022). Aboveground plant-to-plant electrical signaling mediates network acquired acclimation. *The Plant Cell*, *34*(8), 3047–3065.

Vij, S., & Tyagi, A. K. (2007). Emerging trends in the functional genomics of the abiotic stress response in crop plants. *Plant Biotechnology Journal*, *5*(3), 361–380.

Wilby, R., Whitehead, P., Wade, A., Butterfield, D., Davis, R., & Watts, G. (2006). Integrated modelling of climate change impacts on water resources and quality in a lowland catchment: River Kennet, UK. *Journal of Hydrology*, *330*(1–2), 204–220.

World Health Organization. (2022). *The state of food security and nutrition in the world 2022: Repurposing food and agricultural policies to make healthy diets more affordable* (Vol. 2022). Food & Agriculture Org.

Wrzaczek, M., Brosché, M., Salojärvi, J., Kangasjärvi, S., Idänheimo, N., Mersmann, S., Robatzek, S., Karpiński, S., Karpińska, B., & Kangasjärvi, J. (2010). Transcriptional regulation of the CRK/DUF26 group of receptor-like protein kinases by ozone and plant hormones in Arabidopsis. *BMC Plant Biology*, *10*, 1–19.

Zarrin Ghalami, R., Duszyn, M., Kamran, M., Burdiak, P., Gawronski, P., & Karpinski, S. (2025). Transpiration, Photoinhibition and Non-photochemical Quenching Reciprocally Control Foliar Heat Emission. *bioRxiv*, 2025–05.

Zhang, H., Zhao, Y., & Zhu, J.-K. (2020). Thriving under stress: How plants balance growth and the stress response. *Developmental Cell*, *55*(5), 529–543.

Zhang, H., Zhu, J., Gong, Z., & Zhu, J.-K. (2022). Abiotic stress responses in plants. *Nature Reviews Genetics*, *23*(2), 104–119.

Zhao, J., Sun, Y., Li, X., & Li, Y. (2022). CYSTEINE-RICH RECEPTOR-LIKE KINASE5 (CRK5) and CRK22 regulate the response to *Verticillium dahliae* toxins. *Plant Physiology*, *190*(1), 714–731.

Zurbriggen, M. D., Hajirezaei, M.-R., & Carrillo, N. (2010). Engineering the future. Development of transgenic plants with enhanced tolerance to adverse environments. *Biotechnology and Genetic Engineering Reviews*, *27*(1), 33–56.

7. Articles comprising the doctoral thesis

Article 1: Kamran, M., Burdiak, P., & Karpiński, S. (2025a). Crosstalk Between Abiotic and Biotic Stresses Responses and the Role of Chloroplast Retrograde Signaling in the Cross-Tolerance Phenomena in Plants. Cells, 14(3), 176. https://doi.org/10.3390/cells14030176.

(Ministerial score: 140; IF: 5.2)

cells

MDPI

Review

Crosstalk Between Abiotic and Biotic Stresses Responses and the Role of Chloroplast Retrograde Signaling in the Cross-Tolerance Phenomena in Plants

Muhammad Kamran, Paweł Burdiak and Stanisław Karpiński *

Department of Plant Genetics, Breeding and Biotechnology, Institute of Biology, Warsaw University of Life Sciences, Nowoursynowska 159, 02-776 Warsaw, Poland; muhammad_kamran@sggw.edu.pl (M.K.); pawel_burdiak@sggw.edu.pl (P.B.)

* Correspondence: stanislaw_karpinski@sggw.edu.pl

Abstract: In the natural environment, plants are simultaneously exposed to multivariable abiotic and biotic stresses. Typical abiotic stresses are changes in temperature, light intensity and quality, water stress (drought, flood), microelements availability, salinity, air pollutants, and others. Biotic stresses are caused by other organisms, such as pathogenic bacteria and viruses or parasites. This review presents the current state-of-the-art knowledge on programmed cell death in the cross-tolerance phenomena and its conditional molecular and physiological regulators, which simultaneously regulate plant acclimation, defense, and developmental responses. It highlights the role of the absorbed energy in excess and its dissipation as heat in the induction of the chloroplast retrograde phytohormonal, electrical, and reactive oxygen species signaling. It also discusses how systemic- and network-acquired acclimation and acquired systemic resistance are mutually regulated and demonstrates the role of non-photochemical quenching and the dissipation of absorbed energy in excess as heat in the cross-tolerance phenomenon. Finally, new evidence that plants evolved one molecular system to regulate cell death, acclimation, and cross-tolerance are presented and discussed.

Keywords: absorbed energy in excess; electrical signaling; reactive oxygen species; phytohormones; hypersensitive response; systemic acquired resistance; acclimation; non-photochemical quenching; cross-tolerance

Academic Editor: Sara Rinalducci

Received: 27 November 2024
Revised: 14 January 2025
Accepted: 21 January 2025
Published: 23 January 2025

Citation: Kamran, M.; Burdiak, P.; Karpiński, S. Crosstalk Between Abiotic and Biotic Stresses Responses and the Role of Chloroplast Retrograde Signaling in the Cross-Tolerance Phenomena in Plants. *Cells* **2025**, *14*, 176. https://doi.org/10.3390/cells14030176

1. Introduction

One of the most significant challenges of the twenty-first century is feeding a growing world population while dealing with an overheated planet and progressive desertification of fertile land. Climate extremes are becoming more common, disrupting agricultural production and posing continuous dangers of starvation [1,2]. It was estimated that more than 800 million people worldwide did not have access to enough food in 2021 [1,3]. Global warming is anticipated to increase the frequency of extreme weather, endangering agricultural crop production [4]. According to calculations, the desert area will increase from 3 to 10% of the total land area [5]. Crucial agricultural crops experience an overall yield loss of about 70% due to adverse conditions in the fields and from the environment. This means only 30% of the yield was produced compared to its yield from genetic potentials [6–10]. Growing demand for crop production and maintaining yield in adverse global warming conditions necessitates vastly expanded plant stress resistance research initiatives.

The frequency and severity of several environmental stresses, such as absorbed energy in excess (AEE), ultraviolet (UV) radiation, severe droughts, and heat waves, increase due to global warming. When taken as a whole, these stressors cause significant losses in agricultural productivity. Stress response phytohormones and signaling molecules, including ethylene (ET), salicylic acid (SA), abscisic acid (ABA), jasmonic acid (JA), and reactive oxygen species (ROS), have been widely described in terms of the regulation of plant stress responses and productivity. During heat and osmotic stress response, these factors and electrical signaling regulate a mechanism known as programmed cell death (PCD), an eventual completion of the cell cycle. Induced PCD in some cells is required for effective stress response, leading to the acclimatization or induction of disease resistance, thus optimizing plant survival and production under stress conditions [11–20]. Systemic- and network-acquired acclimation (SAA and NAA) processes of chloroplast retrograde signaling happening within and between plants are essential for the induction of PCD [11,12,21–23]. It is important to know that stress response to episodes of AEE stress applied to low light acclimated plants can induce disease resistance to the virulent bacterial pathogen and better tolerance to UV-C irradiation episodes due to the induction of the cellular light memory [22,24]. This memory involves molecular, physiological, biochemical, and biophysical changes that last several days after the stress of absorption of energy in excess (AEE) and is specific to the quantity and quality of AEE. Therefore, the current knowledge and understanding of the cross-tolerance phenomena, plant stress responses (SAA, SAR, NAA), and PCD signaling mechanisms is much better than a decade ago. The knowledge on the role of conditional PCD regulators, such as LESION SIMULATING DISEASE 1 (LSD1), ENHANCED DISEASE SUSCEPTIBILITY 1 (EDS1), and CYSTEINE-RICH RLK (RECEPTOR-LIKE PROTEIN KINASE) 5 (CRK5), METACASPASES (MC4 and MC8), SIGNAL RECOGNITION PARTICLES (cpSRP43 and 54), 22 kDa photosystem II protein (PsbS), Ca^{2+} and other ion channels, transcription factors (WRKY, DREB—CBF2 subfamily A-1 of environment RF/AP2), ROS scavenging/generating enzymes, and others involved in the regulation of NAAs, SAAs, and SAR, has been increased recently [11–16,20–24]. This review contributes to the further understanding of the systemic- and network-acquired acclimation (SAA and NAA) and the cross-tolerance phenomenon. We present and discuss the role of reactive oxygen species (ROS), the redox status of the plastoquinone pool (PQ), phytohormones, electrical signals (ESs), and non-photochemical quenching (NPQ) waves propagated between cells, leaves, and between different plants in the signaling of cross-tolerance phenomenon. The role of transcription factors (TFs) and other proteins as components of the secondary messenger system activated by these complex waves is also presented. Finally, the holistic picture of induced cross-tolerance that makes the plants capable of conditionally optimizing resilience and yield in multivariable environments is presented.

2. Abiotic and Biotic Stresses

In the classical view, environmental stress factors can be classified into two major categories: abiotic and biotic stresses. Plants are constantly exposed to biotic and abiotic stresses, significantly impacting their growth, development, and overall fitness [6,12,25–28]. Abiotic stresses include factors such as changes in temperature extremes (low or high), light intensity and quality (low light, high light, red/blue/UV ratio), water and osmotic stress, heavy metal toxicity (i.e., Hg, Cd, Pb), and nutrient deficiency (organic/inorganic). In the natural environment, abiotic stresses are usually clustered; for example, a shift from low to high light intensity is associated with a sudden increase in UV radiation and an immediate foliar temperature increase due to absorbed energy in excess and its immediate dissipation as heat via the non-photochemical quenching (NPQ) mechanism [29].

This usually can change plant physiology and biochemistry (metabolism), leading to altered retrograde signaling [21,22,24] and changes in gene expression. As a result of NPQ induction, plants increase foliar heat production from photosystems and inhibit stomatal conductance, leading to impaired foliar cooling (transpiration), decreased CO_2 assimilation, water and nutrient uptake, and induced photorespiration [17,19,30–47]. Mittler et al. (2022) [20] discussed the influence of various abiotic stress factors on plant signaling networks, highlighting the role of ROS and stress-responsive genes in stress perception and adaptation.

Abiotic stresses, such as high light, heat, and drought, often combine and are difficult to separate. AEE alone can induce heat shock response due to the higher dissipation of AEE as heat by non-photochemical quenching (NPQ) [29], and *Arabidopsis thaliana* subjected to a combination of high light and heat stress exhibit a unique metabolic response, including an increased accumulation of sugars and amino acids, as well as decreased levels of metabolites participating in the tricarboxylic acid (TCA) cycle [48]. The combined stress in *Arabidopsis* also leads to the accumulation of non-proteinogenic amino acid γ-aminobutyric acid (GABA), which contributes significantly to plants' adaptation to intense light and heat stress, possibly via encouraging autophagy [49]. In *Arabidopsis*, the combination of heat and drought stress led to changes in plant metabolomics and root bacterial microbiota, indicating an orchestrated modulation of the whole holobiont [50]. Wheat crops also experience combined heat and drought stress, which has more detrimental effects on growth and yield than individual stresses. Wheat has developed advanced responses at various levels to tolerate these combined stressors [51]. Due to drought and heat stress, cell wall suberization rises in *Arabidopsis* roots, leading to alterations to the biosynthesis and assembly of primary cell wall components [52].

Biotic stresses, arising from interactions with living organisms, pose significant threats to plant health and agricultural productivity [53,54]. These stressors include pathogens, pests, herbivores, and others, which can cause diseases, nutrient deficiency, physical damage, and induce defense responses in plants [55–62]. Plants have evolved intricate mechanisms to detect and respond to pathogens—a two-branched innate immune system. It means recognition and responsiveness to the molecules of microbes, including non-pathogenic ones. Secondly, they react to the pathogen virulence factors directly or through their effects on host targets [63–67]. Plants do not have mobile "killer" cells (e.g., T4 cells) and a somatic adaptive immune system like animals. Instead, they rely on the innate immunity of each cell, mediated by gene pairs (R genes in plants and avr genes in pathogens) that induce programmed cell death in infected and noninfected cells, known as the hypersensitive disease defense response (HR) and systemic acquired resistance [63–67].

An oxidative burst, manifested by rapid ROS generation, is typically present in conjunction with R gene-mediated resistance and is necessary for HR, a PCD believed to restrict pathogen access to nutrients and water. Stimulating SA-dependent signaling pathways that develop particular pathogenesis-related (PR) proteins is also linked to R gene-mediated resistance. Some defensive mechanisms in plants are regulated by systems that rely on ET and/or JA. Different classes of R proteins, such as those with nucleotide-binding (NB) domains and leucine-rich repeats (LRRs), play crucial roles in basal defense regulation and the resistance to various pathogens, showcasing the diversity of plant immune responses [64,68–70].

Plant pathogens are frequently classified as either necrotrophs or biotrophs based on their life cycle and how they propagate. While necrotrophs live on dead host tissues, biotrophs need live host tissue to propagate [71]. It is interesting to see how SA signaling and R gene-mediated resistance may lead to resistance to avirulent biotrophs [72]. Such pathogens would have no feeding supply due to the HR response due to PCD induction.

However, in the case of necrotrophs, the pathogen would have an easier time existing in the host due to cell death [68]. Studies screening *Arabidopsis* mutants for deficiencies in resistance to different diseases using defective signaling pathways critical in defense gave some evidence to it, like resistance to the necrotrophic fungus *Alternaria brassicicola* is unaffected by the mutation *npr1* and the transgene NahG, which disrupts SA signaling. However, they cause resistance to the biotrophic oomycete *Peronospora parasitica* to be abolished. On the other hand, resistance to the necrotrophic fungus *A. brassicicola* is significantly compromised by the *coi1* mutation, which disrupts JA signaling, whereas resistance to *P. parasitica* is unaffected. These findings showed that plant defensive mechanisms may be modified depending on the pathogen invading, with SA-dependent defenses targeting biotrophs and JA- and ET-dependent responses targeting necrotrophs [68,73–75].

3. Cross-Tolerance

When plants are exposed to one kind of stress, it can trigger shared signals and pathways, which increases their ability to withstand other types of stress. This process is called cross-tolerance and is associated with increased adaptive fitness [17,21,26,76–78]. It can be accomplished by co-activating the plant's innate immune system, which involves a network of non-specific stress-responsive pathways that bridge biotic–abiotic stress borders. Heat, chilling, drought, and salt stress are frequent abiotic stressors with cross-tolerance effects [79,80]. Different stress signaling networks also interact, which might cause plants to develop cross-tolerance. For instance, *Epichloë* endophyte-infected grasses exhibit more excellent resistance to abiotic stressors like drought through increased root biomass and high stomatal conductance with rich accumulation of solutes for osmotic regulation, to cold by the upregulation of unsaturated fatty acids, to salinity by keeping anatomical changes (like increased xylem, phloem, and vascular bundles size), and to pathogen infections by increasing antioxidants and phenolic compounds that are known to be important for plant defenses [81,82]. Capiati et al. (2006) [83] showed that wounding boosts tomato plants' resistance to salt through a mechanism involving the systemins and JA. They showed that calmodulin-like activities are necessary for the downstream signaling events that lead to cross-tolerance between wounding and salt stress. Lima et al. (2018) [84] evaluated cross-tolerance induced by heat stress and water stress in common beans and observed improved germination under osmotic stress following heat stress. These findings suggest that agricultural plants may be engineered or bred to resist many abiotic or biotic stresses [15,26,42].

Cross-tolerance involves chloroplast, mitochondrial retrograde, and nuclear anterograde signaling, which are involved in intra- and intercellular communication between chloroplasts and the nucleus. This signaling pathway is mediated by the redox signal, which regulates the expression of the chloroplast- and nuclear-encoded genes required for appropriate stress responses [11,19–24,26,42,64,77,78,85,86]. The WHIRLY family of proteins and the REDOX-RESPONSIVE TRANSCRIPTION FACTOR 1 (RRTF1) have been identified as potential mediators of chloroplast-to-nucleus retrograde signaling, leading to cross-tolerance [11,19–24,26,42,64,77,78,85,86]. Hydrogen peroxide (H_2O_2), a byproduct of various aerobic pathways, has also been implicated in retrograde signaling and cross-tolerance induction [86]. The chloroplast acts as an environmental sensor and communicates with other cell compartments through retrograde signaling to regulate nuclear gene expression in response to developmental cues and stresses by Ca^{2+} and ROS [11,19–24,26,85,87,88].

On the other hand, retrograde signaling is also essential for mitochondria biogenesis and stress responses. Nevertheless, further research is required to determine if mitochondria and chloroplasts trigger distinct signaling pathways or if they merge into one [88]. The second option is probable given that the energy, metabolism, and redox state are

closely linked to their functioning [89–91]. Nonetheless, mitochondrial retrograde communication is believed to be predominant in non-photosynthetic tissues. This theory was supported by the finding that the overexpression of TF ANAC013 in *Arabidopsis* increased the chloroplast's resistance to photooxidative stress [92,93]. Other reports showed that a strong unfolded protein response (UPR^{mt}) is induced by either mitochondrial translation or protein import. In this process, a quick oxidative burst starts the retrograde signal that activates MPK6 and causes a systemic hormone response that is primarily dependent on ethylene signaling but also involves auxin and JA [94]. As a result, an anterograde response is activated, which assists plants under mitochondrial proteotoxic stress conditions by repairing mitochondrial translation through mitochondrial ribosomal proteins (MRPs) and mitochondrial protein import/folding. However, in this review, mostly chloroplast retrograde signaling is discussed.

The mechanism by which plant chloroplasts sense stress stimuli is the quickest process that happens within pico- or nanoseconds (e.g., AEE dissipation as heat and fluorescence, singlet oxygen production, absorbed energy transfer, electron transfer in photosystem II) (Figure 1). Redox reactions in photosystems, such as electrical charge separation, pH gradient imbalances across thylakoid membranes, or the stimulation of the xanthophyll cycle through NPQ, can be triggered by AEE [77,95]. On an interval of microseconds to seconds, ROS deposits can impact hormones, sugars, ion signaling pathways, and the redox state of the PQ and glutathione/ascorbate pools (Figure 1). Specific regulatory proteins, such as CRK5 and MITOGEN-ACTIVATED PROTEIN KINASE 4

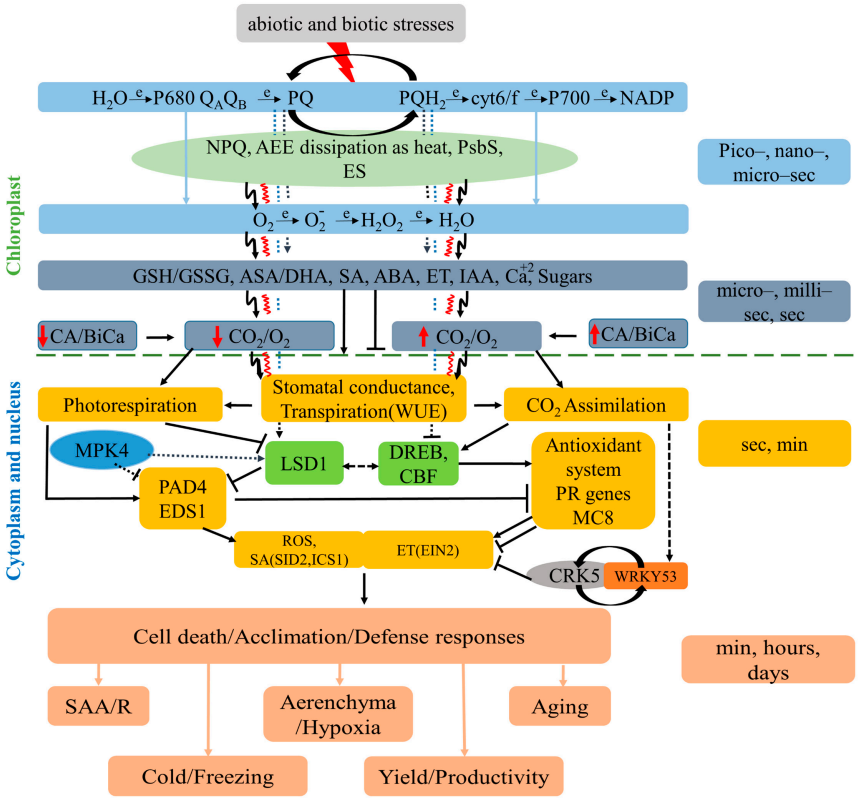


Figure 1. Model of the chloroplast retrograde signaling pathways that regulate cell death (CD) and integrate immune defenses and plant acclimation responses—cross-tolerance to biotic and abiotic stresses. The red lighting-like symbol on the top demonstrates the absorption of energy in excess (AEE) required for electrical charge separation in P680. AEE induces the overproduction of electrons and protons, and thus in non-photochemical quenching (NPQ), heat, and electrical signaling waves.

The excess of electrons leads to increased reactive oxygen species (ROS) production and a higher reduction in the plastoquinone (PQ) pool. This triggers redox changes in the stroma in ascorbate, glutathione, and other redox pair component pools, which regulate carbonic anhydrases (CA) activities, and thus, the CO_2/O_2 ratio near RuBisCO and sugar production. This, in turn, triggers phytohormone precursor synthesis and photorespiration as a photosynthetic electron sink, which further increases ROS production in peroxisomes and mitochondria and regulates stomatal conductance and programmed cell death (PCD). Chloroplast stroma can be directly connected via stormless with the nucleus, and ES-, ROS-, heat-, and other-signaling molecules or dual transcription factors can be directly transduced to the nucleus and specifically alter nuclear-encoded gene expression and cross-tolerance to abiotic and biotic stresses. This happens in most foliar cells, but some cells induce PCD when the chloroplast outer membrane is disintegrated due to AEE, heat, salicylic acid (SA), and ethylene (ET) signaling. Red wavy-like and black lightning-like arrows show heat and electrical signaling waves, respectively. Solid lines show the confirmed regulatory signaling pathways, while dashed lines show hypothetical signaling pathways. Modified version from Karpiński and colleagues (2014) [11] based on new data [15–24,41,42,78,89–96].

(MPK4) regulate stomatal closing within seconds, leading to enhanced photorespiration, triggering ROS generation, and ethylene synthesis [41,96] (Figure 1). This is followed by LSD1-driven regulation, acclimation, and defense reactions and PCD by limiting ROS and ET production [11,17,23,96]. These responses are essential for conditionally optimal plant photosynthesis, transpiration, light acclimation, defense actions, and plant health (Figures 1 and 2) [15–17,22–24,96,97]. Systemic- and network-acquired acclimation (SAA and NAA) and stress memory serve as "naive" non-acclimated plant cells, chloroplasts, and a PSII training process. Peak et al. (2004) [98] suggested that foliar stomata conductance and chlorophyll fluorescence are regulated by the cellular automaton algorithm that relies on collective dynamics and emergent, distributed biological computation in the leaves of plants that, on the contrary, regulates chlorophyll *a* fluorescence and stomata conductance. Later on, studies suggested that ES-, ROS- and NPQ-wave-like-changes propagated in SAA and NAA could be a process regulated in an identical manner like Peak et al. (2004) suggested [22–24,89] (Figures 1 and 2). Plant cells can selectively memorize AEE episodes and the spectral quality of light (either physiologically or molecularly) [22–24]. Overall, the interplay between chloroplast retrograde NPQ- and PQ-dependent signaling and other cellular signaling pathways is crucial for the plant's ability to acclimate and defend, and thus, for cross-tolerance.

Peak et al. (2004) [98] presented confirmation for computation in plans regulating foliar chlorophyll *a* fluorescence and stomatal conductance. In short, under some conditions, stomatal conductance becomes synchronized, and foliar regions with lower chlorophyll *a* fluorescence have higher conductance while regions with higher fluorescence have lower conductance. This regulation can be described by a mathematical cellular automata algorithm [98]. This communication network is controlled and regulated by wavy-like, discrete, and spatially in-time electrical- and ROS-signaling and NPQ changes that depend on the PsbS protein level [22–24,95,99], proton motive force, and chlorophyll *a* fluorescence decay time changes [95]. This hardware controls the fate of absorbed energy in photosystems, thus controlling chloroplast retrograde signaling across the whole plant and between plants. As a result, it discreetly and spatially controls stomatal conductance values over time, causing further NPQ wave-like adjustments, thus regulating AEE dissipation as heat [24,95,99,100] (Figure 1).

Górecka et al. (2020) [24] showed that prior induction of the cellular light memory in response to AEE episodes developed cross-tolerance to subsequent UV-C episodes in a PsbS-dependent manner. Photosystem II subunit S (PsbS) is an essential protein in plants that regulates NPQ and thus regulates the balance between absorbed energy dissipated as heat and is required for photochemistry [24,100–102]. In this study, the *npq4-1* mutant

lacking functional PsbS protein and overexpressing PsbS gene in transgenic *Arabidopsis* line (oePsbS) responded differently to an episode of AEE and in the induction of the cellular light memory and subsequent induction of cross-tolerance to UV-C. After the AEE episode, wild-type and oePsbS but not *npq4-1* plants induced light memory and subsequent cross-tolerance to UV-C. The study revealed a new, significant function for PsbS and NPQ as regulators of chloroplast retrograde signaling and UV cross-tolerance [24].

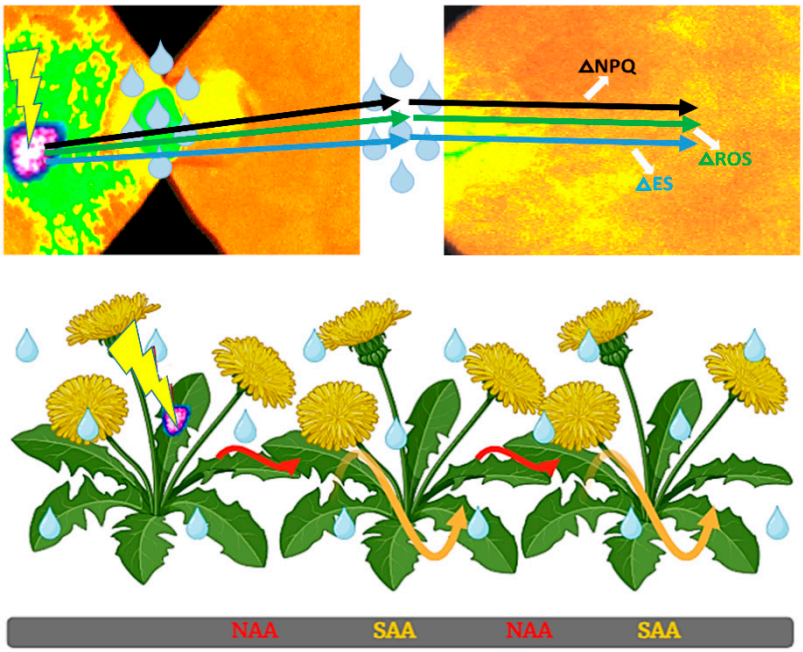


Figure 2. Systemic and network communication of non-photochemical quenching (NPQ) changes between photosystem II and mesophyll cells within and between plants. Electrical signaling (ES) and reactive oxygen species (ROS) waves can induce wavy-like changes in the NPQ value within a chloroplast, between all chloroplasts in the cell, in a whole leaf, in all leaves of a plant, and within the community-dwelling plants, such as the dandelion or *Arabidopsis*, when leaves of neighboring plants are in physical contact [18–24]. ES and ROS waves transmitted from stressed cells across the whole plant and between plants regulate NPQ- and plastoquinone pool-dependent chloroplast retrograde signaling to induce systemic-acquired acclimation (SAA) (yellow-wavy arrows) in a plant and network-acquired acclimation (NAA) (red-wavy arrows) between plants. Physical contact and electrical conductivity (e.g., a drop of water or high relative humidity) are necessary for NAA. In both transmitter and receiver plants, ES causes spatiotemporal variations in energy quenching (NPQ) (solid black line), the following induction of the ROS wave (solid green line), and electrical signaling (ES) (solid blue line). The yellow lighting-like symbol indicates the stress stimuli. Water droplets show the humidity. Interdependent ion fluxes in the plants cause its variable amplitude. (Model modified from Szechyńska-Hebda et al. (2022) [23]).

Systemic acquired acclimation (SAA) is an essential light acclimatory molecular and physiological process that depends on chloroplast retrograde signaling and NPQ [22–24,95,99,103]. Ciszak et al. (2015) [95] studied the time-resolved fluorescence of chlorophyll a in the *npq4-1* mutant in leaves exposed to excess light and in leaves undergoing SAA in ambient light conditions. In this study, leaves undergoing SAA in ambient low light and leaves directly exposed to AEE showed the regulation of fluorescence decay (FD). Wild-type *Arabidopsis* leaves exposed to AEE had a significantly shorter FD time than the control leaves and leaves undergoing SAA in ambient low light. However, SAA leaves showed a more minor but significant decrease in FD time compared to the control low-light leaves. These findings suggest that SAA signaling regulates the quantum-molecular properties of the supramolecular complex of photosystem II and that PsbS-dependent

light memory processing is necessary for the regulation of SAA [95]. It is important to point out that the synthetic (transgenic) improvement of NPQ and its relaxation in tobacco and soybean lead to an improvement of biomass and seed yield productivity in field conditions [101,102].

Electrical signaling (ES) plays a crucial part in cross-tolerance development. It was reported that when a single dandelion leaf is subjected to punctual wounding or high light stress, it generates a surface foliar electrical signal that then spreads to the nearby in-contact plants [23]. This can cause systemic NPQ and physiological alterations in both plants [23,99]. This electrical signaling can be transmitted from plant to plant in a network of plants connected successively by physical touch. This new physiological phenomenon was called network-acquired acclimation (NAA) [23]. These findings strongly imply that the transmission of direct surface electrical signals between the receiver plant (leaves) and the stressed host plant (or one leaf) results in discrete and spatial changes in the absorbed energy fate (NPQ) as well as the induction of common retrograde signaling for defense responses and acclimatization in the host plant and the entire plant community (Figure 2) [23].

4. Signaling Networks During Biotic and Abiotic Stresses

Abiotic factors are more detrimental to crop yield when they occur simultaneously [26,104,105]. It is well known that factors such as drought, heat waves, and salinity affect the development and spread of diseases and insects [104,106–111], as well as plant–pest interactions [107]. Many weeds use water more effectively than crops; in that case, abiotic stress, like drought, can favor these interactions [104,109,112]. The cumulative impact of stressors on crops is not necessarily positive since the final result is often determined by the interplay between these factors [113–117] (Table 1).

Crosstalk between abiotic and biotic stresses occurs on physiological and molecular levels. Reduced photosynthesis, decreased water use efficiency (WUE), and changes in stomatal opening are affected by biotic stresses but are essential for a plant's ability to withstand abiotic stress [118–120]. For instance, it was revealed that Na^+ and Cl^- levels in *Phaseolus vulgaris* shoots increased in salinity after being exposed to a root pathogen [118,121]. ABA signaling, which coordinates plant adaptation to abiotic stress, can be diminished by SA signaling, which is generated following invasion with *Pseudomonas syringae* pv. *tomato* [122], whereas ABA synthesis and signaling depend on zeaxantin, a direct precursor of ABA synthesis triggered by NPQ changes in the chloroplast. In another study, HopAM1, a type III effector of *Pseudomonas syringae* that targets HSP70, is involved in the stress-induced closure of stomata in an ABA-dependent manner [123,124]. In *Arabidopsis*, the HopAM1 overexpressed lines enhance the sensitivity toward ABA for stomatal closure and germination arrest [125]. Tolerance to abiotic stress such as drought and freezing can potentially result from plant–microbe interactions, like exposing plants to a viral strain, which includes *tobacco rattle virus* (TRV), *tobacco mosaic virus* (TMV), *cucumber mosaic virus* (CMV), and *brome mosaic virus* (BMV). The infection of these strains is beneficial against drought stress. On the other hand, the CMV strain works in freezing stress in beet crops due to elevated levels of antioxidants and osmoprotectants [118,126,127]. By interacting with hormonal processes, maintaining water and source–sink relationships, and increasing plant vigor under stress, fungi such as fungal endophytes, non-pathogenic rhizobacteria, and mycorrhizal fungi can benefit plants [118,128–130].

Research of the impacts of the external use of compounds that activate the plant defense mechanism known as priming provides more evidence for the overlap between abiotic and biotic stress signaling pathways [131]. In *Arabidopsis*, increased resilience to heat, drought, and salt stresses, as well as increased resistance to both biotrophic and

necrotrophic fungi, was found when β-aminobutyric acid (β-ABA), SA, and jasmonates were applied [118,132]. Hydrogen peroxide H_2O_2 and nitric oxide NO priming for salt tolerance moderately increased the abundance of oxidized and *S*-nitrosylated proteins, which remained relatively similar after applying stress. Non-treated plants were more sensitive and exhibited increased protein carbonylation, oxidation, and higher antioxidant enzyme activity [133,134]. In cauliflower seedlings, the enrichment of H_2O_2 and superoxide anion is due to the pre-treatment of H_2O_2, which enhances the MDA content. This pre-treatment of H_2O_2 produces changes in antioxidant systems, which include enzymatic (SOD, CAT, GPX, and APX) and non-enzymatic systems (AsA, GSH, and proline) [135]. In durum wheat seeds primed with H_2O_2, resistance against salinity stress is induced by increasing enzymatic and non-enzymatic antioxidant defense systems [136]. Recently, epigenetic modifications, specifically chromatin-regulated gene activation, have been proposed to govern priming responses [137] (Table 1).

Table 1. Factors, phytohormones, and chemical compounds that can trigger cross-tolerance in plants.

Factors	Enhanced Resistance Against	Mechanisms	Examples	References
Drought	Biotic Stress (Weeds)	Drought favors plants with efficient water usage, indirectly suppressing weeds' advantage over crops.	Weeds are more competitive under drought, altering crop–weed dynamics.	[104,109,112]
Salinity (Na^+ and Cl^-)	Root Pathogen (*Macrophomina phaseolina*)	Salinity exposure strengthens resistance mechanisms in infected plants.	*Phaseolus vulgaris* exposed to salinity and *M. phaseolina* shows increased Na^+ and Cl^- accumulation in shoots.	[118,121]
Abscisic Acid (ABA)	Pathogen Infection (*Pseudomonas syringae*)	ABA signaling enhances tolerance through stomatal regulation and water-use efficiency.	*P. syringae* triggers ABA-mediated stomatal closure to limit water loss and pathogen entry.	[123–125]
SA and Jasmonates	Heat, Drought, and Salt Stress	Defense priming activates stress-related pathways and antioxidant systems.	β-aminobutyric acid (β-ABA), SA, and jasmonates increase tolerance to abiotic stresses.	[118,132]
H_2O_2 Priming	Salt Stress	Enhances enzymatic and non-enzymatic antioxidant systems, reducing oxidative damage.	Durum wheat seeds primed with H_2O_2 exhibit higher resistance to salinity.	[135,136]
Nitric Oxide (NO) Priming	Salt Stress	Enhances stress-specific protein modification (e.g., S-nitrosylation) and antioxidant enzyme activities.	NO-primed plants show reduced protein oxidation and better salt tolerance.	[133,134]
Plant–Microbe Interactions and Viral Treatment	Drought and Freezing Stress	Fungi, rhizobacteria, and viral strains enhance hormonal interactions and antioxidant, vigor, and osmoprotectant levels.	Non-pathogenic fungi, rhizobacteria, and viral infection increase antioxidant defenses in beet during freezing stress.	[118,126–130]
Epigenetic Modifications	Multiple Abiotic Stresses	Chromatin remodeling creates stress "memory", enabling faster responses to subsequent stresses.	Epigenetically primed *Arabidopsis* plants show enhanced resistance to heat, drought, and salt stresses.	[137]

4.1. Transcription Factors' Role During Abiotic and Biotic Stress

Because of their functions as key regulators of various stress-related genes, transcription factors (TFs), such as LSD1, EDS1, PAD4, WRKYs, NACs, ERFs, βCAs, DREB, and CBF2, are intriguing prospects for genetic modification [11,130]. The molecular function of LSD1 is currently unknown, but the mutant *lsd1* phenotype is described as runaway cell death (RCD). Runaway cell death (RCD) in LSD1 mutants is usually elicited by AEE, root hypoxia, disturbed stomatal conductance [11,17,138,139], cold [140], drought [11,17], UV radiation [139,141], and pathogen infections [64]. Due to its involvement in different signaling pathways and the regulation of plant CD due to different kinds of stresses (both abiotic and biotic), LSD1 is an important TF in cross-tolerance [11]. Importantly, the RCD of *lsd1* is closely involved in EDS1 and PHYTOALEXIN DEFICIENT 4 (PAD4). These two TFs are important in RCD, as in *eds1/lsd1* and *pad4/lsd1* mutants, RCD was not found even by applying different plant stresses [21,39,64,77,139]. The ablation of LSD1 and EDS1/PAD4 behave differently toward different stresses, and as a result, the enrichments on ROS and SA are also different [11,17,39,140]. Therefore, LSD1 is the native regulator of EDS1- and PAD4-dependent cellular pathways that lead to CD. LSD1 also greatly impacts cell proliferation and modification processes, especially in non-oxidative stress conditions, which helps plants in growth and development [39,142–146] (Table 2).

Transcription factors like WRKY, NAC, ERF, and βCAs have also been related to improved tolerance in agricultural crops and model plants since they initiate defense-responsive gene expression [147]. Previous studies have shown that MPK3/6 phosphorylated the transcription factors WRKY33 and ERF6 to promote the synthesis of camalexin and activate defense genes [148,149]. In another example, the GhMAP3K15-GhMKK4-GhMPK6, a drought stress-activated MAPK cascade phosphorylated in cotton, can then activate GhWRKY59 to control the plant's response to the drought stress [150]. The study by Zhao et al. (2021) [56] found that the ZmWRKY104 phosphorylation by ZmMPK6 is important for its role in ABA-induced antioxidant defense and drought tolerance in maize. Another study reported that when H_2O_2 levels arise, the ANAC017, located in the ER, releases its N-terminus into the nucleus, which controls primary responses [56]. Recently, it has been shown that ANAC017 contributes to aluminum tolerance by regulating genes that change cell wall synthesis [151]. Along with EDS1 and WRKY33, which react to H_2O_2 quickly and transiently, a transcription factor belonging to the ERF family, RRTF1, functions as a key redox signaling regulator [152,153]. In many studies, RRTF1 also showed its role in JA and auxin biosynthesis crosstalk, PCD, salt tolerance, and pathogen resistance [154–157]. Recent studies by Białas et al. (2024) [158] suggested that the simultaneous overexpression of plant β carbonic anhydrases βCA1 and/or βCA2 with PsbS genes leads to improved photoprotection, acclimation to variable light conditions, and WUE. The analysis of *Arabidopsis* mutants showed that in response to low-temperature stress, CBF genes control 134 genes [159]. Six genes implicated in freezing tolerance were identified by transcriptome analysis as being controlled by DREB and ERF/AP2, including CBF1, ERF105, ZAT6, ZAT12, WRKY33, and WRKY40. These findings reveal a novel chloroplast retrograde regulatory hotspot that relies on bicarbonate absorption, βCAs, and PsbS protein relative levels and shows the importance of TFs in cross-tolerance (Table 2).

Table 2. Dual role of transcription factors in the regulation of cross-tolerance.

Transcription Factor	Function	Stress Type	Mechanistic Insights	References
LSD1	Conditional negative regulator of cell death, ROS, hormonal homeostasis, photosynthesis, transpiration, and cell wall synthesis.	Abiotic (cold, drought, UV) and biotic (pathogens).	Balances cell division and cell death under oxidative stress. Key regulator in cross-tolerance pathways.	[11,17,39,64,139,141]
EDS1	Positive regulator of the cell death conditionally depends on LSD1. Mediates defense and acclimation gene activation via the triacylglycerol lipase domain.	Biotic and abiotic.	Works closely with LSD1 and PAD4.	[17,19,21,39,142]
PAD4	Promotes disease resistance and abiotic stress responses. Positive regulator of the cell death conditionally depends on EDS1.	Biotic and abiotic.	Works closely with LSD1 and PAD4.	[142–144]
WRKYs	Activates defense-related gene expression and antioxidant pathways.	Abiotic and biotic.	WRKY33 and WRKY40 are involved in signaling under H_2O_2 and ABA conditions.	[56,148–150,153]
NACs	Regulates responses to oxidative stress, aluminum tolerance, and cell wall synthesis.	Abiotic (drought, salt).	ANAC017 responds to H_2O_2 and activates genes in the nucleus.	[56,151]
ERFs	A key player in redox signaling, salt tolerance, and pathogen resistance.	Abiotic (salt) and biotic (pathogens).	RRTF1 contributes to JA/auxin crosstalk and PCD.	[152–155,157]
βCAs	Improves photoprotection, acclimation, and water use efficiency (WUE).	Abiotic (hypoxia, high light stress).	Enhances bicarbonate-driven stress signaling and chloroplast retrograde pathways.	[158]
DREB-CBF2	Controls freezing tolerance and low-temperature stress responses.	Abiotic (cold, hypoxia).	Regulates numerous downstream genes (e.g., CBF1, ERF105).	[158,159]

4.2. Reactive Oxygen Species Role During Abiotic and Biotic Stress Condition

Higher plants have developed specialized mechanisms to defend themselves from ROS toxicity and to use ROS as signaling molecules. The chloroplast, mitochondria, peroxisomes, and apoplast are the principal ROS-initiating sites under abiotic stress [19,99,160–164]. If left without control, the ROS level would rise in cells and result in oxidative damage [20]. Antioxidant enzymes, such as superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione peroxidase (GPX), and peroxiredoxin (PRX), represent a major ROS-scavenging force. They are crucial for stress tolerance in plants [165].

Environmental factors, such as high light, UV irradiation, drought, cold, high temperatures, and root hypoxia, can disrupt cellular redox homeostasis, resulting in cell death and limiting the amount of produced biomass [15,19]. It is also widely known that ROS play a role in plants' response to a pathogen invasion. Hypersensitive response (HR), triggered by the ROS burst generated by NADPH oxidases or apoplastic enzymes, can cause PCD in different cells at the infection sites. This HR mechanism can limit their invasion because of the biotrophic nature of infections (feeding on live cells). In contrast, the surrounding cells develop the capacity to avoid cell death via the propagation of ROS [66]. Callose deposition and cell wall cross-linking, mediated by apoplastic ROS, strengthen the cell wall and prevent pathogens from penetrating it [166]. Plant NADPH oxidases produce ROS in

the apoplast, but their accumulation has also been shown in mitochondria, chloroplasts, and even nuclei [167]. Additionally, many abiotic stressors can directly or indirectly activate additional signaling pathways to cause ROS generation in the plasma membrane by the Rboh-signaling pathway, in chloroplasts by the Calvin–Benson pathway, in mitochondria through the ubiquinone pathway, and in peroxisomes by the β-oxidation pathway [168]. Cross-tolerance is achieved through crosstalk between ROS signaling mechanisms and other pathways in activating defense responses [169]. Plants respond to abiotic stress by increasing the accumulation of antioxidant defense systems, including secondary metabolites, such as flavonoids, which scavenge excess ROS and help balance the cellular redox state [170]. The interplay between ROS, redox signals, and antioxidative pathways is important for plant acclimation to stress and cross-tolerance acquisition [171]. Additionally, ROS are involved in organelle-to-organelle and cell-to-cell signaling [172]. Crosstalk between ROS and other signaling molecules, such as reactive nitrogen species (RNS), protein kinases, phytohormones, and secondary messengers like calcium, is important for plant responses to environmental stresses [173] (Figure 1).

4.3. Hormonal Response During Abiotic and Biotic Stress

In the control of plant immunological responses, jasmonic acid (JA), salicylic acid (SA), ethylene (ET), and abscisic acid (ABA) are thought to be the key molecules. According to several studies [174–177], the JA signaling pathway always functions as a significant stress hormone route that frequently communicates with various plant hormones to create an extensive signaling network. Several common elements among JA and other plant hormone signaling pathways have recently been discovered. Jasmonic acid (JA) and SA act antagonistically toward each other but often combine to make plants more resilient to stressors [178]. Recently, processes underlying JA–SA crosstalk have been widely studied. Many genes were reported to be involved in SA–JA antagonism, including MYC2, PDF 1.2 (plant defensin 1.2), TGAs (TF family) [179,180], MAPK, NPR1, ERF1, WRKY62, WRKY70, GRX480 (glutaredoxin 480), ORA59 (octadecanoid-responsive *Arabidopsis* AP2/ERF 59), and JAZs [181]. The discovery of the ortholog NPR1 in the ancestor of all terrestrial plants raises the possibility that JA–SA crosstalk occurs in nearly every plant [182].

According to studies, SA reduces the amount of ET produced due to various abiotic factors. For instance, exogenous SA reduces heat sensitivity in heat-stressed plants by decreasing the ACS activity in wheat and promoting proline metabolism while limiting ET production [1,183]. On the contrary, during ozone (O_3) stress, ET and SA work together to control cell death in *Arabidopsis* and tobacco leaves [184–186]. By increasing the transcription of the genes encoding CHORISMATE MUTASE and PAL, O_3-induced ethylene enhances SA biosynthesis [186]. The inhibition of either SA or ethylene biosynthesis in *Arabidopsis* reverses the O_3-induced hypersensitive response phenotype [185]. It is widely known that endogenous SA levels and plant defense mechanisms against biotrophic and hemibiotrophic diseases are positively correlated in plants, as discussed by Glazebrook et al. (2005) [68]. Additionally, the external SA application causes multiple plant species, such as *Fusarium oxysporum*, *Alternaria alternata*, *Magnaporthe grisea*, *Colletotrichum gloeosporides*, and *Xanthomonas* spp., to develop local and systemic acquired resistance against a variety of pathogens [68,187–197].

Jasmonic acid and ethylene play an important, synergistic role in plant resistance to necrotrophic diseases [198,199]. The synergies among ET-stabilized EIN3/EIL1 and JA-activated MYC2 control plant growth and insect resistance. Due to the inhibitory effect of MYC2 on EIN3/EIL1, the transcription of ERF1 (downstream gene of EIN3/EIL1) is suppressed, reducing plant tolerance to necrotrophic fungi [200]. VSP2 and CYP79B3 (wound and herbivore responsive genes, respectively) are suppressed via the JA signaling

pathway when EIN3/EIL1 and MYC2 interact; however, on the other side, weakening MYC2's repression of MYC2 protects against a broad range of herbivores [201,202]. ET and JA can operate oppositely to control several abiotic stress genes, in addition to their combined effects on the functions of EIN3/EIL1, including their targets in rice and *Arabidopsis* [203]. The transcription level of EIN3/EIL1-inducible target genes is decreased in etiolated *Arabidopsis*, which is because the already JA-activated MYC TFs, including MYC2, MYC3, and MYC4), may have actively interacted with EIN3/EIL1 and thus have blocked their ability to bind a DNA target [184,200,203]. The ERF1 is a well-known target of MYC2 and EIN3, modulating responses to various abiotic stressors [204–206]. Studies in *Arabidopsis* showed that ERF1 regulates the synthesis of many genes, including JA-, drought-, salinity-, and high-temperature-responsive genes. It has been demonstrated that plants overexpressing ERF1 are more resilient to salt, heat, and drought stress [204,205]. Also, Chen et al. (2021) [184] discussed a JA-triggered reduction in EIN3 activity at the post-transcriptional level. These findings imply that JA and ET can control plant immunity oppositely and that their role is in defense responses.

Abscisic acid is also widely known for its regulatory function in response to abiotic and biotic stresses [184]. Studies suggest that ABA influences several plant functions, including seed dormancy, seed development, abscission, desiccation tolerance enhancement, and, most importantly, stomatal closure [207,208]. Additionally, ABA may be important in situations that are not stressful. Several ABA-dependent secondary messengers can also help plants adapt to biotic and abiotic stress, including nitric oxide (NO), cytosolic free Ca^{2+}, and ROS [209,210]. By controlling ABA production and vice versa, several substances, including polyamines (PAs), hydrogen sulfide (H_2S), and brassinosteroids (BRs), enhance drought tolerance in plants [211–213]. Other hormones can also assist the effects of ABA [214–216]. A study by Kumar et al. (2016) [217] has reported that ethylene and ABA interact antagonistically, affecting one another's signaling and biosynthesis pathways. According to genetic evidence in *Arabidopsis*, ethylene production and signaling gene knockouts change a plant's sensitivity to ABA, which in turn impacts the plant's ability to withstand abiotic or biotic stresses in several developmental stages [37,216–218]. For instance, during seed germination and seedling growth, the *Arabidopsis ACS7* loss-of-function mutant exhibits increased salt tolerance, accumulates ABA content, and is hypersensitive to ABA due to decreased endogenous ethylene levels [37,216] (Figure 1).

5. Conclusions

In general, environmental stimuli induce foliar stomata closure and the inhibition of photosynthesis, thus inducing AEE and its dissipation as heat in the NPQ mechanism. AEE alone can simultaneously induce five different types of abiotic stress: heat shock stress, photooxidative stress, photoinhibitory stress, photorespiratory stress, and osmotic stress. This, in turn, activates chloroplast retrograde signaling pathways. Chloroplast and cellular redox potential is regulated by glutathione and ascorbate cycles and by other chloroplast redox potential regulators, for example, ferredoxin-$NADP^+$ reductase, thioredoxin, and glutaredoxin. These redox changes induce the synthesis of direct and indirect precursors of phytohormones like ABA, SA, ET, JA, IAA, and other signaling molecules in the chloroplasts of local and directly stressed cells, and in non-directly stressed cells and chloroplasts of systemic cells, tissues, and organs by self-propagating waves of electrical-, ROS-, heat-, and phytohormonal-signaling of SAA and SAR. These signaling waves activate or deactivate various phosphatases, kinase cascades, and transcription factors. As a result, some foliar cells induce PCD due to stomata closure and the induction of the photorespiratory burst of ROS, and other cells induce acclimation and defense responses. All these changes within one plant can be communicated to the other neighboring plants by NAA mechanisms

that propagate SAA and presumably SAR within the plant community. Therefore, cross-tolerance between abiotic and biotic stress responses is possible not only within one stressed plant but also within the plant community.

Given a few leaves per plant and hundreds of plants in contact in a meadow, thousands of cells per leaf, several dozen chloroplasts per cell, and thousands of PSII per chloroplast, which are potentially involved in the network of connections, this reveals the complexity of the SAA and NAA signaling and communication network mechanisms that affects trillions of possible communications routes in several m^2 of a meadow. Plants are environmentally smart and intelligent; they communicate in sophisticated ways globally (within the community of plants), regulate absorbed energy fate, regulate foliar temperature, can count absorbed photons, have quantum-molecular (NPQ-value) memory of AEE episodes, and have physiological (redox) cellular memory; they process this memory differentially at the same time in various foliar cells and tissues to acclimatize and immunize. Thus, plants should be regarded as smart and communicating organisms.

Author Contributions: Conceptualization, S.K.; resources, M.K.; writing—original draft preparation, M.K.; writing—review and editing, P.B. and S.K.; visualization, M.K., P.B. and SK; supervision, S.K.; project administration, S.K.; funding acquisition, S.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Polish National Science Centre (Opus 20 UMO-2020/39/B/NZ3/02103) conceded to S.K.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Peng, P.; Li, R.; Chen, Z.-H.; Wang, Y. Stomata at the Crossroad of Molecular Interaction between Biotic and Abiotic Stress Responses in Plants. *Front. Plant Sci.* **2022**, *13*, 1031891. [CrossRef] [PubMed]
2. Hatfield, J.L.; Prueger, J.H. Temperature Extremes: Effect on Plant Growth and Development. *Weather Clim. Extrem.* **2015**, *10*, 4–10. [CrossRef]
3. World Health Organization. *The State of Food Security and Nutrition in the World 2022: Repurposing Food and Agricultural Policies to Make Healthy Diets More Affordable*; Food & Agriculture Organization: Rome, Italy, 2022; Volume 2022, ISBN 92-5-136499-0.
4. Fedoroff, N.V.; Battisti, D.S.; Beachy, R.N.; Cooper, P.J.; Fischhoff, D.A.; Hodges, C.; Knauf, V.C.; Lobell, D.; Mazur, B.J.; Molden, D. Radically Rethinking Agriculture for the 21st Century. *Science* **2010**, *327*, 833–834. [CrossRef] [PubMed]
5. Wilby, R.; Whitehead, P.; Wade, A.; Butterfield, D.; Davis, R.; Watts, G. Integrated Modelling of Climate Change Impacts on Water Resources and Quality in a Lowland Catchment: River Kennet, UK. *J. Hydrol.* **2006**, *330*, 204–220. [CrossRef]
6. Zhang, H.; Zhao, Y.; Zhu, J.-K. Thriving under Stress: How Plants Balance Growth and the Stress Response. *Dev. Cell* **2020**, *55*, 529–543. [CrossRef] [PubMed]
7. Boyer, J.S. Plant Productivity and Environment. *Science* **1982**, *218*, 443–448. [CrossRef] [PubMed]
8. Tzvi, T. Biochemistry and Molecular Biology of Plants. In *The Quarterly Review of Biology*; Buchanan, B.B., Gruissem, W., Jones, R.L., Eds.; University of Chicago Press: Chicago, IL, USA, 2001; Volume 76, pp. 359–360. [CrossRef]
9. Vij, S.; Tyagi, A.K. Emerging Trends in the Functional Genomics of the Abiotic Stress Response in Crop Plants. *Plant Biotechnol. J.* **2007**, *5*, 361–380. [CrossRef] [PubMed]
10. Zurbriggen, M.D.; Hajirezaei, M.-R.; Carrillo, N. Engineering the Future. Development of Transgenic Plants with Enhanced Tolerance to Adverse Environments. *Biotechnol. Genet. Eng. Rev.* **2010**, *27*, 33–56. [CrossRef]
11. Karpiński, S.; SZECHYŃSKA-HEBDA, M.; Wituszyńska, W.; Burdiak, P. Light Acclimation, Retrograde Signalling, Cell Death and Immune Defences in Plants. *Plant Cell Environ.* **2013**, *36*, 736–744. [CrossRef]
12. Zhang, H.; Zhu, J.; Gong, Z.; Zhu, J.-K. Abiotic Stress Responses in Plants. *Nat. Rev. Genet.* **2022**, *23*, 104–119. [CrossRef]
13. Fahad, S.; Bajwa, A.A.; Nazir, U.; Anjum, S.A.; Farooq, A.; Zohaib, A.; Sadia, S.; Nasim, W.; Adkins, S.; Saud, S. Crop Production under Drought and Heat Stress: Plant Responses and Management Options. *Front. Plant Sci.* **2017**, *8*, 1147. [CrossRef] [PubMed]

14. Minhas, P.S.; Rane, J.; Pasala, R.K. Abiotic Stresses in Agriculture: An Overview. In *Abiotic Stress Management for Resilient Agriculture*; Springer: Singapore, 2017; pp. 3–8.
15. Bernacki, M.J.; Czarnocka, W.; Szechyńska-Hebda, M.; Mittler, R.; Karpiński, S. Biotechnological Potential of LSD1, EDS1, and PAD4 in the Improvement of Crops and Industrial Plants. *Plants* **2019**, *8*, 290. [CrossRef] [PubMed]
16. Gawroński, P.; Burdiak, P.; Scharff, L.B.; Mielecki, J.; Górecka, M.; Zaborowska, M.; Leister, D.; Waszczak, C.; Karpiński, S. CIA2 and CIA2-LIKE Are Required for Optimal Photosynthesis and Stress Responses in *Arabidopsis thaliana*. *Plant J.* **2021**, *105*, 619–638. [CrossRef] [PubMed]
17. Wituszyńska, W.; Ślesak, I.; Vanderauwera, S.; Szechyńska-Hebda, M.; Kornaś, A.; Van Der Kelen, K.; Mühlenbock, P.; Karpińska, B.; Maćkowski, S.; Van Breusegem, F. Lesion Simulating Disease1, Enhanced Disease Susceptibility1, and Phytoalexin Deficient4 Conditionally Regulate Cellular Signaling Homeostasis, Photosynthesis, Water Use Efficiency, and Seed Yield in *Arabidopsis*. *Plant Physiol.* **2013**, *161*, 1795–1805. [CrossRef]
18. Petrov, V.; Hille, J.; Mueller-Roeber, B.; Gechev, T.S. ROS-Mediated Abiotic Stress-Induced Programmed Cell Death in Plants. *Front. Plant Sci.* **2015**, *6*, 69. [CrossRef] [PubMed]
19. Czarnocka, W.; Karpiński, S. Friend or Foe? Reactive Oxygen Species Production, Scavenging and Signaling in Plant Response to Environmental Stresses. *Free Radic. Biol. Med.* **2018**, *122*, 4–20. [CrossRef]
20. Mittler, R.; Zandalinas, S.I.; Fichman, Y.; Van Breusegem, F. Reactive Oxygen Species Signalling in Plant Stress Responses. *Nat. Rev. Mol. Cell Biol.* **2022**, *23*, 663–679. [CrossRef]
21. Muhlenbock, P.; Szechynska-Hebda, M.; Płaszczyca, M.; Baudo, M.; Mateo, A.; Mullineaux, P.M.; Parker, J.E.; Karpinska, B.; Karpiński, S. Chloroplast Signaling and LESION SIMULATING DISEASE1 Regulate Crosstalk between Light Acclimation and Immunity in *Arabidopsis*. *Plant Cell* **2008**, *20*, 2339–2356. [CrossRef] [PubMed]
22. Szechyńska-Hebda, M.; Kruk, J.; Górecka, M.; Karpińska, B.; Karpiński, S. Evidence for Light Wavelength-Specific Photoelectrophysiological Signaling and Memory of Excess Light Episodes in *Arabidopsis*. *Plant Cell* **2010**, *22*, 2201–2218. [CrossRef]
23. Szechyńska-Hebda, M.; Lewandowska, M.; Witoń, D.; Fichman, Y.; Mittler, R.; Karpiński, S.M. Aboveground Plant-to-Plant Electrical Signaling Mediates Network Acquired Acclimation. *Plant Cell* **2022**, *34*, 3047–3065. [CrossRef] [PubMed]
24. Górecka, M.; Lewandowska, M.; Dąbrowska-Bronk, J.; Białasek, M.; Barczak-Brzyżek, A.; Kulasek, M.; Mielecki, J.; Kozłowska-Makulska, A.; Gawroński, P.; Karpiński, S. Photosystem II 22kDa Protein Level-a Prerequisite for Excess Light-inducible Memory, Cross-tolerance to UV-C and Regulation of Electrical Signalling. *Plant Cell Environ.* **2020**, *43*, 649–661. [CrossRef] [PubMed]
25. Fujita, Y.; Fujita, M.; Shinozaki, K.; Yamaguchi-Shinozaki, K. ABA-Mediated Transcriptional Regulation in Response to Osmotic Stress in Plants. *J. Plant Res.* **2011**, *124*, 509–525. [CrossRef] [PubMed]
26. Mittler, R. Abiotic Stress, the Field Environment and Stress Combination. *Trends Plant Sci.* **2006**, *11*, 15–19. [CrossRef] [PubMed]
27. Mohanta, T.K.; Bashir, T.; Hashem, A.; Abd_Allah, E.F. Systems Biology Approach in Plant Abiotic Stresses. *Plant Physiol. Biochem.* **2017**, *121*, 58–73. [CrossRef] [PubMed]
28. Zagorchev, L.; Seal, C.E.; Kranner, I.; Odjakova, M. A Central Role for Thiols in Plant Tolerance to Abiotic Stress. *Int. J. Mol. Sci.* **2013**, *14*, 7405–7432. [CrossRef]
29. Kulasek, M.; Bernacki, M.J.; Ciszak, K.; Witoń, D.; Karpiński, S. Contribution of PsbS Function and Stomatal Conductance to Foliar Temperature in Higher Plants. *Plant Cell Physiol.* **2016**, *57*, 1495–1509. [CrossRef]
30. Kul, R.; Ekinci, M.; Turan, M.; Ors, S.; Yildirim, E. How Abiotic Stress Conditions Affects Plant Roots. In *Plant Roots*; IntechOpen: Rijeka, Croatia, 2020; ISBN 1-83968-276-0.
31. Sanchez, A.; Shin, J.; Davis, S.J. Abiotic Stress and the Plant Circadian Clock. *Plant Signal. Behav.* **2011**, *6*, 223–231. [CrossRef]
32. ElSayed, A.; Rafudeen, M.; Golldack, D. Physiological Aspects of Raffinose Family Oligosaccharides in Plants: Protection against Abiotic Stress. *Plant Biol.* **2014**, *16*, 1–8. [CrossRef] [PubMed]
33. Ziogas, V.; Tanou, G.; Filippou, P.; Diamantidis, G.; Vasilakakis, M.; Fotopoulos, V.; Molassiotis, A. Nitrosative Responses in Citrus Plants Exposed to Six Abiotic Stress Conditions. *Plant Physiol. Biochem.* **2013**, *68*, 118–126. [CrossRef] [PubMed]
34. Mayrose, M.; Kane, N.C.; Mayrose, I.; Dlugosch, K.M.; Rieseberg, L.H. Increased Growth in Sunflower Correlates with Reduced Defences and Altered Gene Expression in Response to Biotic and Abiotic Stress. *Mol. Ecol.* **2011**, *20*, 4683–4694. [CrossRef]
35. Waters, A.J.; Makarevitch, I.; Noshay, J.; Burghardt, L.T.; Hirsch, C.N.; Hirsch, C.D.; Springer, N.M. Natural Variation for Gene Expression Responses to Abiotic Stress in Maize. *Plant J.* **2017**, *89*, 706–717. [CrossRef] [PubMed]
36. Banerjee, P. Plant Abiotic Stress Responses and microRNAs. *Adv. Agric.* **2020**, 118–119. [CrossRef]
37. Dong, S.; Adams, K.L. Differential Contributions to the Transcriptome of Duplicated Genes in Response to Abiotic Stresses in Natural and Synthetic Polyploids. *New Phytol.* **2011**, *190*, 1045–1057. [CrossRef] [PubMed]
38. Sharma, A.; Kumar, V.; Shahzad, B.; Ramakrishnan, M.; Singh Sidhu, G.P.; Bali, A.S.; Handa, N.; Kapoor, D.; Yadav, P.; Khanna, K. Photosynthetic Response of Plants under Different Abiotic Stresses: A Review. *J. Plant Growth Regul.* **2020**, *39*, 509–531. [CrossRef]
39. Rusaczonek, A.; Czarnocka, W.; Kacprzak, S.; Witoń, D.; Ślesak, I.; Szechyńska-Hebda, M.; Gawroński, P.; Karpiński, S. Role of Phytochromes A and B in the Regulation of Cell Death and Acclimatory Responses to UV Stress in *Arabidopsis thaliana*. *J. Exp. Bot.* **2015**, *66*, 6679–6695. [CrossRef]

40. Bourdais, G.; Burdiak, P.; Gauthier, A.; Nitsch, L.; Salojärvi, J.; Rayapuram, C.; Idänheimo, N.; Hunter, K.; Kimura, S.; Merilo, E. Large-Scale Phenomics Identifies Primary and Fine-Tuning Roles for CRKs in Responses Related to Oxidative Stress. *PLoS Genet.* **2015**, *11*, e1005373. [CrossRef]
41. Burdiak, P.; Mielecki, J.; Gawroński, P.; Karpiński, S. The CRK5 and WRKY53 Are Conditional Regulators of Senescence and Stomatal Conductance in *Arabidopsis*. *Cells* **2022**, *11*, 3558. [CrossRef]
42. Wituszyńska, W.; Szechyńska-Hebda, M.; Sobczak, M.; Rusaczonek, A.; Kozłowska-Makulska, A.; Witoń, D.; Karpiński, S. LESION SIMULATING DISEASE 1 and ENHANCED DISEASE SUSCEPTIBILITY 1 Differentially Regulate UV-C-induced Photooxidative Stress Signalling and Programmed Cell Death in *Arabidopsis thaliana*. *Plant Cell Environ.* **2015**, *38*, 315–330. [CrossRef] [PubMed]
43. Gechev, T.; Petrov, V. Reactive Oxygen Species and Abiotic Stress in Plants. *Int. J. Mol. Sci.* **2020**, *21*, 7433. [CrossRef] [PubMed]
44. Bali, A.S.; Sidhu, G.P.S. Abiotic Stress-Induced Oxidative Stress in Wheat. In *Wheat Production in Changing Environments*; Springer: Singapore, 2019; pp. 225–239.
45. Yadav, S.; Modi, P.; Dave, A.; Vijapura, A.; Patel, D.; Patel, M. Effect of Abiotic Stress on Crops. *Sustain. Crop Prod.* **2020**, *3*, 5–16.
46. Liang, B.-W.; Li, C.; Bai, T.-H.; Wang, P. Nutrient Use Efficiency of Plants under Abiotic Stress. *Front. Plant Sci.* **2023**, *14*, 1179842. [CrossRef] [PubMed]
47. Mitra, G. Molecular Approaches to Nutrient Uptake and Cellular Homeostasis in Plants under Abiotic Stress. In *Plant Nutrients and Abiotic Stress Tolerance*; Springer: Singapore, 2018; pp. 525–590.
48. Balfagón, D.; Gómez-Cadenas, A.; Rambla, J.L.; Granell, A.; de Ollas, C.; Bassham, D.C.; Mittler, R.; Zandalinas, S.I. γ-Aminobutyric Acid Plays a Key Role in Plant Acclimation to a Combination of High Light and Heat Stress. *Plant Physiol.* **2022**, *188*, 2026–2038. [CrossRef] [PubMed]
49. Pereira, A. Plant Abiotic Stress Challenges from the Changing Environment. *Front. Plant Sci.* **2016**, *7*, 1123. [CrossRef] [PubMed]
50. Kolb, S.; Senizza, B.; Araniti, F.; Lewin, S.; Wende, S.; Lucini, L. A Multi-Omics Approach to Unravel the Interaction between Heat and Drought Stress in the *Arabidopsis thaliana* Holobiont. *Authorea Prepr.* **2022**. [CrossRef]
51. Jha, A.; Khobra, R.; Masthigowda, M.H.; Dohrey, P.; Wadhwa, Z.; Deswal, K.; Singh, G.; Singh, G.P. Understanding Heat and Drought Stress Adaptation Mechanisms in Wheat: A Combined Approach. *J. Cereal Res.* **2022**, *14*, 42–52. [CrossRef]
52. Leal, A.R.; Belo, J.; Beeckman, T.; Barros, P.M.; Oliveira, M.M. The Combined Effect of Heat and Osmotic Stress on Suberization of *Arabidopsis* Roots. *Cells* **2022**, *11*, 2341. [CrossRef]
53. Gonzalez Guzman, M.; Cellini, F.; Fotopoulos, V.; Balestrini, R.; Arbona, V. New Approaches to Improve Crop Tolerance to Biotic and Abiotic Stresses. *Physiol. Plant.* **2022**, *174*, e13547. [CrossRef]
54. Dresselhaus, T.; Hückelhoven, R. Biotic and Abiotic Stress Responses in Crop Plants. *Agronomy* **2018**, *8*, 267. [CrossRef]
55. Enebe, M.C.; Babalola, O.O. The Impact of Microbes in the Orchestration of Plants' Resistance to Biotic Stress: A Disease Management Approach. *Appl. Microbiol. Biotechnol.* **2019**, *103*, 9–25. [CrossRef]
56. Zhao, D.; Wang, H.; Chen, S.; Yu, D.; Reiter, R.J. Phytomelatonin: An Emerging Regulator of Plant Biotic Stress Resistance. *Trends Plant Sci.* **2021**, *26*, 70–82. [CrossRef]
57. Yan, X.; Talbot, N.J. Investigating the Cell Biology of Plant Infection by the Rice Blast Fungus *Magnaporthe oryzae*. *Curr. Opin. Microbiol.* **2016**, *34*, 147–153. [CrossRef] [PubMed]
58. Andreo-Jimenez, B.; Ruyter-Spira, C.; Bouwmeester, H.J.; Lopez-Raez, J.A. Ecological Relevance of Strigolactones in Nutrient Uptake and Other Abiotic Stresses, and in Plant-Microbe Interactions below-Ground. *Plant Soil* **2015**, *394*, 1–19. [CrossRef]
59. Sundin, G.W.; Castiblanco, L.F.; Yuan, X.; Zeng, Q.; Yang, C. Bacterial Disease Management: Challenges, Experience, Innovation and Future Prospects: Challenges in Bacterial Molecular Plant Pathology. *Mol. Plant Pathol.* **2016**, *17*, 1506–1518. [CrossRef]
60. Zhou, S.; Lou, Y.-R.; Tzin, V.; Jander, G. Alteration of Plant Primary Metabolism in Response to Insect Herbivory. *Plant Physiol.* **2015**, *169*, 1488–1498. [CrossRef]
61. Acevedo, F.E.; Peiffer, M.; Tan, C.-W.; Stanley, B.A.; Stanley, A.; Wang, J.; Jones, A.G.; Hoover, K.; Rosa, C.; Luthe, D. Fall Armyworm-Associated Gut Bacteria Modulate Plant Defense Responses. *Mol. Plant. Microbe Interact.* **2017**, *30*, 127–137. [CrossRef] [PubMed]
62. Wonglom, P.; Ito, S.; Sunpapao, A. Volatile Organic Compounds Emitted from Endophytic Fungus *Trichoderma asperellum* T1 Mediate Antifungal Activity, Defense Response and Promote Plant Growth in Lettuce (*Lactuca sativa*). *Fungal Ecol.* **2020**, *43*, 100867. [CrossRef]
63. Jones, J.D.; Dangl, J.L. The Plant Immune System. *Nature* **2006**, *444*, 323–329. [CrossRef] [PubMed]
64. Rustérucci, C.; Aviv, D.H.; Holt III, B.F.; Dangl, J.L.; Parker, J.E. The Disease Resistance Signaling Components EDS1 and PAD4 Are Essential Regulators of the Cell Death Pathway Controlled by LSD1 in *Arabidopsis*. *Plant Cell* **2001**, *13*, 2211–2224. [CrossRef]
65. Kaur, B.; Bhatia, D.; Mavi, G. Eighty Years of Gene-for-Gene Relationship and Its Applications in Identification and Utilization of R Genes. *J. Genet.* **2021**, *100*, 50. [CrossRef] [PubMed]
66. Wu, L.; Chen, H.; Curtis, C.; Fu, Z.Q. Go in for the Kill: How Plants Deploy Effector-Triggered Immunity to Combat Pathogens. *Virulence* **2014**, *5*, 710–721. [CrossRef]

67. Zhang, S.; Li, C.; Si, J.; Han, Z.; Chen, D. Action Mechanisms of Effectors in Plant-Pathogen Interaction. *Int. J. Mol. Sci.* **2022**, *23*, 6758. [CrossRef] [PubMed]
68. Glazebrook, J. Contrasting Mechanisms of Defense against Biotrophic and Necrotrophic Pathogens. *Annu Rev Phytopathol* **2005**, *43*, 205–227. [CrossRef] [PubMed]
69. Nadarajah, K.K. Defensive Strategies of ROS in Plant–Pathogen Interactions. In *Plant Pathogen Interaction*; Springer: Berlin/Heidelberg, Germany, 2024; pp. 163–183.
70. Ding, L.-N.; Li, Y.-T.; Wu, Y.-Z.; Li, T.; Geng, R.; Cao, J.; Zhang, W.; Tan, X.-L. Plant Disease Resistance-Related Signaling Pathways: Recent Progress and Future Prospects. *Int. J. Mol. Sci.* **2022**, *23*, 16200. [CrossRef] [PubMed]
71. Fei, W.; Liu, Y. Biotrophic Fungal Pathogens: A Critical Overview. *Appl. Biochem. Biotechnol.* **2023**, *195*, 1–16. [CrossRef]
72. Tsuda, K.; Glazebrook, J.; Katagiri, F. The Interplay between MAMP and SA Signaling. *Plant Signal. Behav.* **2008**, *3*, 359–361. [CrossRef]
73. Thomma, B.P.; Eggermont, K.; Penninckx, I.A.; Mauch-Mani, B.; Vogelsang, R.; Cammue, B.P.; Broekaert, W.F. Separate Jasmonate-Dependent and Salicylate-Dependent Defense-Response Pathways in *Arabidopsis* Are Essential for Resistance to Distinct Microbial Pathogens. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 15107–15111. [CrossRef]
74. McDowell, J.M.; Dangl, J.L. Signal Transduction in the Plant Immune Response. *Trends Biochem. Sci.* **2000**, *25*, 79–82. [CrossRef] [PubMed]
75. Ghozlan, M.H.; Eman, E.-A.; Tokgöz, S.; Lakshman, D.K.; Mitra, A. Plant Defense against Necrotrophic Pathogens. *Am. J. Plant Sci.* **2020**, *11*, 2122–2138. [CrossRef]
76. Noctor, G.; Foyer, C.H. Intracellular Redox Compartmentation and ROS-Related Communication in Regulation and Signaling. *Plant Physiol.* **2016**, *171*, 1581–1592. [CrossRef] [PubMed]
77. Mateo, A.; Mühlenbock, P.; Rustérucci, C.; Chang, C.C.-C.; Miszalski, Z.; Karpinska, B.; Parker, J.E.; Mullineaux, P.M.; Karpinski, S. LESION SIMULATING DISEASE 1 Is Required for Acclimation to Conditions That Promote Excess Excitation Energy. *Plant Physiol.* **2004**, *136*, 2818–2830. [CrossRef] [PubMed]
78. Gawroński, P.; Witoń, D.; Vashutina, K.; Bederska, M.; Betliński, B.; Rusaczonek, A.; Karpiński, S. Mitogen-Activated Protein Kinase 4 Is a Salicylic Acid-Independent Regulator of Growth but Not of Photosynthesis in *Arabidopsis*. *Mol. Plant* **2014**, *7*, 1151–1166. [CrossRef] [PubMed]
79. Johnson, R.; Puthur, J.T. Seed Priming as a Cost Effective Technique for Developing Plants with Cross Tolerance to Salinity Stress. *Plant Physiol. Biochem.* **2021**, *162*, 247–257. [CrossRef] [PubMed]
80. Chen, K.; Arora, R. Dynamics of the Antioxidant System during Seed Osmopriming, Post-Priming Germination, and Seedling Establishment in Spinach (*Spinacia oleracea*). *Plant Sci.* **2011**, *180*, 212–220. [CrossRef]
81. Ramegowda, V.; Da Costa, M.V.J.; Harihar, S.; Karaba, N.N.; Sreeman, S.M. Abiotic and Biotic Stress Interactions in Plants: A Cross-Tolerance Perspective. In *Priming-Mediated Stress and Cross-Stress Tolerance in Crop Plants*; Elsevier: Amsterdam, The Netherlands, 2020; pp. 267–302.
82. Lee, K.; Missaoui, A.; Mahmud, K.; Presley, H.; Lonnee, M. Interaction between Grasses and Epichloë Endophytes and Its Significance to Biotic and Abiotic Stress Tolerance and the Rhizosphere. *Microorganisms* **2021**, *9*, 2186. [CrossRef]
83. Capiati, D.A.; País, S.M.; Téllez-Iñón, M.T. Wounding Increases Salt Tolerance in Tomato Plants: Evidence on the Participation of Calmodulin-like Activities in Cross-Tolerance Signalling. *J. Exp. Bot.* **2006**, *57*, 2391–2400. [CrossRef] [PubMed]
84. de Lima, J.D.; Dedido, D.B.; Volpato, L.A.; da Silva, A.P.; Lima, G.O.; Pinheiro, C.R.; da Silva, G.J. Cross Tolerance by Heat and Water Stress at Germination of Common Beans. *Sci. Agrar. Parana.* **2018**, *17*, 515–520.
85. Carvalho, F.E.; Silveira, J.A. H_2O_2-Retrograde Signaling as a Pivotal Mechanism to Understand Priming and Cross Stress Tolerance in Plants. In *Priming-Mediated Stress and Cross-Stress Tolerance in Crop Plants*; Elsevier: Amsterdam, The Netherlands, 2020; pp. 57–78.
86. Jan, M.; Liu, Z.; Rochaix, J.-D.; Sun, X. Retrograde and Anterograde Signaling in the Crosstalk between Chloroplast and Nucleus. *Front. Plant Sci.* **2022**, *13*, 980237. [CrossRef]
87. Corti, F.; Festa, M.; Szabo, I. Mitochondria–Chloroplast Cross Talk: A Possible Role for Calcium and Reactive Oxygen Species? *Bioelectricity* **2023**, *5*, 39–46. [CrossRef]
88. Woodson, J.D.; Chory, J. Coordination of Gene Expression between Organellar and Nuclear Genomes. *Nat. Rev. Genet.* **2008**, *9*, 383–395. [CrossRef]
89. Raghavendra, A.S.; Padmasree, K. Beneficial Interactions of Mitochondrial Metabolism with Photosynthetic Carbon Assimilation. *Trends Plant Sci.* **2003**, *8*, 546–553. [CrossRef]
90. van Lis, R.; Atteia, A. Control of Mitochondrial Function via Photosynthetic Redox Signals. *Photosynth. Res.* **2004**, *79*, 133–148. [CrossRef]
91. Noguchi, K.; Yoshida, K. Interaction between Photosynthesis and Respiration in Illuminated Leaves. *Mitochondrion* **2008**, *8*, 87–99. [CrossRef]

92. De Clercq, I.; Vermeirssen, V.; Van Aken, O.; Vandepoele, K.; Murcha, M.W.; Law, S.R.; Inzé, A.; Ng, S.; Ivanova, A.; Rombaut, D. The Membrane-Bound NAC Transcription Factor ANAC013 Functions in Mitochondrial Retrograde Regulation of the Oxidative Stress Response in *Arabidopsis*. *Plant Cell* **2013**, *25*, 3472–3490. [CrossRef]
93. Mielecki, J.; Gawroński, P.; Karpiński, S. Retrograde Signaling: Understanding the Communication between Organelles. *Int. J. Mol. Sci.* **2020**, *21*, 6173. [CrossRef]
94. Wang, X.; Auwerx, J. Systems Phytohormone Responses to Mitochondrial Proteotoxic Stress. *Mol. Cell* **2017**, *68*, 540–551. [CrossRef]
95. Ciszak, K.; Kulasek, M.; Barczak, A.; Grzelak, J.; Maćkowski, S.; Karpiński, S. PsbS Is Required for Systemic Acquired Acclimation and Post-Excess-Light-Stress Optimization of Chlorophyll Fluorescence Decay Times in *Arabidopsis*. *Plant Signal. Behav.* **2015**, *10*, e982018. [CrossRef]
96. Witoń, D.; Sujkowska-Rybkowska, M.; Dąbrowska-Bronk, J.; Czarnocka, W.; Bernacki, M.; Szechyńska-Hebda, M.; Karpiński, S. MITOGEN-ACTIVATED PROTEIN KINASE 4 Impacts Leaf Development, Temperature, and Stomatal Movement in Hybrid Aspen. *Plant Physiol.* **2021**, *186*, 2190–2204. [CrossRef]
97. Ball, L.; Accotto, G.-P.; Bechtold, U.; Creissen, G.; Funck, D.; Jimenez, A.; Kular, B.; Leyland, N.; Mejia-Carranza, J.; Reynolds, H. Evidence for a Direct Link between Glutathione Biosynthesis and Stress Defense Gene Expression in *Arabidopsis*. *Plant Cell* **2004**, *16*, 2448–2462. [CrossRef]
98. Peak, D.; West, J.D.; Messinger, S.M.; Mott, K.A. Evidence for Complex, Collective Dynamics and Emergent, Distributed Computation in Plants. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 918–922. [CrossRef]
99. Gilroy, S.; Białasek, M.; Suzuki, N.; Górecka, M.; Devireddy, A.R.; Karpiński, S.; Mittler, R. ROS, Calcium, and Electric Signals: Key Mediators of Rapid Systemic Signaling in Plants. *Plant Physiol.* **2016**, *171*, 1606–1615. [CrossRef]
100. Kulheim, C.; Ågren, J.; Jansson, S. Rapid Regulation of Light Harvesting and Plant Fitness in the Field. *Science* **2002**, *297*, 91–93. [CrossRef]
101. Kromdijk, J.; Głowacka, K.; Leonelli, L.; Gabilly, S.T.; Iwai, M.; Niyogi, K.K.; Long, S.P. Improving Photosynthesis and Crop Productivity by Accelerating Recovery from Photoprotection. *Science* **2016**, *354*, 857–861. [CrossRef]
102. De Souza, A.P.; Burgess, S.J.; Doran, L.; Hansen, J.; Manukyan, L.; Maryn, N.; Gotarkar, D.; Leonelli, L.; Niyogi, K.K.; Long, S.P. Soybean Photosynthesis and Crop Yield Are Improved by Accelerating Recovery from Photoprotection. *Science* **2022**, *377*, 851–854. [CrossRef]
103. Karpinski, S.; Reynolds, H.; Karpinska, B.; Wingsle, G.; Creissen, G.; Mullineaux, P. Systemic Signaling and Acclimation in Response to Excess Excitation Energy in *Arabidopsis*. *Science* **1999**, *284*, 654–657. [CrossRef]
104. Pandey, P.; Irulappan, V.; Bagavathiannan, M.V.; Senthil-Kumar, M. Impact of Combined Abiotic and Biotic Stresses on Plant Growth and Avenues for Crop Improvement by Exploiting Physio-Morphological Traits. *Front. Plant Sci.* **2017**, *8*, 537. [CrossRef]
105. Prasad, P.; Pisipati, S.; Momčilović, I.; Ristic, Z. Independent and Combined Effects of High Temperature and Drought Stress during Grain Filling on Plant Yield and Chloroplast EF-Tu Expression in Spring Wheat. *J. Agron. Crop Sci.* **2011**, *197*, 430–441. [CrossRef]
106. Coakley, S.M.; Scherm, H.; Chakraborty, S. Climate Change and Plant Disease Management. *Annu. Rev. Phytopathol.* **1999**, *37*, 399–426. [CrossRef]
107. Seherm, H.; Coakley, S.M. Plant Pathogens in a Changing World. *Australas. Plant Pathol.* **2003**, *32*, 157–165. [CrossRef]
108. McDonald, A.; Riha, S.; DiTommaso, A.; DeGaetano, A. Climate Change and the Geography of Weed Damage: Analysis of US Maize Systems Suggests the Potential for Significant Range Transformations. *Agric. Ecosyst. Environ.* **2009**, *130*, 131–140. [CrossRef]
109. Ziska, L.H.; Tomecek, M.B.; Gealy, D.R. Competitive Interactions between Cultivated and Red Rice as a Function of Recent and Projected Increases in Atmospheric Carbon Dioxide. *Agron. J.* **2010**, *102*, 118–123. [CrossRef]
110. Peters, K.; Breitsameter, L.; Gerowitt, B. Impact of Climate Change on Weeds in Agriculture: A Review. *Agron. Sustain. Dev.* **2014**, *34*, 707–721. [CrossRef]
111. Duveiller, E.; Singh, R.P.; Nicol, J.M. The Challenges of Maintaining Wheat Productivity: Pests, Diseases, and Potential Epidemics. *Euphytica* **2007**, *157*, 417–430. [CrossRef]
112. Valerio, M.; Lovelli, S.; Perniola, M.; Di Tommaso, T.; Ziska, L. The Role of Water Availability on Weed–Crop Interactions in Processing Tomato for Southern Italy. *Acta Agric. Scand. Sect. B–Soil Plant Sci.* **2013**, *63*, 62–68. [CrossRef]
113. Atkinson, N.J.; Urwin, P.E. The Interaction of Plant Biotic and Abiotic Stresses: From Genes to the Field. *J. Exp. Bot.* **2012**, *63*, 3523–3543. [CrossRef]
114. Prasch, C.M.; Sonnewald, U. Simultaneous Application of Heat, Drought, and Virus to *Arabidopsis* Plants Reveals Significant Shifts in Signaling Networks. *Plant Physiol.* **2013**, *162*, 1849–1866. [CrossRef] [PubMed]
115. Pandey, P.; Ramegowda, V.; Senthil-Kumar, M. Shared and Unique Responses of Plants to Multiple Individual Stresses and Stress Combinations: Physiological and Molecular Mechanisms. *Front. Plant Sci.* **2015**, *6*, 723. [CrossRef]

116. Choudhary, A.; Pandey, P.; Senthil-Kumar, M. Tailored Responses to Simultaneous Drought Stress and Pathogen Infection in Plants. In *Drought Stress Tolerance in Plants*; Springer: Cham, Switzerland, 2016; Volume 1, pp. 427–438.
117. Ramu, V.S.; Paramanantham, A.; Ramegowda, V.; Mohan-Raju, B.; Udayakumar, M.; Senthil-Kumar, M. Transcriptome Analysis of Sunflower Genotypes with Contrasting Oxidative Stress Tolerance Reveals Individual-and Combined-Biotic and Abiotic Stress Tolerance Mechanisms. *PLoS ONE* **2016**, *11*, e0157522. [CrossRef]
118. Kissoudis, C.; van de Wiel, C.; Visser, R.G.; van der Linden, G. Enhancing Crop Resilience to Combined Abiotic and Biotic Stress through the Dissection of Physiological and Molecular Crosstalk. *Front. Plant Sci.* **2014**, *5*, 207. [CrossRef]
119. Bilgin, D.D.; Zavala, J.A.; Zhu, J.; Clough, S.J.; Ort, D.R.; DeLUCIA, E.H. Biotic Stress Globally Downregulates Photosynthesis Genes. *Plant Cell Environ.* **2010**, *33*, 1597–1613. [CrossRef]
120. Grimmer, M.K.; John Foulkes, M.; Paveley, N.D. Foliar Pathogenesis and Plant Water Relations: A Review. *J. Exp. Bot.* **2012**, *63*, 4321–4331. [CrossRef]
121. You, M.P.; Colmer, T.D.; Barbetti, M.J. Salinity Drives Host Reaction in *Phaseolus vulgaris* (Common Bean) to *Macrophomina phaseolina*. *Funct. Plant Biol.* **2011**, *38*, 984–992. [CrossRef]
122. Kim, T.-H.; Hauser, F.; Ha, T.; Xue, S.; Böhmer, M.; Nishimura, N.; Munemasa, S.; Hubbard, K.; Peine, N.; Lee, B. Chemical Genetics Reveals Negative Regulation of Abscisic Acid Signaling by a Plant Immune Response Pathway. *Curr. Biol.* **2011**, *21*, 990–997. [CrossRef]
123. Jelenska, J.; Van Hal, J.A.; Greenberg, J.T. Pseudomonas Syringae Hijacks Plant Stress Chaperone Machinery for Virulence. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 13177–13182. [CrossRef]
124. Clément, M.; Leonhardt, N.; Droillard, M.-J.; Reiter, I.; Montillet, J.-L.; Genty, B.; Lauriere, C.; Nussaume, L.; Noël, L.D. The Cytosolic/Nuclear HSC70 and HSP90 Molecular Chaperones Are Important for Stomatal Closure and Modulate Abscisic Acid-Dependent Physiological Responses in *Arabidopsis*. *Plant Physiol.* **2011**, *156*, 1481–1492. [CrossRef]
125. Goel, A.K.; Lundberg, D.; Torres, M.A.; Matthews, R.; Akimoto-Tomiyama, C.; Farmer, L.; Dangl, J.L.; Grant, S.R. The *Pseudomonas syringae* Type III Effector HopAM1 Enhances Virulence on Water-Stressed Plants. *Mol. Plant. Microbe Interact.* **2008**, *21*, 361–370. [CrossRef]
126. Xu, P.; Chen, F.; Mannas, J.P.; Feldman, T.; Sumner, L.W.; Roossinck, M.J. Virus Infection Improves Drought Tolerance. *New Phytol.* **2008**, *180*, 911–921. [CrossRef]
127. Reusche, M.; Thole, K.; Janz, D.; Truskina, J.; Rindfleisch, S.; Drübert, C.; Polle, A.; Lipka, V.; Teichmann, T. Verticillium Infection Triggers VASCULAR-RELATED NAC DOMAIN7–Dependent de Novo Xylem Formation and Enhances Drought Tolerance in *Arabidopsis*. *Plant Cell* **2012**, *24*, 3823–3837. [CrossRef]
128. Dodd, I.C.; Pérez-Alfocea, F. Microbial Amelioration of Crop Salinity Stress. *J. Exp. Bot.* **2012**, *63*, 3415–3428. [CrossRef]
129. Márquez, L.M.; Redman, R.S.; Rodriguez, R.J.; Roossinck, M.J. A Virus in a Fungus in a Plant: Three-Way Symbiosis Required for Thermal Tolerance. *Science* **2007**, *315*, 513–515. [CrossRef]
130. Berendsen, R.L.; Pieterse, C.M.; Bakker, P.A. The Rhizosphere Microbiome and Plant Health. *Trends Plant Sci.* **2012**, *17*, 478–486. [CrossRef]
131. Goellner, K.; Conrath, U. Priming: It's All the World to Induced Disease Resistance. In *Sustainable Disease Management in a European Context*; Springer: Dordrecht, The Netherlands, 2008; pp. 233–242.
132. Kissoudis, C. Genetics and Regulation of Combined Abiotic and Biotic Stress Tolerance in Tomato. Ph.D. Thesis, Wageningen University and Research, Wageningen, The Netherlands, 2016. [CrossRef]
133. Karimi, Z.; Khara, J.; Habibi, G. Combined Hydrogen Peroxide and Nitric Oxide Priming Modulate Salt Stress Tolerance in Acclimated and Non-Acclimated Oilseed Rape (*Brassica napus* L.) Plants. *J. Plant Physiol. Breed.* **2020**, *10*, 27–43.
134. Zulfiqar, F.; Nafees, M.; Chen, J.; Darras, A.; Ferrante, A.; Hancock, J.T.; Ashraf, M.; Zaid, A.; Latif, N.; Corpas, F.J. Chemical Priming Enhances Plant Tolerance to Salt Stress. *Front. Plant Sci.* **2022**, *13*, 946922. [CrossRef]
135. Ellouzi, H.; Oueslati, S.; Hessini, K.; Rabhi, M.; Abdelly, C. Seed-Priming with H_2O_2 Alleviates Subsequent Salt Stress by Preventing ROS Production and Amplifying Antioxidant Defense in Cauliflower Seeds and Seedlings. *Sci. Hortic.* **2021**, *288*, 110360. [CrossRef]
136. Momeni, M.M.; Kalantar, M.; Dehghani-Zahedani, M. H_2O_2 Seed Priming Improves Tolerance to Salinity Stress in Durum Wheat. *Cereal Res. Commun.* **2023**, *51*, 391–401. [CrossRef]
137. Pillay, L.C.; Nekati, L.; Makhwitine, P.J.; Ndlovu, S.I. Epigenetic Activation of Silent Biosynthetic Gene Clusters in Endophytic Fungi Using Small Molecular Modifiers. *Front. Microbiol.* **2022**, *13*, 815008. [CrossRef]
138. Baillo, E.H.; Kimotho, R.N.; Zhang, Z.; Xu, P. Transcription Factors Associated with Abiotic and Biotic Stress Tolerance and Their Potential for Crops Improvement. *Genes* **2019**, *10*, 771. [CrossRef]
139. Muhlenbock, P.; Plaszczyca, M.; Plaszczyca, M.; Mellerowicz, E.; Karpinski, S. Lysigenous Aerenchyma Formation in *Arabidopsis* Is Controlled by LESION SIMULATING DISEASE1. *Plant Cell* **2007**, *19*, 3819–3830. [CrossRef]
140. Huang, X.; Li, Y.; Zhang, X.; Zuo, J.; Yang, S. The Arabidopsis LSD1 Gene Plays an Important Role in the Regulation of Low Temperature-dependent Cell Death. *New Phytol.* **2010**, *187*, 301–312. [CrossRef]

141. Czarnocka, W.; Van Der Kelen, K.; Willems, P.; Szechyńska-Hebda, M.; Shahnejat-Bushehri, S.; Balazadeh, S.; Rusaczonek, A.; Mueller-Roeber, B.; Van Breusegem, F.; Karpiński, S. The Dual Role of LESION SIMULATING DISEASE 1 as a Condition-dependent Scaffold Protein and Transcription Regulator. *Plant Cell Environ.* **2017**, *40*, 2644–2662. [CrossRef]
142. Bernacki, M.J.; Mielecki, J.; Antczak, A.; Drożdżek, M.; Witoń, D.; Dąbrowska-Bronk, J.; Gawroński, P.; Burdiak, P.; Marchwicka, M.; Rusaczonek, A. Biotechnological Potential of the Stress Response and Plant Cell Death Regulators Proteins in the Biofuel Industry. *Cells* **2023**, *12*, 2018. [CrossRef]
143. Ślesak, I.; Szechyńska-Hebda, M.; Fedak, H.; Sidoruk, N.; Dąbrowska-Bronk, J.; Witoń, D.; Rusaczonek, A.; Antczak, A.; Drożdżek, M.; Karpińska, B. PHYTOALEXIN DEFICIENT 4 Affects Reactive Oxygen Species Metabolism, Cell Wall and Wood Properties in Hybrid Aspen (*Populus tremula* L.× *P. tremuloides*). *Plant Cell Environ.* **2015**, *38*, 1275–1284. [CrossRef]
144. Bernacki, M.J.; Czarnocka, W.; Witoń, D.; Rusaczonek, A.; Szechyńska-Hebda, M.; Ślesak, I.; Dąbrowska-Bronk, J.; Karpiński, S. ENHANCED DISEASE SUSCEPTIBILITY 1 (EDS1) Affects Development, Photosynthesis, and Hormonal Homeostasis in Hybrid Aspen (*Populus tremula* L. × P. tremuloides). *J. Plant Physiol.* **2018**, *226*, 91–102. [CrossRef]
145. Bernacki, M.J.; Rusaczonek, A.; Czarnocka, W.; Karpiński, S. Salicylic Acid Accumulation Controlled by LSD1 Is Essential in Triggering Cell Death in Response to Abiotic Stress. *Cells* **2021**, *10*, 962. [CrossRef]
146. Bernacki, M.J.; Rusaczonek, A.; Gołębiewska, K.; Majewska-Fala, A.B.; Czarnocka, W.; Karpiński, S.M. METACASPASE8 (MC8) Is a Crucial Protein in the LSD1-Dependent Cell Death Pathway in Response to Ultraviolet Stress. *Int. J. Mol. Sci.* **2024**, *25*, 3195. [CrossRef]
147. Wang, H.; Wang, H.; Shao, H.; Tang, X. Recent Advances in Utilizing Transcription Factors to Improve Plant Abiotic Stress Tolerance by Transgenic Technology. *Front. Plant Sci.* **2016**, *7*, 67. [CrossRef]
148. Mao, G.; Meng, X.; Liu, Y.; Zheng, Z.; Chen, Z.; Zhang, S. Phosphorylation of a WRKY Transcription Factor by Two Pathogen-Responsive MAPKs Drives Phytoalexin Biosynthesis in *Arabidopsis*. *Plant Cell* **2011**, *23*, 1639–1653. [CrossRef] [PubMed]
149. Meng, X.; Xu, J.; He, Y.; Yang, K.-Y.; Mordorski, B.; Liu, Y.; Zhang, S. Phosphorylation of an ERF Transcription Factor by *Arabidopsis* MPK3/MPK6 Regulates Plant Defense Gene Induction and Fungal Resistance. *Plant Cell* **2013**, *25*, 1126–1142. [CrossRef]
150. Li, F.; Li, M.; Wang, P.; Cox Jr, K.L.; Duan, L.; Dever, J.K.; Shan, L.; Li, Z.; He, P. Regulation of Cotton (*Gossypium hirsutum*) Drought Responses by Mitogen-activated Protein (MAP) Kinase Cascade-mediated Phosphorylation of Gh WRKY 59. *New Phytol.* **2017**, *215*, 1462–1475. [CrossRef]
151. Tao, Y.; Wan, J.X.; Liu, Y.S.; Yang, X.Z.; Shen, R.F.; Zhu, X.F. The NAC Transcription Factor ANAC017 Regulates Aluminum Tolerance by Regulating the Cell Wall-Modifying Genes. *Plant Physiol.* **2022**, *189*, 2517–2534. [CrossRef]
152. Khandelwal, A.; Elvitigala, T.; Ghosh, B.; Quatrano, R.S. Arabidopsis Transcriptome Reveals Control Circuits Regulating Redox Homeostasis and the Role of an AP2 Transcription Factor. *Plant Physiol.* **2008**, *148*, 2050–2058. [CrossRef]
153. Matsuo, M.; Johnson, J.M.; Hieno, A.; Tokizawa, M.; Nomoto, M.; Tada, Y.; Godfrey, R.; Obokata, J.; Sherameti, I.; Yamamoto, Y.Y. High REDOX RESPONSIVE TRANSCRIPTION FACTOR1 Levels Result in Accumulation of Reactive Oxygen Species in *Arabidopsis thaliana* Shoots and Roots. *Mol. Plant* **2015**, *8*, 1253–1273. [CrossRef] [PubMed]
154. Cai, X.-T.; Xu, P.; Zhao, P.-X.; Liu, R.; Yu, L.-H.; Xiang, C.-B. Arabidopsis ERF109 Mediates Cross-Talk between Jasmonic Acid and Auxin Biosynthesis during Lateral Root Formation. *Nat. Commun.* **2014**, *5*, 5833. [CrossRef]
155. Bahieldin, A.; Atef, A.; Edris, S.; Gadalla, N.O.; Ali, H.M.; Hassan, S.M.; Al-Kordy, M.A.; Ramadan, A.M.; Makki, R.M.; Al-Hajar, A.S. Ethylene Responsive Transcription Factor ERF109 Retards PCD and Improves Salt Tolerance in Plant. *BMC Plant Biol.* **2016**, *16*, 216. [CrossRef] [PubMed]
156. Cantabella, D.; Karpinska, B.; Teixidó, N.; Dolcet-Sanjuan, R.; Foyer, C.H. Non-Volatile Signals and Redox Mechanisms Are Required for the Responses of *Arabidopsis* Roots to *Pseudomonas oryzihabitans*. *J. Exp. Bot.* **2022**, *73*, 6971–6982. [CrossRef]
157. Singh, D.; Patil, V.; Kumar, R.; Gautam, J.K.; Singh, V.; Nandi, A.K. RSI1/FLD and Its Epigenetic Target RRTF1 Are Essential for the Retention of Infection Memory in *Arabidopsis thaliana*. *Plant J.* **2023**, *115*, 662–677. [CrossRef]
158. Białas, A.; Dąbrowska-Bronk, J.; Gawroński, P.; Karpiński, S. The New Role of Carbonic Anhydrases and Bicarbonate in Acclimatization and Hypoxia Stress Responses in *Arabidopsis thaliana*. *bioRxiv* **2024**. [CrossRef]
159. Jia, Y.; Ding, Y.; Shi, Y.; Zhang, X.; Gong, Z.; Yang, S. The Cbfs Triple Mutants Reveal the Essential Functions of CBF s in Cold Acclimation and Allow the Definition of CBF Regulons in *Arabidopsis*. *New Phytol.* **2016**, *212*, 345–353. [CrossRef] [PubMed]
160. Dietz, K.-J.; Turkan, I.; Krieger-Liszkay, A. Redox-and Reactive Oxygen Species-Dependent Signaling into and out of the Photosynthesizing Chloroplast. *Plant Physiol.* **2016**, *171*, 1541–1550. [CrossRef] [PubMed]
161. Huang, S.; Van Aken, O.; Schwarzländer, M.; Belt, K.; Millar, A.H. The Roles of Mitochondrial Reactive Oxygen Species in Cellular Signaling and Stress Response in Plants. *Plant Physiol.* **2016**, *171*, 1551–1559. [CrossRef]
162. Kerchev, P.; Waszczak, C.; Lewandowska, A.; Willems, P.; Shapiguzov, A.; Li, Z.; Alseekh, S.; Mühlenbock, P.; Hoeberichts, F.A.; Huang, J. Lack of GLYCOLATE OXIDASE1, but Not GLYCOLATE OXIDASE2, Attenuates the Photorespiratory Phenotype of CATALASE2-Deficient *Arabidopsis*. *Plant Physiol.* **2016**, *171*, 1704–1719. [CrossRef]

163. Rodríguez-Serrano, M.; Romero-Puertas, M.C.; Sanz-Fernández, M.; Hu, J.; Sandalio, L.M. Peroxisomes Extend Peroxules in a Fast Response to Stress via a Reactive Oxygen Species-Mediated Induction of the Peroxin PEX11a. *Plant Physiol.* **2016**, *171*, 1665–1674. [CrossRef] [PubMed]
164. Takagi, D.; Takumi, S.; Hashiguchi, M.; Sejima, T.; Miyake, C. Superoxide and Singlet Oxygen Produced within the Thylakoid Membranes Both Cause Photosystem I Photoinhibition. *Plant Physiol.* **2016**, *171*, 1626–1634. [CrossRef]
165. Mittler, R.; Vanderauwera, S.; Gollery, M.; Van Breusegem, F. Reactive Oxygen Gene Network of Plants. *Trends Plant Sci.* **2004**, *9*, 490–498. [CrossRef] [PubMed]
166. O'Brien, J.A.; Daudi, A.; Butt, V.S.; Paul Bolwell, G. Reactive Oxygen Species and Their Role in Plant Defence and Cell Wall Metabolism. *Planta* **2012**, *236*, 765–779. [CrossRef]
167. Van Breusegem, F.; Bailey-Serres, J.; Mittler, R. Unraveling the Tapestry of Networks Involving Reactive Oxygen Species in Plants. *Plant Physiol.* **2008**, *147*, 978–984. [CrossRef] [PubMed]
168. Corpas, F.J.; Gupta, D.K.; Palma, J.M. Production Sites of Reactive Oxygen Species (ROS) in Organelles from Plant Cells. In *Reactive Oxygen Species and Oxidative Damage in Plants Under Stress*; Springer: Cham, Switzerland, 2015; pp. 1–22.
169. Imran, Q.M.; Shahid, M.; Hussain, A.; Yun, B.-W. NO and ROS Crosstalk and Acquisition of Abiotic Stress Tolerance. In *Nitric Oxide in Plant Biology*; Elsevier: Amsterdam, The Netherlands, 2022; pp. 477–491.
170. Chen, Z.; Raji, M. Role of Reactive Oxygen Species in Modulating Cross Tolerance in Plants via Flavonoids. In *Priming-Mediated Stress and Cross-Stress Tolerance in Crop Plants*; Elsevier: Amsterdam, The Netherlands, 2020; pp. 203–214.
171. Locato, V.; Cimini, S.; De Gara, L. ROS and Redox Balance as Multifaceted Players of Cross-Tolerance: Epigenetic and Retrograde Control of Gene Expression. *J. Exp. Bot.* **2018**, *69*, 3373–3391. [CrossRef]
172. Mhamdi, A.; Van Breusegem, F. Reactive Oxygen Species in Plant Development. *Development* **2018**, *145*, dev164376. [CrossRef] [PubMed]
173. Nafees, M.; Fahad, S.; Shah, A.N.; Bukhari, M.A.; Maryam; Ahmed, I.; Ahmad, S.; Hussain, S. Reactive Oxygen Species Signaling in Plants. In *Plant Abiotic Stress Tolerance*; Springer: Cham, Switzerland, 2019; pp. 259–272.
174. Kazan, K.; Manners, J.M. Jasmonate Signaling: Toward an Integrated View. *Plant Physiol.* **2008**, *146*, 1459–1468. [CrossRef] [PubMed]
175. Raza, A.; Charagh, S.; Zahid, Z.; Mubarik, M.S.; Javed, R.; Siddiqui, M.H.; Hasanuzzaman, M. Jasmonic Acid: A Key Frontier in Conferring Abiotic Stress Tolerance in Plants. *Plant Cell Rep.* **2021**, *40*, 1513–1541. [CrossRef] [PubMed]
176. Wasternack, C.; Strnad, M. Jasmonate Signaling in Plant Stress Responses and Development–Active and Inactive Compounds. *New Biotechnol.* **2016**, *33*, 604–613. [CrossRef] [PubMed]
177. Santner, A.; Estelle, M. Recent Advances and Emerging Trends in Plant Hormone Signalling. *Nature* **2009**, *459*, 1071–1078. [CrossRef]
178. Yang, Y.-X.; Ahammed, G.J.; Wu, C.; Fan, S.; Zhou, Y.-H. Crosstalk among Jasmonate, Salicylate and Ethylene Signaling Pathways in Plant Disease and Immune Responses. *Curr. Protein Pept. Sci.* **2015**, *16*, 450–461. [CrossRef]
179. Gatz, C. From Pioneers to Team Players: TGA Transcription Factors Provide a Molecular Link between Different Stress Pathways. *Mol. Plant. Microbe Interact.* **2013**, *26*, 151–159. [CrossRef] [PubMed]
180. Wang, W.; Wang, X.; Lv, Z.; Khanzada, A.; Huang, M.; Cai, J.; Zhou, Q.; Huo, Z.; Jiang, D. Effects of Cold and Salicylic Acid Priming on Free Proline and Sucrose Accumulation in Winter Wheat under Freezing Stress. *J. Plant Growth Regul.* **2022**, *41*, 2171–2184. [CrossRef]
181. Cristina, M.S.; Petersen, M.; Mundy, J. Mitogen-Activated Protein Kinase Signaling in Plants. *Annu. Rev. Plant Biol.* **2010**, *61*, 621–649. [CrossRef]
182. de Vries, S.; de Vries, J.; von Dahlen, J.K.; Gould, S.B.; Archibald, J.M.; Rose, L.E.; Slamovits, C.H. On Plant Defense Signaling Networks and Early Land Plant Evolution. *Commun. Integr. Biol.* **2018**, *11*, 1–14. [CrossRef] [PubMed]
183. Khan, M.I.R.; Iqbal, N.; Masood, A.; Per, T.S.; Khan, N.A. Salicylic Acid Alleviates Adverse Effects of Heat Stress on Photosynthesis through Changes in Proline Production and Ethylene Formation. *Plant Signal. Behav.* **2013**, *8*, e26374. [CrossRef]
184. Chen, H.; Bullock Jr, D.A.; Alonso, J.M.; Stepanova, A.N. To Fight or to Grow: The Balancing Role of Ethylene in Plant Abiotic Stress Responses. *Plants* **2021**, *11*, 33. [CrossRef] [PubMed]
185. Rao, M.V.; Lee, H.; Davis, K.R. Ozone-induced Ethylene Production Is Dependent on Salicylic Acid, and Both Salicylic Acid and Ethylene Act in Concert to Regulate Ozone-induced Cell Death. *Plant J.* **2002**, *32*, 447–456. [CrossRef]
186. Ogawa, D.; Nakajima, N.; Sano, T.; Tamaoki, M.; Aono, M.; Kubo, A.; Kanna, M.; Ioki, M.; Kamada, H.; Saji, H. Salicylic Acid Accumulation under O3 Exposure Is Regulated by Ethylene in Tobacco Plants. *Plant Cell Physiol.* **2005**, *46*, 1062–1072. [CrossRef] [PubMed]
187. Koo, Y.M.; Heo, A.Y.; Choi, H.W. Salicylic Acid as a Safe Plant Protector and Growth Regulator. *Plant Pathol. J.* **2020**, *36*, 1. [CrossRef] [PubMed]
188. Daw, B.; Zhang, L.; Wang, Z. Salicylic Acid Enhances Antifungal Resistance to *Magnaporthe grisea* in Rice Plants. *Australas. Plant Pathol.* **2008**, *37*, 637–644. [CrossRef]

189. Esmailzadeh, M.; Soleimani, M.; Rouhani, H. Exogenous Applications of Salicylic Acid for Inducing Systemic Acquired Resistance against Tomato Stem Canker Disease. *J. Biol. Sci.* **2008**, *8*, 1039–1044. [CrossRef]
190. Jendoubi, W.; Harbaoui, K.; Hamada, W. Salicylic Acid-Induced Resistance against *Fusarium oxysporumf. s. pradicis lycopercisi* in Hydroponic Grown Tomato Plants. *J. New Sci.* **2015**, *21*, 5.
191. Kundu, S.; Chakraborty, D.; Pal, A. Proteomic Analysis of Salicylic Acid Induced Resistance to Mungbean Yellow Mosaic India Virus in *Vigna mungo*. *J. Proteomics* **2011**, *74*, 337–349. [CrossRef] [PubMed]
192. Le Thanh, T.; Thumanu, K.; Wongkaew, S.; Boonkerd, N.; Teaumroong, N.; Phansak, P.; Buensanteai, N. Salicylic Acid-Induced Accumulation of Biochemical Components Associated with Resistance against *Xanthomonas oryzae Pv. oryzae* in Rice. *J. Plant Interact.* **2017**, *12*, 108–120. [CrossRef]
193. Mohan Babu, R.; Sajeena, A.; Vijaya Samundeeswari, A.; Sreedhar, A.; Vidhyasekaran, P.; Seetharaman, K.; Reddy, M. Induktion Einer Systemischen Resistenz Gegen *Xanthomonas oryzae Pv. oryzae* Durch Salicylsäure in *Oryza sativa* (L.). *J. Plant Dis. Prot.* **2003**, *110*, 419–431. [CrossRef]
194. Radwan, D.E.M.; Fayez, K.A.; Mahmoud, S.Y.; Hamad, A.; Lu, G. Physiological and Metabolic Changes of Cucurbita Pepo Leaves in Response to Zucchini Yellow Mosaic Virus (ZYMV) Infection and Salicylic Acid Treatments. *Plant Physiol. Biochem.* **2007**, *45*, 480–489. [CrossRef] [PubMed]
195. Saikia, R.; Singh, T.; Kumar, R.; Srivastava, J.; Srivastava, A.K.; Singh, K.; Arora, D.K. Role of Salicylic Acid in Systemic Resistance Induced by *Pseudomonas fluorescens* against *Fusarium oxysporum f.* sp. *ciceri* in Chickpea. *Microbiol. Res.* **2003**, *158*, 203–213. [CrossRef] [PubMed]
196. Wang, Y.; Liu, J.-H. Exogenous Treatment with Salicylic Acid Attenuates Occurrence of Citrus Canker in Susceptible Navel Orange (*Citrus sinensis osbeck*). *J. Plant Physiol.* **2012**, *169*, 1143–1149. [CrossRef]
197. Wang Jun, W.J.; Chen ShaoHong, C.S.; Huang YongFang, H.Y.; Sun Si, S.S. Induced Resistance to Anthracnose of *Camellia oleifera* by Salicylic Acid. *For. Res.* **2006**, *19*, 629–632.
198. Erb, M.; Meldau, S.; Howe, G.A. Role of Phytohormones in Insect-Specific Plant Reactions. *Trends Plant Sci.* **2012**, *17*, 250–259. [CrossRef] [PubMed]
199. Wang, C.; Liu, Y.; Li, S.-S.; Han, G.-Z. Insights into the Origin and Evolution of the Plant Hormone Signaling Machinery. *Plant Physiol.* **2015**, *167*, 872–886. [CrossRef] [PubMed]
200. Song, S.; Huang, H.; Gao, H.; Wang, J.; Wu, D.; Liu, X.; Yang, S.; Zhai, Q.; Li, C.; Qi, T. Interaction between MYC2 and ETHYLENE INSENSITIVE3 Modulates Antagonism between Jasmonate and Ethylene Signaling in *Arabidopsis*. *Plant Cell* **2014**, *26*, 263–279. [CrossRef] [PubMed]
201. Zhu, Z.; Lee, B. Friends or Foes: New Insights in Jasmonate and Ethylene Co-Actions. *Plant Cell Physiol.* **2015**, *56*, 414–420. [CrossRef]
202. Wasternack, C.; Song, S. Jasmonates: Biosynthesis, Metabolism, and Signaling by Proteins Activating and Repressing Transcription. *J. Exp. Bot.* **2017**, *68*, 1303–1321. [CrossRef] [PubMed]
203. Zhang, X.; Zhu, Z.; An, F.; Hao, D.; Li, P.; Song, J.; Yi, C.; Guo, H. Jasmonate-Activated MYC2 Represses ETHYLENE INSENSITIVE3 Activity to Antagonize Ethylene-Promoted Apical Hook Formation in *Arabidopsis*. *Plant Cell* **2014**, *26*, 1105–1117. [CrossRef]
204. Cheng, M.-C.; Liao, P.-M.; Kuo, W.-W.; Lin, T.-P. The *Arabidopsis* ETHYLENE RESPONSE FACTOR1 Regulates Abiotic Stress-Responsive Gene Expression by Binding to Different Cis-Acting Elements in Response to Different Stress Signals. *Plant Physiol.* **2013**, *162*, 1566–1582. [CrossRef] [PubMed]
205. Lorenzo, O.; Piqueras, R.; Sánchez-Serrano, J.J.; Solano, R. ETHYLENE RESPONSE FACTOR1 Integrates Signals from Ethylene and Jasmonate Pathways in Plant Defense. *Plant Cell* **2003**, *15*, 165–178. [CrossRef]
206. Zander, M.; Lewsey, M.G.; Clark, N.M.; Yin, L.; Bartlett, A.; Saldierna Guzmán, J.P.; Hann, E.; Langford, A.E.; Jow, B.; Wise, A. Integrated Multi-Omics Framework of the Plant Response to Jasmonic Acid. *Nat. Plants* **2020**, *6*, 290–302. [CrossRef]
207. Lim, C.W.; Baek, W.; Jung, J.; Kim, J.-H.; Lee, S.C. Function of ABA in Stomatal Defense against Biotic and Drought Stresses. *Int. J. Mol. Sci.* **2015**, *16*, 15251–15270. [CrossRef] [PubMed]
208. Bharath, P.; Gahir, S.; Raghavendra, A.S. Abscisic Acid-Induced Stomatal Closure: An Important Component of Plant Defense against Abiotic and Biotic Stress. *Front. Plant Sci.* **2021**, *12*, 615114. [CrossRef]
209. León, J.; Castillo, M.C.; Coego, A.; Lozano-Juste, J.; Mir, R. Diverse Functional Interactions between Nitric Oxide and Abscisic Acid in Plant Development and Responses to Stress. *J. Exp. Bot.* **2014**, *65*, 907–921. [CrossRef] [PubMed]
210. Huang, H.; Ullah, F.; Zhou, D.-X.; Yi, M.; Zhao, Y. Mechanisms of ROS Regulation of Plant Development and Stress Responses. *Front. Plant Sci.* **2019**, *10*, 800. [CrossRef] [PubMed]
211. Ha, C.V.; Leyva-González, M.A.; Osakabe, Y.; Tran, U.T.; Nishiyama, R.; Watanabe, Y.; Tanaka, M.; Seki, M.; Yamaguchi, S.; Dong, N.V. Positive Regulatory Role of Strigolactone in Plant Responses to Drought and Salt Stress. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 851–856. [CrossRef] [PubMed]

212. Jin, Z.; Xue, S.; Luo, Y.; Tian, B.; Fang, H.; Li, H.; Pei, Y. Hydrogen Sulfide Interacting with Abscisic Acid in Stomatal Regulation Responses to Drought Stress in *Arabidopsis*. *Plant Physiol. Biochem.* **2013**, *62*, 41–46. [CrossRef] [PubMed]
213. Adamipour, N.; Khosh-Khui, M.; Salehi, H.; Razi, H.; Karami, A.; Moghadam, A. Role of Genes and Metabolites Involved in Polyamines Synthesis Pathways and Nitric Oxide Synthase in Stomatal Closure on *Rosa damascena* Mill. under Drought Stress. *Plant Physiol. Biochem.* **2020**, *148*, 53–61. [CrossRef] [PubMed]
214. Robert-Seilaniantz, A.; Grant, M.; Jones, J.D. Hormone Crosstalk in Plant Disease and Defense: More than Just Jasmonate-Salicylate Antagonism. *Annu. Rev. Phytopathol.* **2011**, *49*, 317–343. [CrossRef] [PubMed]
215. Wang, X.; Kong, L.; Zhi, P.; Chang, C. Update on Cuticular Wax Biosynthesis and Its Roles in Plant Disease Resistance. *Int. J. Mol. Sci.* **2020**, *21*, 5514. [CrossRef] [PubMed]
216. Marczak, M.; Cieśla, A.; Janicki, M.; Kasprowicz-Maluśki, A.; Kubiak, P.; Ludwików, A. Protein Phosphatases Type 2C Group A Interact with and Regulate the Stability of ACC Synthase 7 in Arabidopsis. *Cells* **2020**, *9*, 978. [CrossRef] [PubMed]
217. Kumar, D.; Hazra, S.; Datta, R.; Chattopadhyay, S. Transcriptome Analysis of *Arabidopsis* Mutants Suggests a Crosstalk between ABA, Ethylene and GSH against Combined Cold and Osmotic Stress. *Sci. Rep.* **2016**, *6*, 36867. [CrossRef]
218. Wang, Y.; Liu, C.; Li, K.; Sun, F.; Hu, H.; Li, X.; Zhao, Y.; Han, C.; Zhang, W.; Duan, Y. *Arabidopsis* EIN2 Modulates Stress Response through Abscisic Acid Response Pathway. *Plant Mol. Biol.* **2007**, *64*, 633–644. [CrossRef] [PubMed]

Warsaw, 12.03.2026

Muhammad Kamran

muhammad_kamran@sggw.edu.pl

Department of Plant Genetics, Breeding and Biotechnology, Institute of Biology

Warsaw University of Life Sciences

Biological Sciences Discipline Counsel

of the Warsaw University

of Life Sciences

Statement

I hereby declare that in the publication:

Kamran, M., Burdiak, P., & Karpiński, S. (2025). Crosstalk Between Abiotic and Biotic Stresses Responses and the Role of Chloroplast Retrograde Signaling in the Cross-Tolerance Phenomena in Plants. Cells, 14(3), 176. https://doi.org/10.3390/cells14030176. **(Publication 1)**

My participation constitutes:

- participation in the experimental design of the study
- resource collection for the article
- visualization of the data (preparing figures and tables)
- drafting of the original manuscript, and the review and editing of the final version.

I estimate my contribution at: 70%

Signature

Warsaw, 12.03.2026

Paweł Burdiak

pawel_burdiak@sggw.edu.pl

Department of Plant Genetics, Breeding and Biotechnology, Institute of Biology

Warsaw University of Life Sciences

Biological Sciences Discipline Counsel

of the Warsaw University

of Life Sciences

Statement

regarding co-authorship in the publication with Muhammad Kamran, which is the basis of his doctoral thesis

I hereby declare that I am aware that the publication:

Kamran, M., Burdiak, P., & Karpiński, S. (2025). Crosstalk Between Abiotic and Biotic Stresses Responses and the Role of Chloroplast Retrograde Signaling in the Cross-Tolerance Phenomena in Plants. Cells, 14(3), 176. https://doi.org/10.3390/cells14030176 **(Publication 1),** of which I am a co-author, has been included in Muhammad Kamran's doctoral thesis.

I contributed to:

- participated in the visualization of the data
- review and editing of the final version.

I estimate my contribution at: 10%

Signature

Paweł Burdiak

Warsaw, 12.03.2026

Stanisław Karpinski

stanislaw_karpinski@sggw.edu.pl

Department of Plant Genetics, Breeding and Biotechnology, Institute of Biology

Warsaw University of Life Sciences

Biological Sciences Discipline Counsel

of the Warsaw University

of Life Sciences

Statement

regarding co-authorship in the publication with Muhammad Kamran, which is the basis of his doctoral thesis

I hereby declare that I am aware that the publication:

Kamran, M., Burdiak, P., & Karpiński, S. (2025). Crosstalk Between Abiotic and Biotic Stresses Responses and the Role of Chloroplast Retrograde Signaling in the Cross-Tolerance Phenomena in Plants. Cells, 14(3), 176. https://doi.org/10.3390/cells14030176 **(Publication 1),** of which I am a co-author, has been included in Muhammad Kamran's doctoral thesis.

I contributed to:

- securing research funding
- conceptualization of the study
- participated in the visualization of the data
- participated in writing the manuscript and the review and editing of the final version.

I estimate my contribution at: 20%

S. Karpiński

Signature

Article 2: **Kamran, M**., Burdiak, P., Zarrin Ghalami, R., Rusaczonek, A., Duszyn, M., Gołębiewska, K., Gawroński, P., Karpiński, SM (2026a). The kinase CRK5 regulates dark-induced senescence and dissipation of energy as heat by inhibiting salicylic acid signaling, *Plant Physiology*, Volume 200, Issue 2, February 2026, kiag046, https://doi.org/10.1093/plphys/kiag046.

(Ministerial score: 140; IF: 6.9)

Plant Physiology, 2026, 200, 1–12
https://doi.org/10.1093/plphys/kiag046
Published: 5 February 2026
Research article

Plant Physiology® OXFORD

The kinase CRK5 regulates dark-induced senescence and dissipation of energy as heat by inhibiting salicylic acid signaling

Muhammad Kamran[1], Paweł Burdiak[1], Roshanak Zarrin Ghalami[1], Anna Rusaczonek[2], Maria Duszyn[1], Kinga Gołębiewska[1], Piotr Gawroński[1], and Stanisław M. Karpiński[1]*

[1]Department of Plant Genetics, Breeding and Biotechnology, Institute of Biology, Warsaw University of Life Sciences, Nowoursynowska Street 159, Warsaw 02-776, Poland

[2]Department of Botany and Plant Physiology, Institute of Biology, Warsaw University of Life Sciences, Nowoursynowska 159, Warsaw 02-776, Poland

*Corresponding author: Department of Plant Genetics, Breeding and Biotechnology, Institute of Biology, Warsaw University of Life Sciences, Nowoursynowska Street 159, Warsaw 02-776, Poland. Email: Stanislaw_Karpinski@sggw.edu.pl

The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (https://academic.oup.com/plphys/pages/General-Instructions) is Stanisław M. Karpiński.

Abstract

CYSTEINE-RICH RECEPTOR-LIKE KINASE 5 (CRK5) is a membrane-localized signaling protein implicated in developmental and stress-responsive pathways. Its promoter contains multiple W-box motifs, suggesting regulation by WRKY transcription factors and a potential role in salicylic acid (SA)-dependent signaling. Since SA simultaneously promotes dark-induced senescence and modulates photo-protective dissipation of absorbed energy in excess (AEE) as heat through its effects on non-photochemical quenching (NPQ), stomatal behavior, and leaf temperature, how these SA-driven processes are coordinated remains unclear. Here, we address the unresolved question of whether CRK5 links SA-signaling to the regulation of both senescence and the dissipation of AEE as heat. We demonstrated that loss of CRK5 function leads to increased SA-accumulation, accelerated dark-induced senescence, reduced NPQ, lower foliar temperature, and impaired photosynthetic performance in Arabidopsis (*Arabidopsis thaliana*). Transcriptomic analysis revealed extensive deregulation of senescence-associated, SA-responsive, and WRKY genes in *crk5*, particularly under extended darkness. Crucially, introduction of SA-induction-deficient-2 (*sid2*) or transgenic line (NahG) into the *crk5* background fully reverted these phenotypes, whereas disruption of ethylene signaling, ethylene-insensitive-2 (*ein2*), did not, demonstrating that CRK5 acts specifically through SA-dependent pathways. A line with constitutively enhanced SA levels, constitutive expressor of *PR* genes 1 (*cpr1*), showed similar phenotypes to *crk5*, and exogenous SA further reduced NPQ and leaf temperature across genotypes, confirming that SA negatively regulates foliar AEE dissipation as heat and photosynthetic efficiency. Together, our results identify CRK5 as a key negative regulator of the SA-signaling pathway, which delays dark-induced senescence while positively regulating photosynthesis, NPQ, and thermal dissipation of AEE as heat. This work reveals a previously unrecognized role of CRK5 in coordinating SA-mediated senescence and photo-protective energy management.

Introduction

Light intensity and photoperiod length play major roles in regulating normal plant physiology. Foliar mesophyll tissues are functionally organized to absorb light as efficiently as possible to optimize anabolic output relative to absorbed energy input. The ability of leaves to dissipate absorbed energy in excess (AEE) as heat primarily through non-photochemical quenching (NPQ) and other quenching mechanisms is essential for preventing photo-oxidative damage to photosystems and the photosynthetic apparatus (Murakami et al. 2024). This foliar heat production originates largely from NPQ, a protective process involving the PSII antenna complexes and the xanthophyll cycle (Kaňa and Vass 2008; Ruban et al. 2012).

On the other hand, limited light availability, like severe shade or darkness, triggers leaf senescence in numerous plant species (Liebsch and Keech 2016; Paluch-Lubawa et al. 2021). Leaf senescence is a coordinated process characterized by the degradation of proteins, RNA, membrane lipids, chlorophyll, and other pigments to retrieve resources and recycle from aging to young organs and tissues. It is accompanied by profound changes in cell structure, metabolism,

Received: July 21, 2025. **Accepted:** December 10, 2025

and gene expression (Himelblau and Amasino 2001; Maillard et al. 2015). Several phytohormones, including ethylene (ET), abscisic acid (ABA), jasmonic acid (JA), and salicylic acid (SA), play central roles in controlling the onset and progression of senescence (Jing et al. 2005; Yoshimoto et al. 2009; Li et al. 2013; Koyama 2014; Lee et al. 2015; Zhao et al. 2016; Zhuo et al. 2020). Apoptosis-like processes and leaf aging can be induced by extended darkness, but also by seasonal variations in photoperiod length, light intensity, and its spectral quality (Muhlenbock et al. 2008; Schippers 2015).

Although extended darkness and high light represent opposite environmental extremes, both conditions require the plant to adjust energy metabolism and stress responses. In darkness, chloroplasts gradually lose photochemical activity, dismantling the photosynthetic apparatus and mobilizing resources, whereas high light causes chlorophyll over-excitation and necessitates increased NPQ and heat dissipation to prevent damage. Thus, both conditions involve stress responses triggered by an imbalance between absorbed light energy and the leaf's capacity to use it safely (Li and Kim 2022). In each case, the chloroplast acts as a signaling hub, serving as the initial site of stress perception and subsequent signal release (Foyer and Hanke 2022).

Salicylic acid (SA) plays a multifaceted role in plant signaling, particularly under temperature stress (Hassan et al. 2022). As a key endogenous signaling molecule, SA helps plants fine-tune metabolic and physiological responses to temperature fluctuations through signaling networks involving reactive oxygen species (ROS), calcium ions (Ca^{2+}), nitric oxide (NO), and interaction with other hormonal pathways (Devireddy et al. 2021). SA enhances thermo-tolerance and protection of PSII under high light by stimulating heat shock proteins (HSPs), promoting ROS scavenging, and optimizing photosynthetic performance (Khan et al. 2013; Sangwan et al. 2022). Exogenous SA application is a sustainable way to reduce heat stress damage in plants (Hassan et al. 2022; Sangwan et al. 2022; Kavulych et al. 2023). (Moustakas et al. 2022) demonstrated that SA reduces chlorophyll content and suppresses NPQ, resulting in more absorbed light energy being used for photochemistry rather than dissipated as heat.

SA also plays a significant role in regulating cell death and senescence in plants (Morris et al. 2000; Bernacki et al. 2021). It is involved in chlorophyll degradation, regulates expression of senescence-associated genes such as *SAG12* and contributes in stages of senescence characterized by programmed cell death (Morris et al. 2000; Hsu et al. 2022). Excessive SA can lead to hydrogen peroxide accumulation, triggering cell death (Liu et al. 2015). Mutants defective in SA signaling exhibit delayed leaf yellowing and reduced necrosis, supporting the central role of SA in age-related senescence (Morris et al. 2000; Lee et al. 2020). SA accumulation increases in aging leaves and enhances the expression of senescence-associated and defense-related genes (Khan et al. 2013; Zhang et al. 2013). SA biosynthesis and signaling pathways are tightly regulated and integrate multiple upstream signals, including those perceived by receptor-like kinases (RLKs) at the cell surface (Wrzaczek et al. 2010; Bourdais et al. 2015).

Receptor-like kinases (RLKs) form a large group of transmembrane proteins in plants that contain an extracellular domain at the N-terminus for ligand binding and an intracellular kinase domain at the C-terminus to phosphorylate downstream components. They regulate diverse processes, including hormone sensing, self-incompatibility, growth, development, and responses to abiotic and biotic stresses (Wrzaczek et al. 2010; Antolín-Llovera et al. 2014; Bourdais et al. 2015; Burdiak et al. 2015, 2022; Ye et al. 2017). Cysteine-rich receptor-like kinases (CRKs) constitute the largest family of RLKs, with 44 members in Arabidopsis (*Arabidopsis thaliana*). Their extracellular domain contains two conserved cysteine-rich motifs (C-8X-C-2X-C, known as DUF26), forming disulfide bridges (Wrzaczek et al. 2010). Although DUF26 domains were initially proposed to function as ROS or redox sensors, recent structural data indicate that the disulfide bonds likely contribute to structural stability rather than direct redox sensing (Vaattovaara et al. 2019). CRKs play roles in plant immunity since they interact with other RLKs to form pattern-recognition receptor (PRR) complexes that initiate pattern-triggered immunity (Zhang et al. 2023b). Many CRKs are strongly induced by pathogens or exogenous SA, and CRK loss-of-function mutants frequently display altered stress responses (Wrzaczek et al. 2010; Burdiak et al. 2015). For instance, CRK2 regulates ROS production by modulating NADPH oxidase RBOHD activity (Kimura et al. 2020), while CRK36 interacts with BIK1 to regulate both immunity and aging (Lee et al. 2017; Zhang et al. 2023b).

Among CRK family members, CRK5, has been identified as a positive regulator of plant growth and a negative regulator of stomatal conductance, cell death, and foliar senescence (Chen et al. 2003, 2004; Bourdais et al. 2015; Burdiak et al. 2015, 2022). The *CRK5* overexpression was linked to enhanced ABA sensitivity and drought tolerance (Lu et al. 2016). Conversely, *crk5* mutant shows accelerated senescence, with elevated foliar SA, ET, and ROS levels (Burdiak et al. 2015). The *CRK5* promoter is notable for its unusually large number of W-box *cis*-elements (nine within 1,248 bp), more than any other CRK promoter (Burdiak et al. 2022). W-boxes (TTGACT/C) are characteristic of WRKY transcription factors (TFs) binding sites and are enriched in promoters of genes associated with SA-mediated immunity (Muhlenbock et al. 2008; Arndt et al. 2022). Many WRKY TFs are important regulators of SA-biosynthesis and signaling (Rushton et al. 2010; van Verk et al. 2011). Recent studies demonstrated that CRK5 regulates SA-signaling by activating MITOGEN-ACTIVATED PROTEIN KINASE (MPK3) and (MPK6) (Zhao et al. 2022). Based on these observations and the dual role of SA in dark-induced aging and photoprotection under excess light, we hypothesized that CRK5 may participate in SA-mediated coordination between senescence progression and photo-protective responses.

In this study, we show that CRK5 acts as a negative regulator of dark-induced senescence through SA-signaling pathway. To test this hypothesis, we used single and double mutant lines impaired in *CRK5* activity and deficient in SA-synthesis or catabolism (*sid2* and NahG). The constitutive expresser of *PR* genes 1 (*cpr1*) mutant, which accumulates high SA levels and constitutively expresses PR genes due to a defect in a ubiquitin ligase component, served as a comparison line because it displays elevated SA-dependent defense responses (Clarke et al. 2000; Furlan et al. 2012). Since SA and ET have been reported to synergistically regulate leaf senescence through interactions between their signaling components, for example, physical interaction between ETHYLENE-INSENSITIVE-3 (EIN3) and NON-EXPRESSOR OF PR GENES 1 (NPR1) (Wang et al. 2021), we also included ET-signaling mutants to assess the contribution of ET to the *crk5* phenotype. We found that introducing SA-synthesis or catabolism deficiencies, but not ET-signaling deficiencies, into *crk5* background reverted the dark-induced accelerated senescence phenotype. RNA-seq analysis supported the involvement of CRK5 in SA-dependent regulation of senescence. Moreover, our foliar fluorescence and thermal imaging studies revealed a reduced

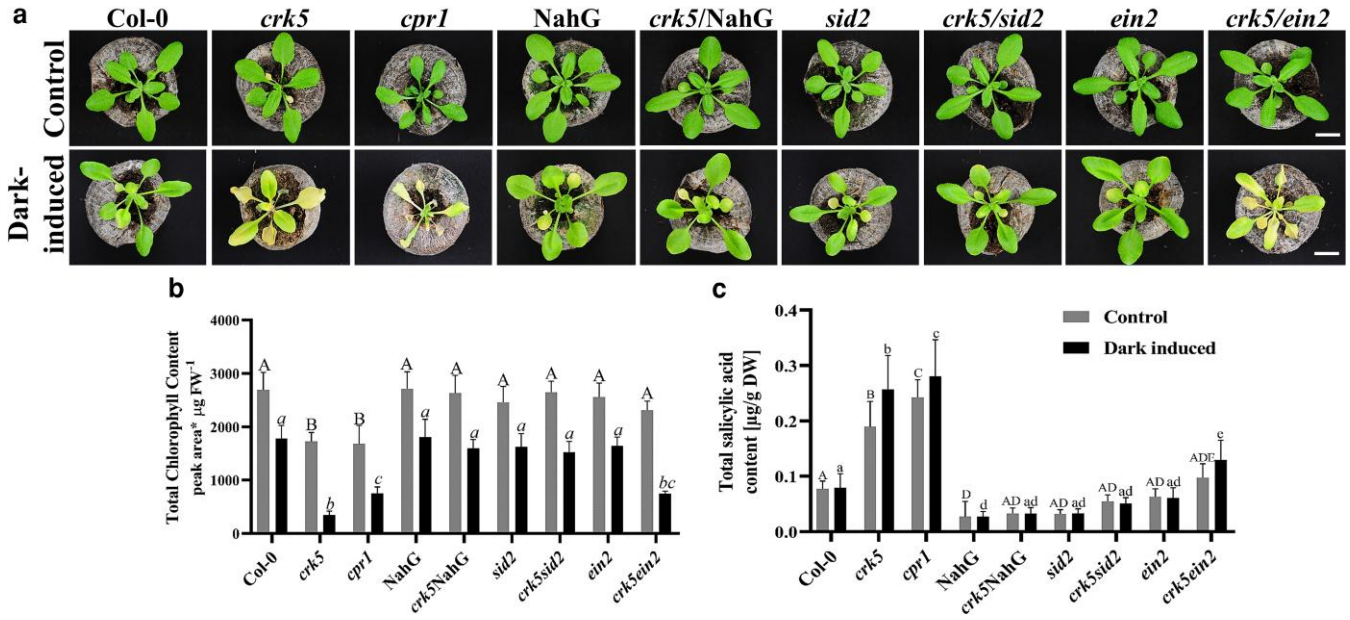


Figure 1 Morphology, total foliar chlorophyll, and salicylic acid (SA) content of the Arabidopsis salicylic acid-synthesis or catabolism and ethylene-signaling mutants in control and after exposure for 4 days to permanent darkness. The picture shows a phenotype of 4-week-old plants growing under ambient light (120 μmol m^{-2} s^{-1}) conditions and exposed to four days of permanent darkness **a)**, Total chlorophyll content **b)**, and Total SA content **c)**. White scale bars indicate 1 cm. Mean values (±SD) were derived from 9 plants ($n = 9$). Statistical analysis was performed according to a *t*-test at a level of $P < 0.05$. Letters A, B, C, D, E, and a, b, c, d, e above the bars indicate homogenous groups, and values sharing common labels (letters) are not significantly different from each other.

NPQ and lower foliar temperature in SA-accumulating lines (*crk5* and *cpr1*) compared to the wild type. These results are consistent with the established effects of SA on reducing NPQ and modifying photo-protective responses.

Taken together, our data indicate that CRK5 simultaneously functions as a negative regulator of SA-mediated dark-induced senescence and as a positive regulator of NPQ and foliar temperature during episodes of dissipation AEE as heat. This dual regulatory activity positions CRK5 as a key mediator in balancing senescence and photo-protection mechanisms to maintain leaf homeostasis under stress conditions.

Results

CRK5 is a negative regulator of SA signaling during dark-induced senescence

In order to confirm previous studies showing that CRK5 negatively regulates dark-induced senescence, we performed microscopic analysis of chloroplast membrane stability in dark-treated leaves. We found a significant acceleration in the chloroplast rupture in *crk5* mutant compared to wild type plants (Fig. S1). Based on previous studies, salicylic acid (SA) appeared as the most promising candidate involved in CRK5-dependent signaling, while ethylene (ET) was found to act synergistically with SA in the regulation of leaf aging (Wang et al. 2021). Therefore, to get a closer insight into the hormonal pathways involved in the progression of dark-induced senescence, we crossed several mutants disturbed in SA-synthesis or catabolism and ET-signaling pathways with *crk5* and checked their phenotypic features under ambient light and after 4 days in the darkness. We observed that the introduction of SA-synthesis or catabolism deficiencies (*sid2* and NahG), but not ET-signaling deficiency (*ein2*), into *crk5* background reverted decreased chlorophyll content and accelerated dark-induced senescence of the *crk5* plants to the wild type phenotype (Fig. 1a, b). In order to confirm that disturbed CRK5 activity in *crk5* mutant was directly associated with enhanced leaf aging in the darkness, we also included complementation lines used in our previous study (Burdiak et al. 2022) and found a reversion of the observed phenotype (Fig. S2). The *cpr1* mutant with constitutive SA-dependent signaling, used as a positive control, also showed an accelerated dark-induced aging phenotype and lowered chlorophyll content (Fig. 1a, b). The chlorophyll *a/b* ratio was higher, specifically in those genotypes showing accelerated dark-induced senescence (Fig. S3). The phenotype of mutants showing accelerated leaf aging under darkness (*crk5*, *cpr1*, *crk5ein2*) correlated with significantly increased SA level, but did not correlate with ET content (Fig. 1c and Fig. S4), and it was reverted in *crk5sid2* and *crk5*NahG, supporting a role of the SA pathway in the regulation of this process. On the basis of these studies, the CRK5 appeared as a negative regulator of SA-signaling and dark-induced senescence.

SA signaling negatively regulates foliar temperature, photosynthesis, and excess energy dissipation

Taking into consideration that CRK5 acts as a negative regulator of dark-induced senescence in a SA-dependent manner, we hypothesized that it may function as a broader regulator of stress responses through its role in SA-signaling. Since SA participates both in senescence and in photo-protective responses, we tested whether CRK5 also contributes to the regulation of excess light responses. Therefore, we focused on estimating whether CRK5 could be involved in the regulation of foliar temperature during dissipation of AEE as heat. The analysis performed with the use of a thermal imaging infrared camera revealed a significant reduction in the delta foliar

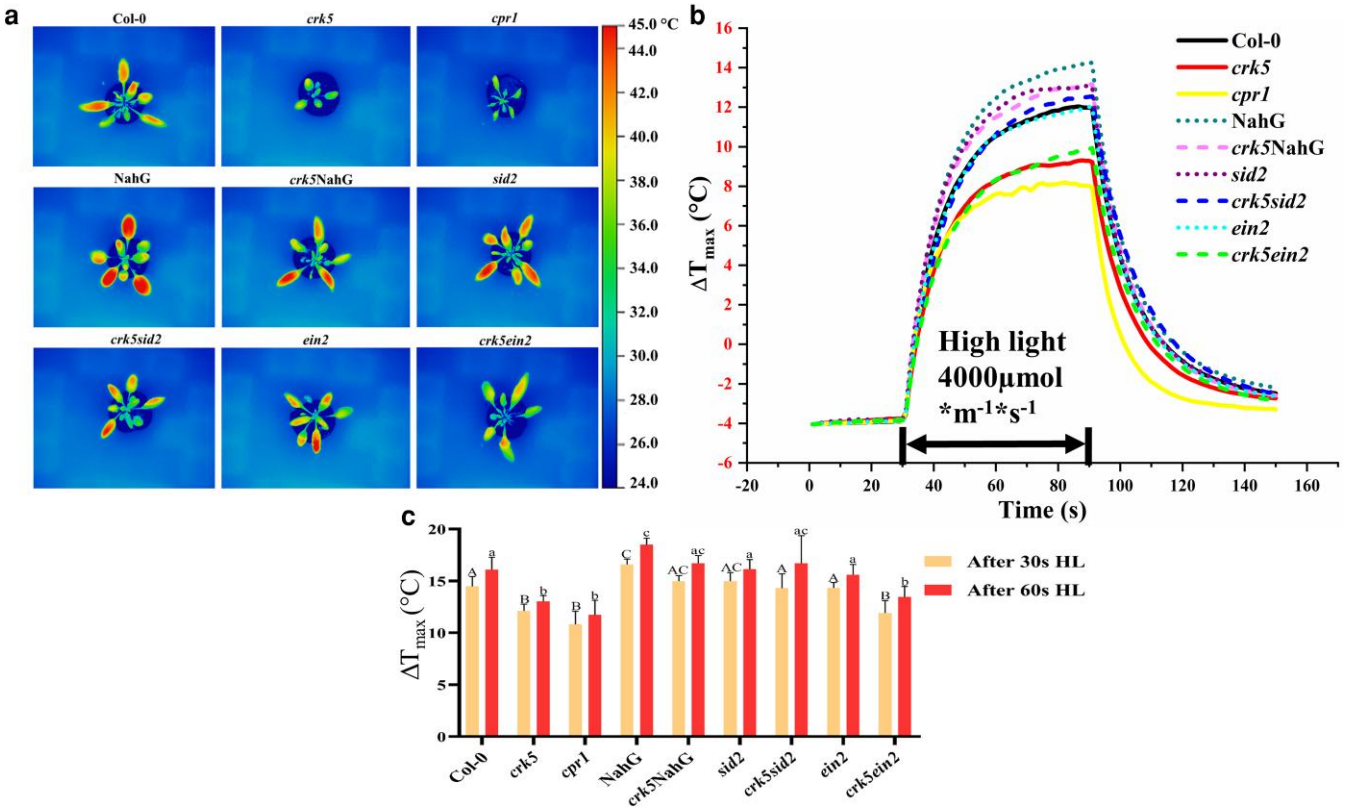


Figure 2 Analysis of foliar temperature under variable light conditions in Arabidopsis salicylic acid-synthesis or catabolism and ethylene-signaling mutants. The thermograms displaying whole rosettes of various Arabidopsis genotypes after 30 seconds of high light (4,000 μmol photons m^{-2} s^{-1}) **a)**. The plot displays dynamic changes in delta foliar maximal temperature (ΔT_{max}) under varying light levels relative to the white background on which the plants were placed for the measurement. The white surface reflected light, and exposure to intense light only caused a 1.5 °C rise in temperature. The following was the program's setup: 30 s of ambient light (150 μmol photons m^{-2} s^{-1}), followed by 60 s of blue LED light (4,000 μmol photons m^{-2} s^{-1}), and lastly by 60 s of ambient light (150 μmol photons m^{-2} s^{-1}) **b)**. The average leaf temperature changes after 30 and 60 s of high light exposure **c)**. For temperature analysis, data represent mean values ± SD of 3 different leaves from 9 plants ($n = 27$). Statistical analysis was performed according to a *t*-test at a level of $P < 0.05$. Letters A, B, C, and a, b, c above the bars indicate homogenous groups, and values sharing common labels (letters) are not significantly different from each other.

maximal temperature (ΔT_{max}) in *crk5* and *cpr1* plants, compared to wild type, during episodes of AEE as heat (Fig. 2a–c). ΔT_{max} refers to the difference between the foliar temperature measured in ambient low-light laboratory conditions and the maximal foliar temperature in the AEE dissipation as heat in a short episode. Keeping the plants in prolonged darkness triggered a reduction of ΔT_{max} in all analyzed lines, but the pattern of ΔT_{max} changes within genotypes remained the same (Fig. S5). We observed that the introduction of SA-synthesis or catabolism deficiencies (*sid2* and NahG), but not ET-signaling deficiency (*ein2*), into the *crk5* background reverted lowered ΔT_{max} to the wild type (Fig. 2a–c). It indicates that, unlike ET, the SA-signaling is predominantly involved in the regulation of foliar temperature in a CRK5-dependent manner. In order to get a closer insight into the role of SA in the foliar temperature regulation process, exogenous SA was applied to all analyzed genotypes, which led to a significant decrease in the ΔT_{max}. The most significant reduction was observed in the SA-synthesis or catabolism mutants, NahG and *sid2*, which further supports the negative effect of SA on foliar temperature regulation (Fig. S6). Lower ΔT_{max} in SA-accumulating lines (*crk5*, *cpr1*), reversion of this phenotype in *crk5sid2* and *crk5*NahG lines, and higher ΔT_{max} in SA-catabolism mutant line (NahG) clearly indicate that endogenous SA level in Arabidopsis negatively correlates with foliar temperature (Fig. S7a, b). The Pearson's correlation analysis showed that for each 0.01 μg ΔSA content, there is a 0.30 °C reduction in $\Delta\Delta T_{max}$ in control and a 0.16 °C reduction in constant darkness conditions (Fig. S7a, b), supporting a negative correlation between SA and foliar temperature. Then we performed the gas exchange analysis and noticed that the *crk5* and *cpr1* showed the opposite stomatal conductance rates, compared to the wild type. The *crk5* plants showed significantly higher stomatal conductance (reflecting stomatal opening/closure) and evapotranspiration (reflecting the actual flux of water vapor leaving the leaf and entering the air stream), similar to SA-synthesis or catabolism mutants, while *cpr1* displayed the opposite phenotype (Fig. S8a, b). It revealed that in our experimental setup, stomatal conductance and evapotranspiration rate were not directly influenced by SA-signaling, since they did not correlate with observed differences in foliar temperature (Fig. S9a, b).

Our studies revealed that SA-accumulating lines (*crk5* and *cpr1*), which showed lower foliar temperature, had also disturbed the NPQ mechanism, which regulates AEE dissipation as heat (Fig. 3a). The Pearson's correlation analysis proved that a positive linear correlation exists between ΔNPQ and $\Delta\Delta T_{max}$ in control and under dark conditions, as it shows that for each 0.01 ΔNPQ increase, there is a 0.23 °C increase in control and a 0.11 °C increase in dark-induced $\Delta\Delta T_{max}$ °C (Fig. S10a, b). In line with that, when we calculated our data for the correlation between ΔNPQ and ΔSA, we observed a

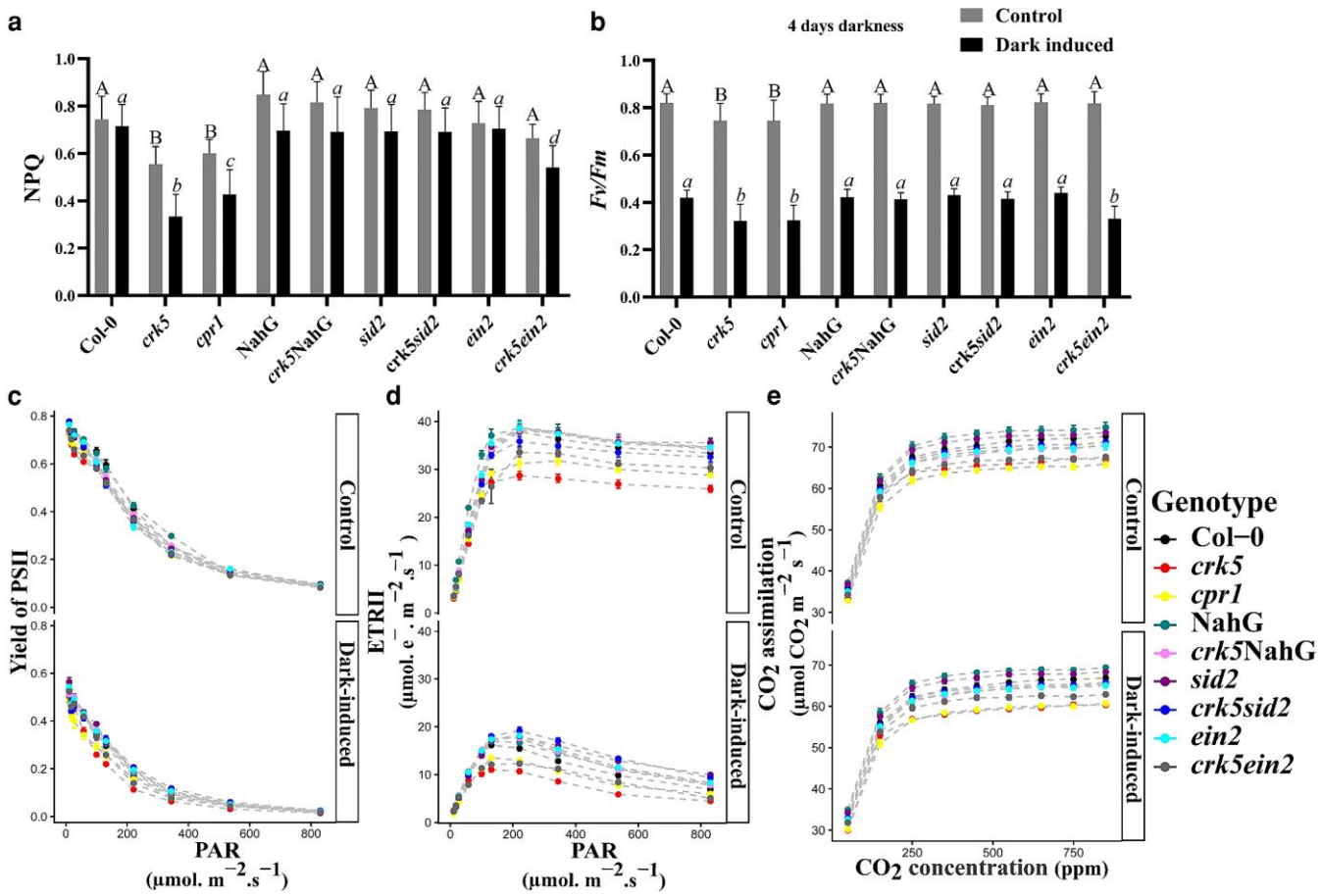


Figure 3 Photosynthetic and foliar gas exchange parameters analysis of the Arabidopsis salicylic acid-synthesis or catabolism and ethylene-signaling mutants in control and after exposure for 4 days to permanent darkness. Non-photochemical quenching (NPQ) **a)**, maximal photochemical efficiency of photosystem II (*Fv/Fm*) **b)**, yield of PSII **c)**, electron transport rate of PSII **d)**, and CO_2 assimilation **e)**. For **a)** and **b)** panels, mean values were derived from 9 plants ± SD ($n = 9$), and statistical analysis was performed according to a *t*-test at a level of $P < 0.05$. Letters A, B, and a, b, c, d, above the bars indicate homogenous groups, and values sharing common labels (letters) are not significantly different from each other. For **c)**, **d)**, and **e)** panels, the mean values were derived from 9 plants ± SD ($n = 9$) and statistical analysis was performed according to a *t*-test at a level of $P < 0.05$(*), $P < 0.005$ (**), or $P < 0.001$ (***) relative to Col-0 as shown in Table S1.

negative correlation between ΔNPQ and ΔSA in all analyzed genotypes. For each 0.01 ΔSA content (µg) increase, there was a 0.012 ΔNPQ decrease in control and a 0.013 decrease under darkness (Fig. S10c, d).

Further, we measured chlorophyll fluorescence and CO_2 assimilation rate in all analyzed lines in order to investigate the role of SA-signaling in the regulation of photosynthesis. The maximum quantum yield of PSII (*Fv/Fm*) and the operating quantum efficiency of PSII (ΦPSII) under variable light conditions were significantly reduced in *crk5* and *cpr1* plants, both under control and dark-induced conditions. Opposite to that, double mutant lines with disrupted SA-synthesis or catabolism (*crk5sid2* and *crk5*NahG) reverted this phenotype (Fig. 3b, c). The photosystem II electron transport rate (ETRII), which is directly related to ΦPSII and reflects actual photochemical activity in PSII, was also reduced in both analyzed SA-accumulating mutant lines (Fig. 3d). Since electrons generated by PSII electron transport are ultimately used for the reduction of CO_2 in the Calvin cycle, we also analyzed CO_2 assimilation rate. In line with the observed differences in chlorophyll fluorescence parameters, the photosynthetic CO_2 uptake efficiency was also reduced in *crk5* and reverted in SA-synthesis or catabolism *crk5sid2*, *crk5*NahG double lines (Fig. 3e and Table S1). These results suggest that enhanced SA-signaling negatively affects the amount of quantum light absorbed by chlorophyll during photochemistry and leads to impaired thermal dissipation of excess excitation energy (Fig. 3), which is correlated to decreased foliar temperature in plants with enhanced SA content (Figs. 1c and 2).

CRK5-regulated transcriptomic changes reveal a key role of salicylic acid in dark-induced senescence

To get a closer insight into signaling pathways driven by CRK5, we performed whole transcriptome sequencing for the analyzed genotypes under ambient light and after four days of darkness. In control conditions, a 4-week-old SA-synthesis mutant (*sid2*) and SA-accumulating mutant (*cpr1*) showed the most pronounced changes in the number of differentially expressed genes, supporting the essential role of SA-signaling in dark-induced senescence (Fig. S11a, b). Interestingly, SA-accumulating lines (*crk5* and *cpr1*) showed a clear deregulation of many SA-related genes compared to wild type, particularly pathogenesis-related (*PR1* and *PR5*) and *WRKY38*, whereas SA-synthesis or catabolism-deficient lines demonstrated the opposite gene expression pattern (Fig. 4b). As expected, the transcriptional

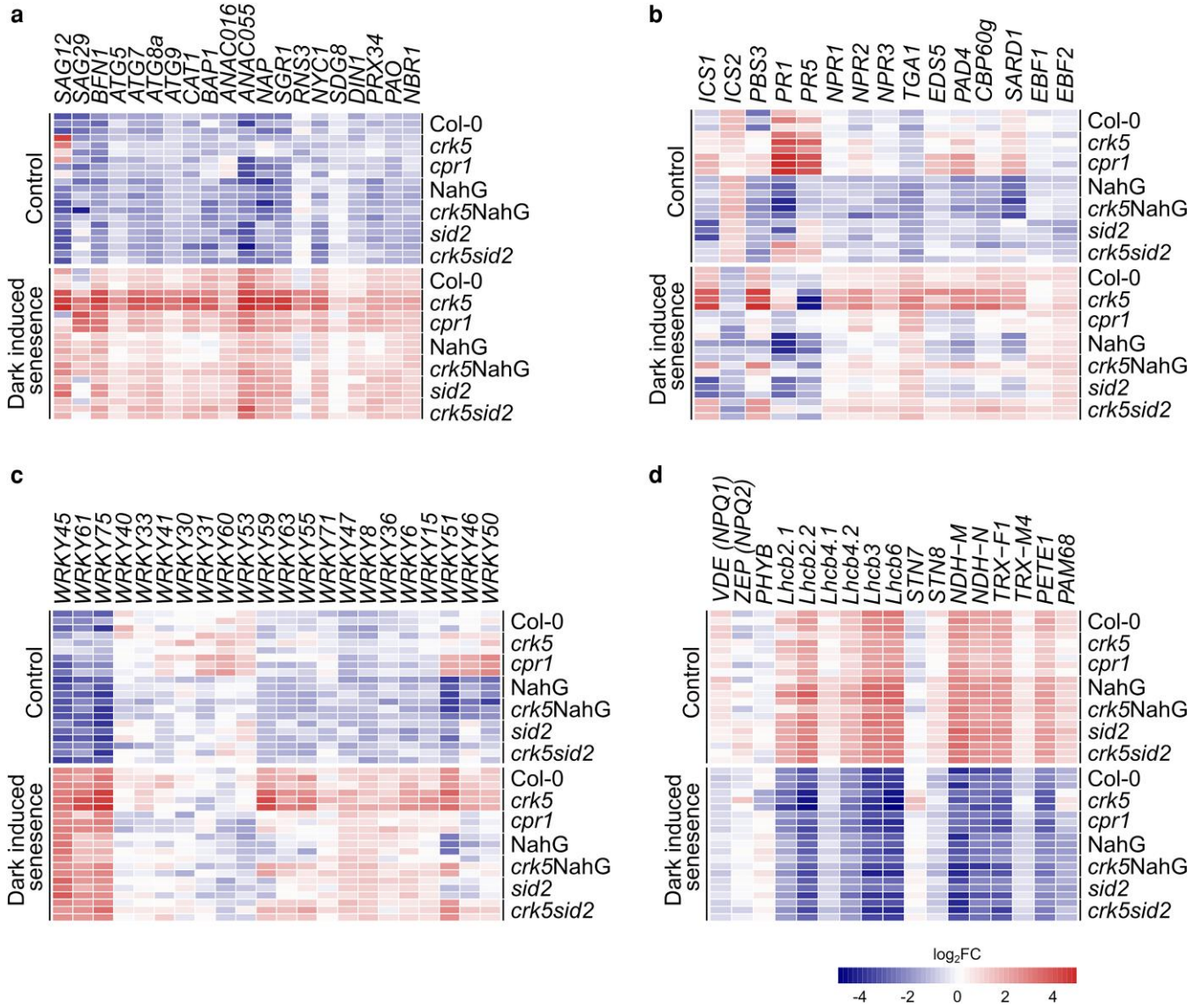


Figure 4 RNA seq analysis in control and after exposure for 4 days to permanent darkness. In total, 7 genotypes were used for this analysis: Col-0, *crk5*, *cpr1*, NahG, *sid2*, and two double mutant lines, *crk5*NahG and *crk5sid2*. Heat map shows the expression patterns of selected senescence marker genes **a)**, SA signaling pathway genes **b)**, WRKY transcription factors **c)**, and NPQ and photosynthesis-related genes **d)**.

changes in senescence markers were not particularly relevant during ambient light, except for *crk5*, which showed upregulation of *SAG12*, as the effect of premature onset of aging processes (Fig. 4a). In accordance with that, the gene ontology (GO) enrichment analysis demonstrated that GO terms related to chlorophyll and other pigments catabolic processes, senescence, and response to SA were the most significantly overrepresented among genes induced in *crk5* (Fig. S12a). Keeping the plants for 4 days in constant darkness resulted in upregulation of many senescence-associated and SA-related genes (Fig. 4a, b). Under these conditions, the *crk5* plants showed clearly the most significant alterations in transcriptomic profiles, with more than 3,500 differentially expressed genes, compared to the wild type (Fig. S11c, d). The GO term analysis in *crk5* revealed overrepresentation of induced genes involved in pigment catabolic process, plant organ senescence, and response to light intensity (Fig. S12b). In this mutant line, we observed especially profound changes in the expression of senescence-related genes, such as *SAG12, BFN1*, *ANAC055*, *NAP*, *SGR1*, *RNS3*, *NYC1*, as well as autophagy-related genes *ATG7*, *ATG8*, and *ATG9* (Fig. 4a). The analysis of dark-exposed *crk5* plants also revealed an increased transcript abundance of an array of SA-associated genes as well as WRKYs, and a clear deregulation of NPQ- and photosynthesis-related genes, suggesting that SA-signaling might play a role in the development of accelerated foliar aging phenotype (Fig. 4b–d). Surprisingly, in spite of clear foliar senescence symptoms in *cpr1* mutant under darkness (Fig. 1a), the transcriptional changes were not so pronounced as in the case of *crk5*, suggesting that CRK5 might act as a major regulator of SA-related pathways during dark-induced senescence, upstream of CPR1. Gene expression changes observed during dark-induced senescence in the *crk5* plants were almost completely reverted in SA-synthesis or catabolism double mutants (*crk5sid2*, *crk5*NahG), supporting the role of SA-signaling in the development of aging processes under darkness.

Transcriptomic results of selected genes were also supported by Western blot. It showed a substantially higher accumulation of

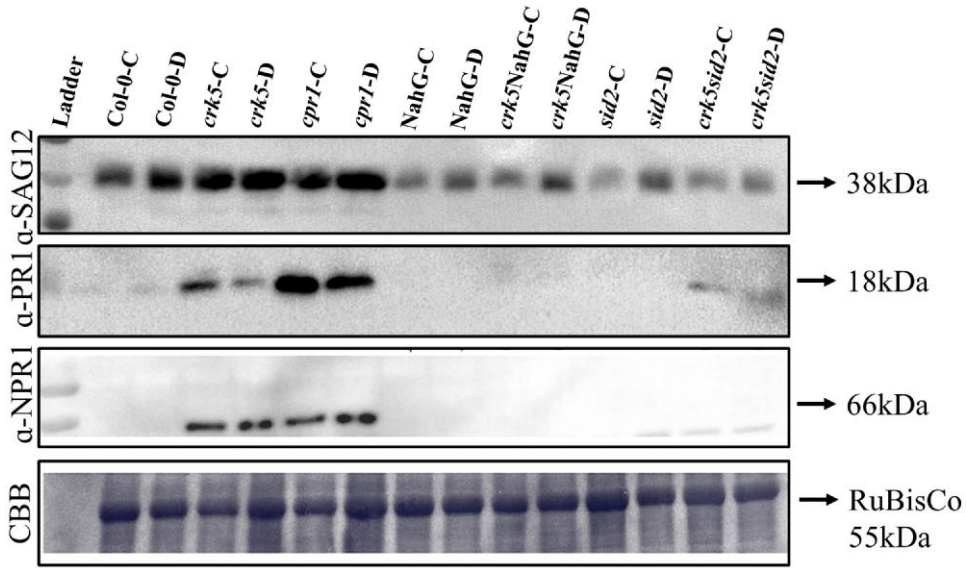


Figure 5 Level of SAG, PR1, and NPR1 proteins in control and after exposure for 4 days to continuous darkness and SA accumulation. Western blot analysis on gels is shown here, and the relative intensity for the bands is shown in Fig. S13. CBB staining was used as a loading control.

NPR1 and PR1 proteins in *crk5* and *cpr1* under both ambient light and darkness conditions compared to wild type, which was reverted in *crk5sid*, *crk5*NahG double mutant plants. The protein level of SAG12 was also enhanced in aging *crk5* and *cpr1*. It was relatively high in wild type plants as well, but almost completely abolished in SA-synthesis or catabolism-deficient mutants (Fig. 5, Fig. S13).

Discussion

Previous studies have shown that CRK5 acts as a negative regulator of foliar senescence and a modulator of stomatal conductance, biomass production, and leaf growth (Chen et al. 2003; Bourdais et al. 2015; Burdiak et al. 2022) In the present study, we provided genetic, molecular, and physiological evidence that CRK5 delays dark-induced senescence in plants through inhibition of the SA-signaling pathway and, at the same time, promotes photosynthesis and energy dissipation as heat (Fig. 6).

In accordance with earlier reports, we found a significant increase in SA content, a decrease in total chlorophyll content, and an increased chlorophyll *a*/*b* ratio in dark-treated *crk5*, *cpr1,* and *crk5ein2* compared to Col-0. Introduction of SA-synthesis or catabolism deficiencies (*sid2* and NahG) but not ET-signaling deficiency (*ein2*) into the *crk5* background reverted its accelerated dark-induced senescence phenotype (Fig. 1a, b and Fig. S3). Previous reports showed that keeping plants in continuous darkness for longer times can lead to the development of premature aging processes, induce a rapid reduction of photosynthetic pigments, and increase in chlorophyll *a*/*b* ratio due to the breakdown of light-harvesting complexes for PSII (LHCB), which are enriched in chlorophyll *b*, thereby reducing antenna size and altering photosynthetic efficiency (Yoo et al. 2003; Lichtenthaler and Babani 2021; Zhang et al. 2023a).

Previous studies have demonstrated that SA induces the production of HSPs, which act as molecular chaperones protecting proteins from heat-induced denaturation, contributing to basal thermotolerance in plants such as Arabidopsis. Endogenous SA levels positively correlate with heat tolerance during both heat shock and recovery phases (Clarke et al. 2004). Consistent with these findings, our results show that SA-accumulating lines *crk5* and *cpr1* exhibit lower foliar temperature and reduced NPQ during AEE as heat stress than SA-synthesis or catabolism mutants (*sid2* and NahG) lines (Figs. 2 and 3a). Exogenous application of SA reduced the foliar temperature across all genotypes, confirming that SA-signaling negatively modulates this process (Figs. S6 and S7). These data suggest that elevated SA levels reduce NPQ induction, potentially serving as a protective mechanism to prevent leaf overheating in response to the dissipation of AEE as heat (Fig. S6).

It is well established that foliar temperature regulation depends on stomatal conductance through transpiration (Urban et al. 2017).

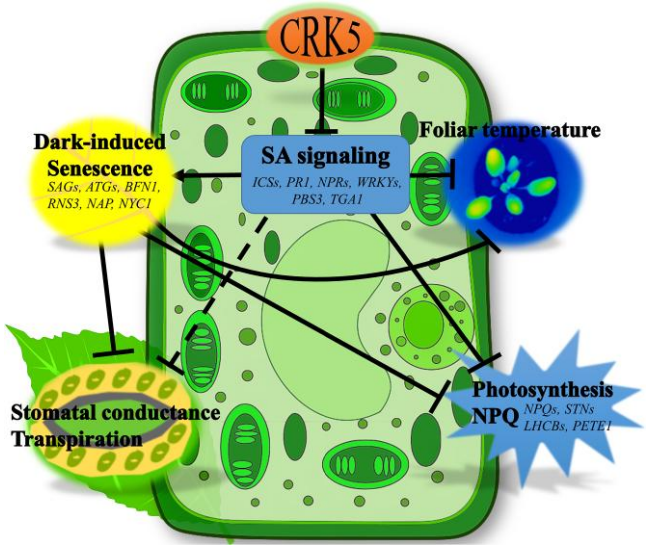


Figure 6 A model summarizing the negative regulatory role of CRK5 in the salicylic acid signaling. The CRK5-driven inhibition of SA-signaling leads to the induction of the foliar temperature, photosynthesis, and non-photochemical quenching and delays the onset of aging processes.

However, foliar temperature may also be elevated due to increased heat production, due to higher NPQ during AEE (Witoń et al. 2021; Burdiak et al. 2022; Zarrin Ghalami et al. 2025, 2026). Thermal imaging studies reveal that NPQ amplitude correlates strongly with adaxial (upper), while stomatal conductance with abaxial (lower) leaf surface temperature (Trojak and Skowron 2023). Hence, NPQ and stomatal conductance together intricately regulate leaf thermal balance. While our data show a consistent positive correlation between changes in NPQ and ΔT_{max} in the genotypes tested, we acknowledge that correlation does not imply causation. NPQ is a photo-protective mechanism that converts excess absorbed light energy into heat (Ruban 2016). However, whether the heat released by NPQ significantly increases whole-leaf temperature depends on the scale of heat production relative to heat loss from the leaf (via transpiration and radiative/conductive exchange) and on leaf thermal conductance. It was reported that energy dissipated by NPQ is usually small and would typically change leaf temperature by < 0.1 °C (Sobejano-Paz et al. 2023; Murakami et al. 2024). However, in our experiments, plants were exposed to brief but extreme AEE as heat episodes together with genotypes that differ in chloroplast energy handling and SA-synthesis or catabolism. Under such conditions, the relative contribution of energy dissipation as heat in the chloroplast to leaf thermal balance increases, which can explain why NPQ-related parameters correlate with ΔT_{max} in our dataset. Importantly, these findings do not decrease the recognized role of stomatal conductance and evapotranspiration as primary regulators of leaf temperature in many contexts. They rather indicate that chloroplast-level heat production can be an important, sometimes dominant, additional factor when transpiration is restricted, or thermal conductance is low.

Altered SA levels are known to impair photochemical efficiency, as SA-accumulating lines *dnd1* and *cpr5* exhibited lower *Fv/Fm* and NPQ (Mateo et al. 2006). Within our experimental conditions, ΔNPQ negatively correlated with ΔSA (Fig. S10c, d), supporting that SA-accumulation disrupts PSII efficiency. This aligns with previous studies showing that SA slows PSII electron transport *via* direct or indirect mechanisms, including ROS accumulation, pH modulation, and glutathione levels (Ball et al. 2004; Mateo et al. 2006; Janda et al. 2012). Our data revealed that *crk5* mutant exhibits reduced ETRII, lowered CO_2 assimilation, and decreased ΦPSII (Fig. 3), which aligns with reported inhibitory effects of SA on photosynthetic parameters under stress (Mateo et al. 2006), supporting the hypothesis that CRK5 influences photosynthetic performance partly *via* SA-mediated pathways.

Our transcriptomic analysis provides strong molecular evidence that CRK5 regulates dark-induced senescence through SA-dependent transcriptional networks. Consistent with previous reports showing that SA contributes to developmental and stress-induced leaf aging (Morris et al. 2000; Jibran et al. 2013; Kanojia et al. 2020), our results extend this understanding by positioning CRK5 upstream of SA-mediated transcriptional reprogramming in response to darkness. Under ambient conditions, the most pronounced transcriptional divergence was observed in *sid2* (SA-synthesis) and *cpr1* (SA-accumulating) mutants, indicating that SA-signaling is essential during the vegetative-to-senescent transition (Fig. S11a, b).

Under exposure to constant darkness, the *crk5* plants exhibited the largest transcriptomic shift. Notably, genes associated with macromolecular degradation and autophagy were among the most induced in *crk5* (Fig. 4a), which is in accordance with previous studies describing autophagy as an important mechanism in nutrient remobilization, active catabolic remodeling, and senescence (Guiboileau et al. 2012; Avila-Ospina et al. 2014). This overlap between SA-responsive and autophagy-related genes in *crk5* points to a coordinated mechanism in which CRK5 balances SA-driven senescence and autophagy to optimize stress responses (Fig. 4b). The GO enrichment analysis revealed that *crk5*-induced genes are closely associated with chlorophyll catabolism, senescence, and light responses, reflecting physiological decline observed in aging leaves, which was consistent with a previous report on dramatic changes in transcripts during the senescence process (Fig. S12) (Breeze et al. 2011).

The *cpr1* mutant, which constitutively accumulates SA (Clarke et al. 2000; Jirage et al. 2001; Hedtmann et al. 2017), served as a positive control, reinforcing the central role of SA in dark-induced senescence. Consistent with its elevated SA levels, *cpr1* displayed reduced chlorophyll content, increased NPQ, lower foliar temperature, and strong induction of SA-responsive genes, in line with previous reports describing CPR1 as a pleiotropic regulator of senescence (Jing et al. 2007). Surprisingly, despite obvious changes in morphological phenotype after darkness, *cpr1* did not show significant transcriptional reprogramming of SA-related genes after darkness, suggesting that CRK5 functions upstream of CPR1 in orchestrating SA-dependent dark-induced senescence.

In summary, CRK5 functions as a central regulator of salicylic acid (SA)-dependent transcription factors, including WRKY family members and key components of the SA-signaling pathway, thereby CRK5 negatively regulate the expression of downstream senescence effectors. Our data further demonstrate that CRK5 positively modulates foliar temperature, NPQ, and photosynthetic efficiency, supporting its role as a key signaling kinase that maintains leaf longevity through precise regulation of SA-mediated senescence and photoprotective processes.

Materials and methods

Plant material and growing conditions

All Arabidopsis (*Arabidopsis thaliana*) plants used in this research were in the Col-0 background. The generation of double mutant lines was achieved by crossing, while the generation of complementation lines was previously described in (Burdiak et al. 2015). All primers used for genotyping are shown in Table S2. After stratification (3 days at 4 °C), plants were placed in the growing chamber and grown on Jiffy Pots for 4 to 5 weeks in long-day photoperiod (16 h/8 h) under the constant white light of 120 µmol photons m^{-2} sec^{-1} at 22 °C, relative humidity of $70 \pm 5\%$. For dark-induced senescence studies, 4-week-old plants were kept in continuous darkness for four days.

Confocal microscopic analysis of chloroplast rupture

The study involved Arabidopsis plants expressing enhanced green fluorescence protein (EGFP) fused to a transit peptide of ferredoxin NADP(H) oxidoreductase (FNR) anchored to chloroplast stroma (Marques et al. 2004; Schattat et al. 2011; Górecka et al. 2020), which were crossed with the *crk5* mutant.

The material for analysis was obtained from plants kept for 4 days in the dark. Sections from central regions next to the mid-vein and tip of the leaves were taken for confocal microscopy observations using an LSM700 microscope equipped with 20× EC Plan-Neofluar objectives (Zeiss, Germany). We used SP555 and BP652-682 filters for GFP and chlorophyll auto-fluorescence signals, respectively. The induction of fluorescence was conducted with a 488-nm laser.

Pigment content analysis

20–50 mg of frozen tissue was homogenized in a Mixer Mill MM 400 (Retsch, Düsseldorf, Germany) (5 min, 4 °C, 30 Hz) with 1 ml of cold acetone (−20 °C). The homogenate was evaporated using Savant DNA120 SpeedVac (Thermo Scientific, Waltham, MA, USA), dissolved in cold solvent A (acetonitrile: methanol; 90:10; v/v), and re-homogenized for 1 min. The extract was filtered through a 0.2 μm nylon filter (Whatman) into an auto-sampler vial, capped, and stored in the dark at −80 °C for HPLC analysis (Shimadzu, Kyoto, Japan). The pigments were separated on a Synergi*TM* 4 μm MAX-RP 80 Å LC Column 250 × 4.6 mm (Phenomenex, Torrance, CA, USA) at 30 °C. Solvent A was used for 10 min to elute all xanthophylls, followed by solvent B (methanol: ethyl acetate; 68:32; v/v) for 10 min at a flow rate of 1 ml/min. The results are given as the peak area per μg of fresh weight, according to the protocol previously used (Rusaczonek et al. 2015, 2021).

Determination of SA content

Determination of SA was performed as described by Meuwly and Métraux (1993). 2-Methoxybenzoic acid and 3-hydroxybenzoic acid were used as the internal standards. SA was eluted on a Luna 5uC18(2)100A 150 × 4.6 mm column (Phenomenex, Torrance, CA, USA) at 30 °C for 15 min using a Shimadzu HPLC System (Columbia, MD, USA). A low-pressure gradient system was used with 20 mmol phosphate buffer (pH 2.5; adjusted with 8 M HCl) and acetonitrile (75:25; v/v) at a flow rate of 1 mL min^{-1}. The results were calculated as micrograms of SA per gram of dry weight.

Determination of ethylene content

The ethylene production was assessed using a previously described method (Burdiak et al. 2015; Małachowska and Tomala 2022). A gas sample (1 mL) was collected using a syringe. The measurement was conducted using gas chromatography (HP 5890, Hewlett-Packard, Palo Alto, CA, USA). Measurements were made in at least four biological and three technical replicates. The results of ethylene production are expressed in $\mu L \cdot kg^{-1} \cdot h^{-1}$ per fresh weight.

Determination of foliar temperature under variable light conditions

The analysis was performed on whole soil-grown *Arabidopsis* rosettes as described by Burdiak et al. (2022).

Gas exchange analysis

Stomatal conductance (*gs*), evapotranspiration (E), and net CO_2 assimilation rate were measured using a portable gas-exchange system CIRAS-3 (PP Systems, Amesbury, MA, USA) in fixed conditions of CO_2 concentration (400 μmol CO_2 m^{-2} s^{-1}). During measurements, the CO_2 concentration in the leaf cuvette was maintained (according to Ci Ramp protocol), humidity at 80%, light intensity at 200 μmol m^{-2} s^{-1}, and temperature at 25 °C.

Chlorophyll *a* fluorescence

Chlorophyll *a* fluorescence parameters were determined on whole rosettes using a pulse amplitude-modulated FluorCam 800 MF with the associated software (Photon Systems Instruments, Drasov, Czech Republic) and Dual PAM-100 (Heinz Walz, Effeltrich, Germany) equipped with DUAL-DB module for measuring the fluorescence. Before measurements, the plants were kept in darkness for 30 min to determine F_0 and F_m, and then exposed to 5 min of actinic red light (90 μmol m^{-2} s^{-1}) to determine F_m'. The maximum photosystem II (PSII) quantum yield $Fv/Fm = (F_m - F_0)/F_m$, and non-photochemical quenching $NPQ = [(F_m/F_m') - 1]$ were determined as described earlier (Kramer et al. 2004; Baker 2008). The effective photosystem II (PSII) quantum yield (ΦPSII) $Y(II) = (F_m' - Fs)/F_m'$ and electron transport rate through PSII ($ETRII = \Phi PSII \times PAR \times 0.5 \times \alpha$), where 0.5 is the assumed proportion of absorbed light reaching PSII and α is the assumed absorbance usually taken as 0.84 as described earlier by (Cun et al. 2021).

Library preparation and RNA-Seq analysis

For RNA isolation, 4- or 4.5-week-old plants were used. The experiment was performed on three biological replicates. Each replicate consisted of several pooled detached leaves from at least three individual plants. Frozen leaves were ground using a mortar and pestle in liquid nitrogen, and RNA was isolated using the Spectrum™ Plant Total RNA Kit (Sigma, St. Louis, MO, USA). mRNA fraction was isolated using Oligo d(T)25 Magnetic Beads (NEB, S1419S) from 20 ug of total RNA. RNA-seq libraries were self-prepared according to the (Veeranagouda et al. 2019). RNA-seq libraries were sequenced in 150-bp paired-end reads using the Aviti High flowcell platform (Element Biosciences), conducted by Genomed (Warsaw, Poland). Unique Molecular Identifiers (UMIs) were extracted from R2 reads using UMI-tools extract (Smith et al. 2017). Subsequently, R1 reads underwent adapter trimming and quality filtering to remove low-quality sequences using Trim Galore (https://github.com/FelixKrueger/TrimGalore). The cleaned reads were then aligned using STAR (Dobin et al. 2013) to the *Arabidopsis thaliana* reference genome (Ensembl Plants release 60). To eliminate PCR duplicates, UMI-tools dedup was employed, leveraging both UMI sequences and mapping positions. Gene-level read counts were generated using htseq-count (Putri et al. 2022). The resulting count data were imported into R, where a count matrix was constructed. Differential expression analysis was performed using the DESeq2 package (Love et al. 2014). Significantly enriched GO terms were identified in up-/downregulated gene sets using the package (v4.10.1) (Wu et al. 2021).

Protein extraction and western blot analysis

Total plant protein extraction was performed as described by Bernacki et al. (2024) with some modifications. Proteins were extracted from 100 mg of ground leaf tissue. The powder was re-suspended in 500 μL of 2× Laemmli buffer (4% SDS, 20% glycerol, 0.12 M Tris-HCl pH 7.0, 0.02% bromophenol blue, and 0.7 M β-mercaptoethanol) and incubated for 10 min at 95 °C, followed by incubation for 10 min on ice (Laemmli 1970). The re-suspended samples were centrifuged at 12,000× *g* for 5 min at 4 °C, and the supernatants were used for further steps. Total protein concentration was determined using the RC-DC protein assay kit II (Bio-Rad, Hercules, CA, USA, 5000122). A total of 20 μg of total protein extract was used for 12% SDS-PAGE. Next, the proteins were electro-transferred to an Immobilon P PVDF membrane (Merck, Darmstadt, Germany) using the Trans-Blot Turbo Transfer System (Bio-Rad). The membrane was blocked in 5% skim milk in TBS-T buffer (1× Tris-buffered Saline, 0.1% Tween-20) for at least 1 h and incubated with primary antibodies (diluted at a ratio of 1:10,000, 5% skim milk in TBS-T buffer) SAG12 (Phy3591A, PhytoAB, California, USA) and PR1 (AS10687, Agrisera, Vännäs, Sweden) for 1 h at room temperature, and NPR1 (Phy3594A, PhytoAB, California, USA) overnight at 4 °C. Incubation

with the goat anti-rabbit horseradish peroxidase-conjugated secondary antibodies (Thermofisher, QG221919; diluted at a ratio of 1:10,000) was performed for 1 h at room temperature. Protein bands were immunodetected using SuperSignal West Dura Extended Duration Substrate (Thermo Scientific, 34075) according to the manufacturer's recommendations, visualized with the ChemiDoc XRS + System (Bio-Rad), and analyzed with ImageLab Software 5.2.1 (Bio-Rad). Total protein staining of membranes was conducted as described previously (Goldman et al. 2016).

Statistical analysis

The statistical analysis of chlorophyll *a* and *b* fluorescence, non-photochemical quenching, gas exchange, pigment content, SA and ET content, and foliar temperature was performed using GraphPad Prism 8 software.

Accession numbers

Accession numbers of all genes in Fig. 4a and b are given in Table S3, and in Fig. 4c and d in Table S4.

Author contributions

SK and PB proposed the hypothesis, and SK, PB, and MK designed the research. MK, PB, AR, RZG, and MD performed the research. PG and KG analyzed the RNA-seq data. MK, PB, and SK analyzed the data holistically. MK, PB, and SK wrote and revised the manuscript.

Supplementary material

Supplementary material is available at *Plant Physiology* online.

Funding

This research was funded by the Narodowe Centrum Nauki (National Science Centre, Opus 20 UMO-2020/39/B/NZ3/02103), granted to S.K.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

The raw RNA-seq (fastq) data are deposited in the NCBI database (BioProject: PRJNA1389866).

References

Antolín-Llovera M *et al.* 2014. Knowing your friends and foes–plant receptor-like kinases as initiators of symbiosis or defence. *New Phytol.* 204:791–802. https://doi.org/10.1111/nph.13117.

Arndt LC *et al.* 2022. Genomic distribution and context dependent functionality of novel WRKY transcription factor binding sites. *BMC Genomics*. 23:673. https://doi.org/10.1186/s12864-022-08877-y.

Avila-Ospina L, Moison M, Yoshimoto K, Masclaux-Daubresse C. 2014. Autophagy, plant senescence, and nutrient recycling. *J Exp Bot.* 65: 3799–3811. https://doi.org/10.1093/jxb/eru039.

Baker NR. 2008. Chlorophyll fluorescence: a probe of photosynthesis in vivo. *Annu Rev Plant Biol.* 59:89–113. https://doi.org/10.1146/annurev.arplant.59.032607.092759.

Ball L *et al.* 2004. Evidence for a direct link between glutathione biosynthesis and stress defense gene expression in Arabidopsis. *Plant Cell.* 16: 2448–2462. https://doi.org/10.1105/tpc.104.022608.

Bernacki MJ *et al.* 2024. METACASPASE8 (MC8) is a crucial protein in the LSD1-dependent cell death pathway in response to ultraviolet stress. *Int J Mol Sci.* 25:3195. https://doi.org/10.3390/ijms25063195.

Bernacki MJ, Rusaczonek A, Czarnocka W, Karpiński S. 2021. Salicylic acid accumulation controlled by LSD1 is essential in triggering cell death in response to abiotic stress. *Cells*. 10:962. https://doi.org/10.3390/cells10040962.

Bourdais G *et al.* 2015. Large-scale phenomics identifies primary and fine-tuning roles for CRKs in responses related to oxidative stress. *PLoS Genet.* 11:e1005373. https://doi.org/10.1371/journal.pgen.1005373.

Breeze E *et al.* 2011. High-resolution temporal profiling of transcripts during Arabidopsis leaf senescence reveals a distinct chronology of processes and regulation. *Plant Cell.* 23:873–894. https://doi.org/10.1105/tpc.111.083345.

Burdiak P, Mielecki J, Gawroński P, Karpiński S. 2022. The CRK5 and WRKY53 are conditional regulators of senescence and stomatal conductance in Arabidopsis. *Cells*. 11:3558. https://doi.org/10.3390/cells11223558.

Burdiak P, Rusaczonek A, Witoń D, Głów D, Karpiński S. 2015. Cysteine-rich receptor-like kinase CRK5 as a regulator of growth, development, and ultraviolet radiation responses in Arabidopsis thaliana. *J Exp Bot.* 66: 3325–3337. https://doi.org/10.1093/jxb/erv143.

Chen K, Du L, Chen Z. 2003. Sensitization of defense responses and activation of programmed cell death by a pathogen-induced receptor-like protein kinase in Arabidopsis. *Plant Mol Biol.* 53:61–74. https://doi.org/10.1023/B:PLAN.0000009265.72567.58.

Chen K, Fan B, Du L, Chen Z. 2004. Activation of hypersensitive cell death by pathogen-induced receptor-like protein kinases from Arabidopsis. *Plant Mol Biol.* 56:271–283. https://doi.org/10.1007/s11103-004-3381-2.

Clarke JD, Volko SM, Ledford H, Ausubel FM, Dong X. 2000. Roles of salicylic acid, jasmonic acid, and ethylene in cpr-induced resistance in Arabidopsis. *Plant Cell.* 12:2175–2190. https://doi.org/10.1105/tpc.12.11.2175.

Clarke SM, Mur LA, Wood JE, Scott IM. 2004. Salicylic acid dependent signaling promotes basal thermotolerance but is not essential for acquired thermotolerance in Arabidopsis thaliana. *Plant J.* 38:432–447. https://doi.org/10.1111/j.1365-313X.2004.02054.x.

Cun Z, Zhang J-Y, Wu H-M, Zhang L, Chen J-W. 2021. High nitrogen inhibits photosynthetic performance in a shade-tolerant and N-sensitive species Panax notoginseng. *Photosynth Res.* 147:283–300. https://doi.org/10.1007/s11120-021-00823-5.

Devireddy AR, Tschaplinski TJ, Tuskan GA, Muchero W, Chen J-G. 2021. Role of reactive oxygen species and hormones in plant responses to temperature changes. *Int J Mol Sci.* 22:8843. https://doi.org/10.3390/ijms22168843.

Dobin A *et al.* 2013. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics*. 29:15–21. https://doi.org/10.1093/bioinformatics/bts635.

Foyer CH, Hanke G. 2022. ROS production and signalling in chloroplasts: cornerstones and evolving concepts. *Plant J.* 111:642–661. https://doi.org/10.1111/tpj.15856.

Furlan G, Klinkenberg J, Trujillo M. 2012. Regulation of plant immune receptors by ubiquitination. *Front Plant Sci.* 3:238. https://doi.org/10.3389/fpls.2012.00238.

Goldman A, Harper S, Speicher DW. 2016. Detection of proteins on blot membranes. *Curr Protoc Protein Sci.* 86:10–18. https://doi.org/10.1002/cpps.15.

Górecka M *et al.* 2020. Photosystem II 22 kDa protein level-a prerequisite for excess light-inducible memory, cross-tolerance to UV-C and

regulation of electrical signalling. *Plant Cell Environ.* 43:649–661. https://doi.org/10.1111/pce.13686.

Guiboileau A *et al.* 2012. Autophagy machinery controls nitrogen remobilization at the whole-plant level under both limiting and ample nitrate conditions in Arabidopsis. *New Phytol.* 194:732–740. https://doi.org/10.1111/j.1469-8137.2012.04084.x.

Hassan MU *et al.* 2022. Salicylic acid-mediated physiological and molecular mechanisms in plants under heat stress. In: *Managing plant stress using salicylic acid: physiological and molecular aspects*. Wiley Online Library. p. 163–182. https://doi.org/10.1002/9781119671107.ch9.

Hedtmann C *et al.* 2017. The plant immunity regulating F-Box protein CPR1 supports plastid function in absence of pathogens. *Front Plant Sci.* 8: 1650. https://doi.org/10.3389/fpls.2017.01650.

Himelblau E, Amasino RM. 2001. Nutrients mobilized from leaves of Arabidopsis thaliana during leaf senescence. *J Plant Physiol.* 158: 1317–1323. https://doi.org/10.1078/0176-1617-00608.

Hsu C-Y *et al.* 2022. Chloroplast protein Tic55 involved in dark-induced senescence through AtbHLH/AtWRKY-ANAC003 controlling pathway of Arabidopsis thaliana. *Genes (Basel).* 13:308. https://doi.org/10.3390/genes13020308.

Janda K, Hideg É, Szalai G, Kovács L, Janda T. 2012. Salicylic acid may indirectly influence the photosynthetic electron transport. *J Plant Physiol.* 169:971–978. https://doi.org/10.1016/j.jplph.2012.02.020.

Jibran R, Hunter DA, Dijkwel PP. 2013. Hormonal regulation of leaf senescence through integration of developmental and stress signals. *Plant Mol Biol.* 82:547–561. https://doi.org/10.1007/s11103-013-0043-2.

Jing H-C, Anderson L, Sturre MJ, Hille J, Dijkwel PP. 2007. Arabidopsis CPR5 is a senescence-regulatory gene with pleiotropic functions as predicted by the evolutionary theory of senescence. *J Exp Bot.* 58:3885–3894. https://doi.org/10.1093/jxb/erm237.

Jing H-C, Schippers JH, Hille J, Dijkwel PP. 2005. Ethylene-induced leaf senescence depends on age-related changes and OLD genes in Arabidopsis. *J Exp Bot.* 56:2915–2923. https://doi.org/10.1093/jxb/eri287.

Jirage D *et al.* 2001. Constitutive salicylic acid-dependent signaling in cpr1 and cpr6 mutants requires PAD4. *Plant J.* 26:395–407. https://doi.org/10.1046/j.1365-313X.2001.2641040.x.

Kaňa R, Vass I. 2008. Thermoimaging as a tool for studying light-induced heating of leaves: correlation of heat dissipation with the efficiency of photosystem II photochemistry and non-photochemical quenching. *Environ Exp Bot.* 64:90–96. https://doi.org/10.1016/j.envexpbot.2008.02.006.

Kanojia A *et al.* 2020. Developmentally controlled changes during Arabidopsis leaf development indicate causes for loss of stress tolerance with age. *J Exp Bot.* 71:6340–6354. https://doi.org/10.1093/jxb/eraa347.

Kavulych Y, Kobyletska M, Romanyuk N, Terek O. 2023. Stress-protective and regulatory properties of salicylic acid and prospects of its use in plant production. *Stud Biol.* 17:173–200. https://doi.org/10.30970/sbi.1702.718.

Khan MIR, Iqbal N, Masood A, Per TS, Khan NA. 2013. Salicylic acid alleviates adverse effects of heat stress on photosynthesis through changes in proline production and ethylene formation. *Plant Signal Behav.* 8: e26374. https://doi.org/10.4161/psb.26374.

Kimura S *et al.* 2020. CRK2 and C-terminal phosphorylation of NADPH oxidase RBOHD regulate reactive oxygen species production in Arabidopsis. *Plant Cell.* 32:1063–1080. https://doi.org/10.1105/tpc.19.00525.

Koyama T. 2014. The roles of ethylene and transcription factors in the regulation of onset of leaf senescence. *Front Plant Sci.* 5:650. https://doi.org/10.3389/fpls.2014.00650.

Kramer DM, Johnson G, Kiirats O, Edwards GE. 2004. New fluorescence parameters for the determination of QA redox state and excitation energy fluxes. *Photosynth Res.* 79:209–218. https://doi.org/10.1023/B:PRES.0000015391.99477.0d.

Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*. 227:680–685. https://doi.org/10.1038/227680a0.

Lee DS, Kim YC, Kwon SJ, Ryu C-M, Park OK. 2017. The Arabidopsis cysteine-rich receptor-like kinase CRK36 regulates immunity through interaction with the cytoplasmic kinase BIK1. *Front Plant Sci.* 8:1856. https://doi.org/10.3389/fpls.2017.01856.

Lee H-N, Lee K-H, Kim CS. 2015. Abscisic acid receptor PYRABACTIN RESISTANCE-LIKE 8, PYL8, is involved in glucose response and dark-induced leaf senescence in Arabidopsis. *Biochem Biophys Res Commun.* 463:24–28. https://doi.org/10.1016/j.bbrc.2015.05.010.

Lee S *et al.* 2020. Glycosyltransferase-like RSE1 negatively regulates leaf senescence through salicylic acid signaling in Arabidopsis. *Front Plant Sci.* 11:551. https://doi.org/10.3389/fpls.2020.00551.

Li M, Kim C. 2022. Chloroplast ROS and stress signaling. *Plant Commun.* 3: 100264. https://doi.org/10.1016/j.xplc.2021.100264.

Li Z, Peng J, Wen X, Guo H. 2013. Ethylene-insensitive3 is a senescence-associated gene that accelerates age-dependent leaf senescence by directly repressing miR164 transcription in Arabidopsis. *Plant Cell.* 25:3311–3328. https://doi.org/10.1105/tpc.113.113340.

Lichtenthaler H, Babani F. 2021. Contents of photosynthetic pigments and ratios of chlorophyll a/b and chlorophylls to carotenoids (a+ b)/(x+ c) in C4 plants as compared to C3 plants. *Photosynthetica.* 60:3–9. https://doi.org/10.32615/ps.2021.041.

Liebsch D, Keech O. 2016. Dark-induced leaf senescence: new insights into a complex light-dependent regulatory pathway. *New Phytol.* 212: 563–570. https://doi.org/10.1111/nph.14217.

Liu J, Xu Y, Zhang Z, Wei J. 2015. Hydrogen peroxide promotes programmed cell death and salicylic acid accumulation during the induced production of sesquiterpenes in cultured cell suspensions of Aquilaria sinensis. *Funct Plant Biol.* 42:337–346. https://doi.org/10.1071/FP14189.

Love MI, Huber W, Anders S. 2014. Moderated estimation of fold change and dispersion for RNA-Seq data with DESeq2. *Genome Biol.* 15:550. https://doi.org/10.1186/s13059-014-0550-8.

Lu K *et al.* 2016. Overexpression of an Arabidopsis cysteine-rich receptor-like protein kinase, CRK5, enhances abscisic acid sensitivity and confers drought tolerance. *J Exp Bot.* 67:5009–5027. https://doi.org/10.1093/jxb/erw266.

Maillard A *et al.* 2015. Leaf mineral nutrient remobilization during leaf senescence and modulation by nutrient deficiency. *Front Plant Sci.* 6: 317. https://doi.org/10.3389/fpls.2015.00317.

Małachowska M, Tomala K. 2022. Effect of preharvest and postharvest application of 1-MCP on the quality of Gala Schniga® SchniCo Red (s) apples during long-term storage. *Agriculture*. 12:2073. https://doi.org/10.3390/agriculture12122073.

Marques JP, Schattat MH, Hause G, Dudeck I, Klösgen RB. 2004. In vivo transport of folded EGFP by the ΔpH/TAT-dependent pathway in chloroplasts of Arabidopsis thaliana. *J Exp Bot.* 55:1697–1706. https://doi.org/10.1093/jxb/erh191.

Mateo A *et al.* 2006. Controlled levels of salicylic acid are required for optimal photosynthesis and redox homeostasis. *J Exp Bot.* 57:1795–1807. https://doi.org/10.1093/jxb/erj196.

Meuwly P, Métraux J-P. 1993. Ortho-anisic acid as internal standard for the simultaneous quantitation of salicylic acid and its putative biosynthetic precursors in cucumber leaves. *Anal Biochem.* 214:500–505. https://doi.org/10.1006/abio.1993.1529.

Morris K *et al.* 2000. Salicylic acid has a role in regulating gene expression during leaf senescence. *Plant J.* 23:677–685. https://doi.org/10.1046/j.1365-313x.2000.00836.x.

Moustakas M *et al.* 2022. Harnessing the role of foliar applied salicylic acid in decreasing chlorophyll content to reassess photosystem II photoprotection in crop plants. *Int J Mol Sci.* 23:7038. https://doi.org/10.3390/ijms23137038.

Muhlenbock P *et al.* 2008. Chloroplast signaling and LESION SIMULATING DISEASE1 regulate crosstalk between light acclimation and immunity in Arabidopsis. *Plant Cell.* 20:2339–2356. https://doi.org/10.1105/tpc.108.059618.

Murakami A, Kim E, Minagawa J, Takizawa K. 2024. How much heat does non-photochemical quenching produce? *Front Plant Sci.* 15:1367795. https://doi.org/10.3389/fpls.2024.1367795.

Paluch-Lubawa E, Stolarska E, Sobieszczuk-Nowicka E. 2021. Dark-induced barley leaf senescence–a crop system for studying senescence and autophagy mechanisms. *Front Plant Sci.* 12:635619. https://doi.org/10.3389/fpls.2021.635619.

Putri GH, Anders S, Pyl PT, Pimanda JE, Zanini F. 2022. Analysing high-throughput sequencing data in Python with HTSeq 2.0. *Bioinformatics*. 38:2943–2945. https://doi.org/10.1093/bioinformatics/btac166.

Ruban AV. 2016. Nonphotochemical chlorophyll fluorescence quenching: mechanism and effectiveness in protecting plants from photodamage. *Plant Physiol.* 170:1903–1916. https://doi.org/10.1104/pp.15.01935.

Ruban AV, Johnson MP, Duffy CD. 2012. The photoprotective molecular switch in the photosystem II antenna. *Biochim Biophys Acta*. 1817:167–181. https://doi.org/10.1016/j.bbabio.2011.04.007.

Rusaczonek A *et al.* 2015. Role of phytochromes A and B in the regulation of cell death and acclimatory responses to UV stress in Arabidopsis thaliana. *J Exp Bot.* 66:6679–6695. https://doi.org/10.1093/jxb/erv375.

Rusaczonek A *et al.* 2021. Phototropin 1 and 2 influence photosynthesis, UV-C induced photooxidative stress responses, and cell death. *Cells*. 10:200. https://doi.org/10.3390/cells10020200.

Rushton PJ, Somssich IE, Ringler P, Shen QJ. 2010. WRKY transcription factors. *Trends Plant Sci.* 15:247–258. https://doi.org/10.1016/j.tplants.2010.02.006.

Sangwan S *et al.* 2022. Role of salicylic acid in combating heat stress in plants: insights into modulation of vital processes. *Front Biosci (Landmark Ed)*. 27:310. https://doi.org/10.31083/j.fbl2711310.

Schattat M, Barton K, Baudisch B, Klösgen RB, Mathur J. 2011. Plastid stromule branching coincides with contiguous endoplasmic reticulum dynamics. *Plant Physiol.* 155:1667–1677. https://doi.org/10.1104/pp.110.170480.

Schippers JH. 2015. Transcriptional networks in leaf senescence. *Curr Opin Plant Biol.* 27:77–83. https://doi.org/10.1016/j.pbi.2015.06.018.

Smith T, Heger A, Sudbery I. 2017. UMI-tools: modeling sequencing errors in unique molecular identifiers to improve quantification accuracy. *Genome Res.* 27:491–499. https://doi.org/10.1101/gr.209601.116.

Sobejano-Paz V *et al.* 2023. Heat dissipation from photosynthesis contributes to maize thermoregulation under suboptimal temperature conditions [preprint]. bioRxiv. https://doi.org/10.1101/2023.01.27.525868.

Trojak M, Skowron E. 2023. Growth light quality influences leaf surface temperature by regulating the rate of non-photochemical quenching thermal dissipation and stomatal conductance. *Int J Mol Sci.* 24:16911. https://doi.org/10.3390/ijms242316911.

Urban J, Ingwers M, McGuire MA, Teskey RO. 2017. Stomatal conductance increases with rising temperature. *Plant Signal Behav.* 12:e1356534. https://doi.org/10.1080/15592324.2017.1356534.

Vaattovaara A *et al.* 2019. Mechanistic insights into the evolution of DUF26-containing proteins in land plants. *Commun Biol.* 2:56. https://doi.org/10.1038/s42003-019-0306-9.

van Verk MC, Bol JF, Linthorst HJ. 2011. WRKY transcription factors involved in activation of SA biosynthesis genes. *BMC Plant Biol.* 11:89. https://doi.org/10.1186/1471-2229-11-89.

Veeranagouda Y, Remaury A, Guillemot J, Didier M. 2019. RNA fragmentation and sequencing (RF-seq): cost-effective, time-efficient, and high-throughput 3′ mRNA sequencing library construction in a single tube. *Curr Protoc Mol Biol.* 129:e109. https://doi.org/10.1002/cpmb.109.

Wang C *et al.* 2021. Ethylene and salicylic acid synergistically accelerate leaf senescence in Arabidopsis. *J Integr Plant Biol.* 63:828–833. https://doi.org/10.1111/jipb.13075.

Witoń D *et al.* 2021. MITOGEN-ACTIVATED PROTEIN KINASE 4 impacts leaf development, temperature, and stomatal movement in hybrid aspen. *Plant Physiol.* 186:2190–2204. https://doi.org/10.1093/plphys/kiab186.

Wrzaczek M *et al.* 2010. Transcriptional regulation of the CRK/DUF26 group of receptor-like protein kinases by ozone and plant hormones in Arabidopsis. *BMC Plant Biol.* 10:95. https://doi.org/10.1186/1471-2229-10-95.

Wu T *et al.* 2021. clusterProfiler 4.0: a universal enrichment tool for interpreting omics data. *The Innovation*. 2:100141. https://doi.org/10.1016/j.xinn.2021.100141.

Ye Y *et al.* 2017. The role of receptor-like protein kinases (RLKs) in abiotic stress response in plants. *Plant Cell Rep.* 36:235–242. https://doi.org/10.1007/s00299-016-2084-x.

Yoo SD, Greer DH, Laing WA, McManus MT. 2003. Changes in photosynthetic efficiency and carotenoid composition in leaves of white clover at different developmental stages. *Plant Physiol Biochem.* 41:887–893. https://doi.org/10.1016/S0981-9428(03)00138-4.

Yoshimoto K *et al.* 2009. Autophagy negatively regulates cell death by controlling NPR1-dependent salicylic acid signaling during senescence and the innate immune response in Arabidopsis. *Plant Cell.* 21:2914–2927. https://doi.org/10.1105/tpc.109.068635.

Zarrin Ghalami R *et al.* 2025. Transpiration, Photoinhibition and Non-photochemical Quenching Reciprocally Control Foliar Heat Emission [Preprint]. bioRxiv. https://doi.org/10.1101/2025.05.27.656286.

Zarrin Ghalami R, Duszyn M, Karpiński S. 2026. Absorption of energy in excess, photoinhibition, transpiration, and foliar heat emission feedback loops during global warming. *Cells*. 15:75. https://doi.org/10.3390/cells15010075.

Zhang B *et al.* 2023a. Salicylic acid accelerates carbon starvation-induced leaf senescence in Arabidopsis thaliana by inhibiting autophagy through Nonexpressor of pathogenesis-related genes 1. *Plant Sci.* 336:111859. https://doi.org/10.1016/j.plantsci.2023.111859.

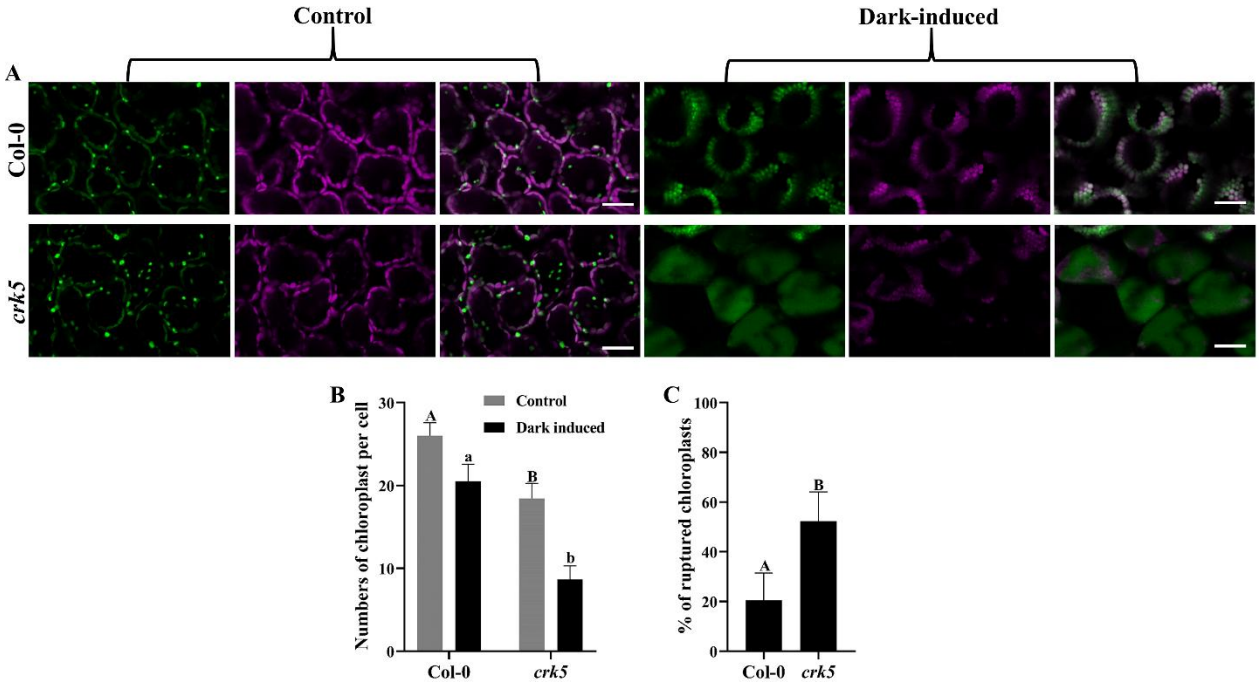
Zhang K, Halitschke R, Yin C, Liu C-J, Gan S-S. 2013. Salicylic acid 3-hydroxylase regulates Arabidopsis leaf longevity by mediating salicylic acid catabolism. *Proc Natl Acad Sci U S A.* 110:14807–14812. https://doi.org/10.1073/pnas.1302702110.

Zhang Y *et al.* 2023b. Cysteine-rich receptor-like protein kinases: emerging regulators of plant stress responses. *Trends Plant Sci.* 28:776–794. https://doi.org/10.1016/j.tplants.2023.03.028.

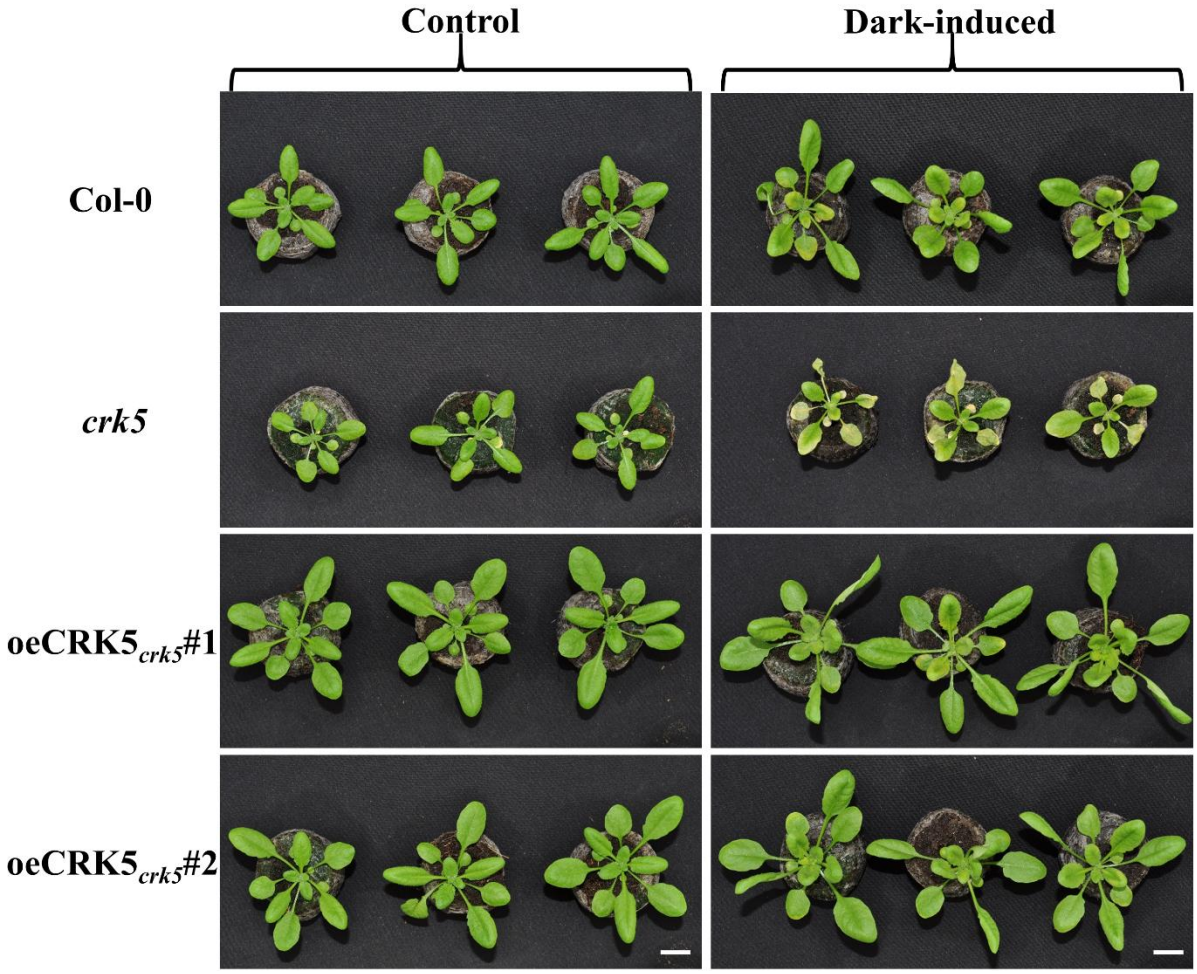
Zhao J, Sun Y, Li X, Li Y. 2022. CYSTEINE-RICH RECEPTOR-LIKE KINASE5 (CRK5) and CRK22 regulate the response to Verticillium dahliae toxins. *Plant Physiol.* 190:714–731. https://doi.org/10.1093/plphys/kiac277.

Zhao Y *et al.* 2016. ABA receptor PYL9 promotes drought resistance and leaf senescence. *Proc Natl Acad Sci U S A.* 113:1949–1954. https://doi.org/10.1073/pnas.1522840113.

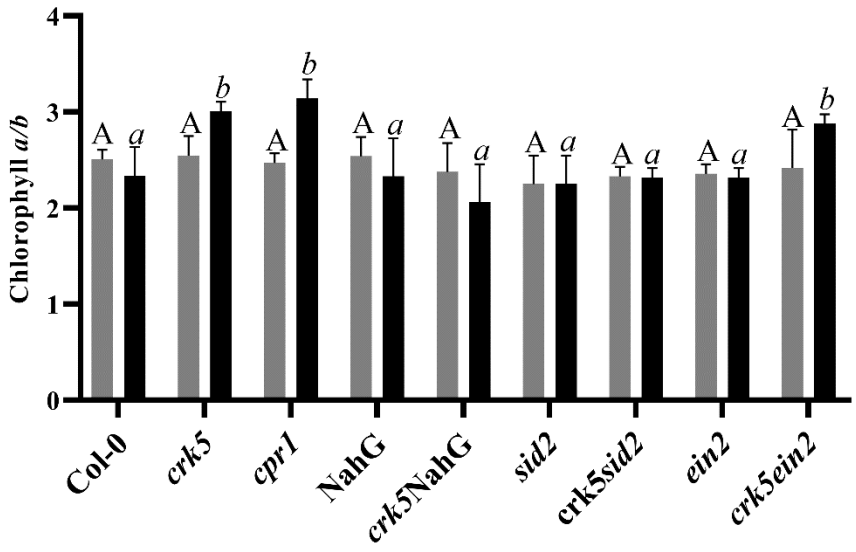
Zhuo M, Sakuraba Y, Yanagisawa S. 2020. A jasmonate-activated MYC2–Dof2.1–MYC2 transcriptional loop promotes leaf senescence in Arabidopsis. *Plant Cell.* 32:242–262. https://doi.org/10.1105/tpc.19.00297.



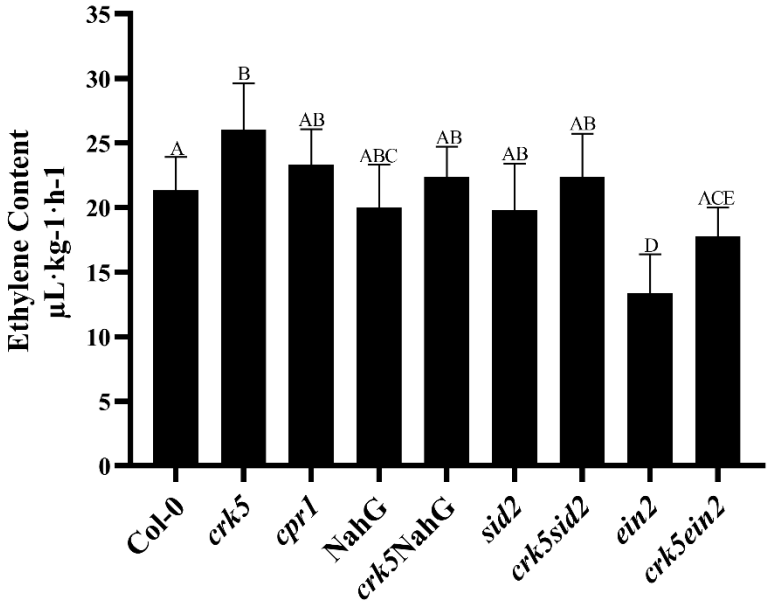
Supplementary Figure S1. Confocal microscopic analysis of Arabidopsis wild type and *crk5* mutant in control and dark-induced conditions. Arabidopsis plants expressing enhanced green fluorescence protein (EGFP) fused to a transit peptide of ferredoxin NADP(H) oxidoreductase (FNR) anchored to the chloroplast stroma for the chloroplast membrane stability, and the purple fluorescence corresponds to the chlorophyll fluorescence in the chloroplasts of mesophyll cells in Col-0 and *crk5* (**A**). The number of chloroplast per cell (**B**) and the percentage of ruptured chloroplasts (**C**) is calculated. Photographs were taken of plants growing under ambient light (120 µmol m^{-2} s^{-1}) conditions and then transferred for four days to continuous darkness. The white bar represents 50µm. Mean values (±SD) were derived from 12-15 cells for (**B**) and from 30 chloroplasts for (**C**). Statistical analysis was performed according to a *t*-test at a level of $p < 0.05$. Letters A, B, and a, b above the bars indicate homogenous groups, and values sharing common labels (letters) are not significantly different from each other.



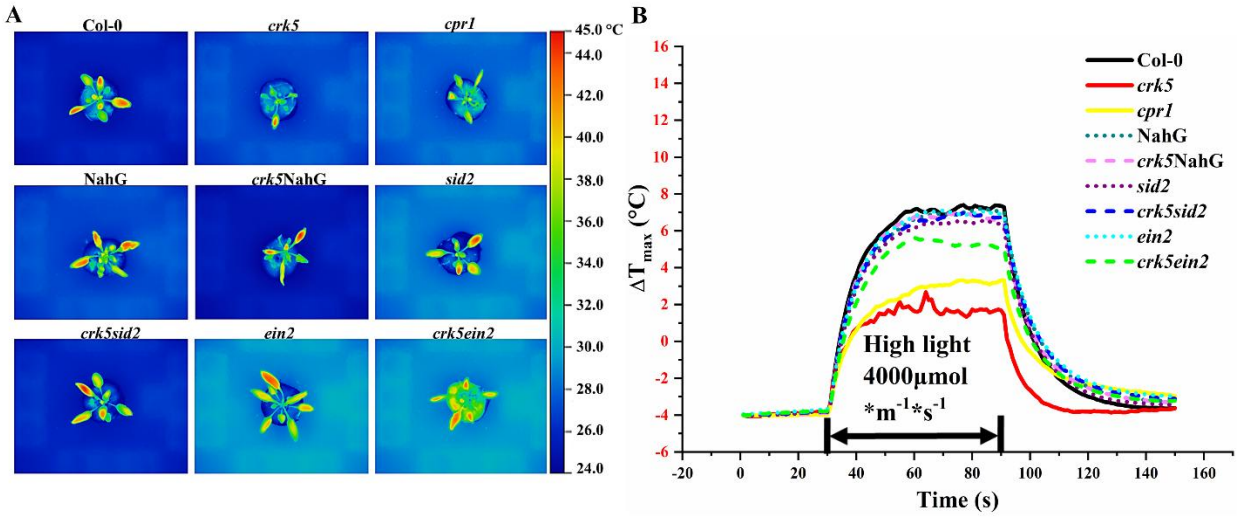
Supplementary Figure S2. Morphology of the Arabidopsis wild type, *crk5* mutant, and complementation lines in control and after exposure for 4 days to permanent darkness. The picture shows a phenotype of 4-week-old plants growing under ambient light (120 μmol m^{-2} s^{-1}) conditions and exposed to four days of permanent darkness. White scale bars indicate 1 cm.



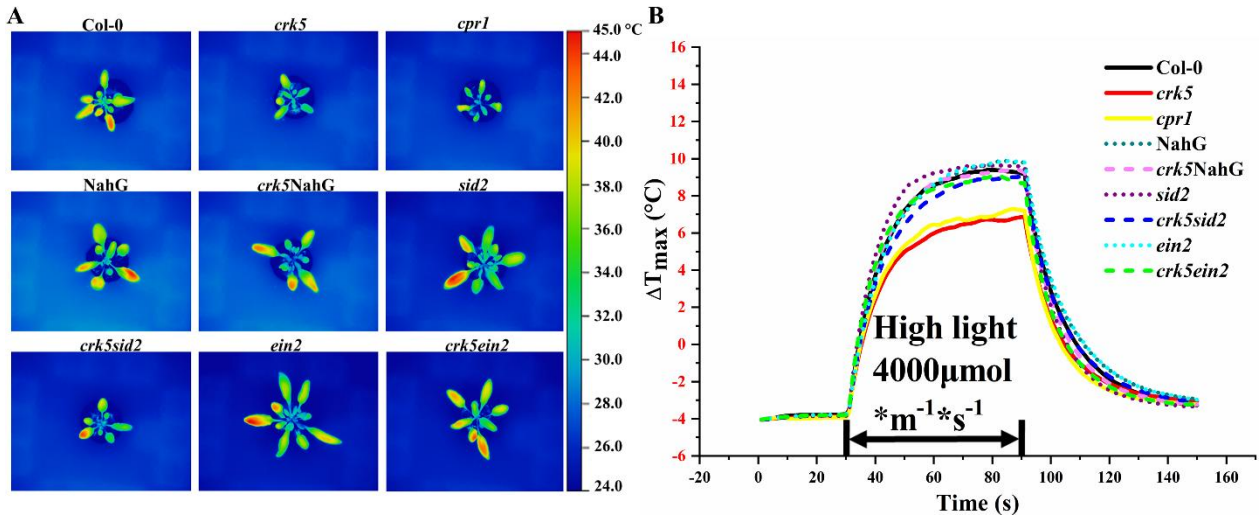
Supplementary Figure S3. Chlorophyll *a/b* ratio of the Arabidopsis salicylic acid-synthesis or catabolism and ethylene-signaling mutants in control and after exposure for 4 days to permanent darkness. Mean values (±SD) were derived from 9 plants (n = 9). Statistical analysis was performed according to a *t*-test at a level of $p < 0.05$. Letters A, B, and a, b, c above the bars indicate homogenous groups, and values sharing common labels (letters) are not significantly different from each other.



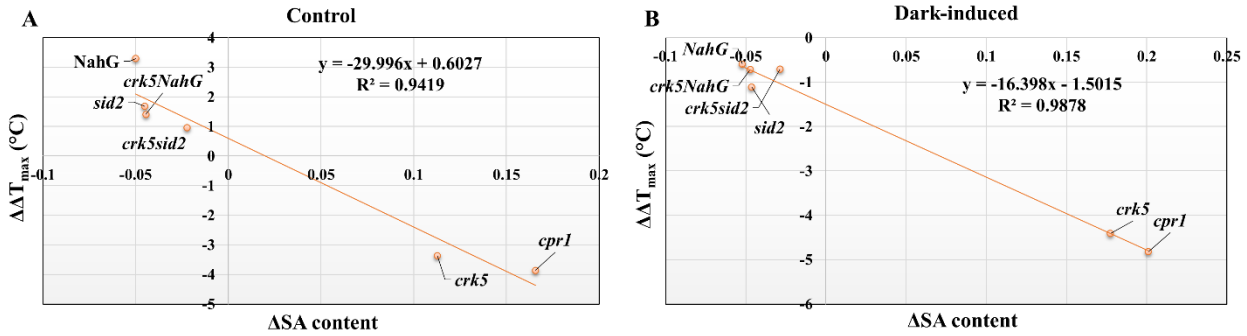
Supplementary Figure S4. Ethylene content in the Arabidopsis salicylic acid-synthesis or catabolism and ethylene-signaling mutants. Mean values (±SD) were derived from 9 plants (n = 9). Statistical analysis was performed according to a t-test at a level of $p < 0.05$. Letters A, B, C, D, and E above the bars indicate homogenous groups, and values sharing common labels (letters) are not significantly different from each other.



Supplementary Figure S5. Foliar temperature analysis in the analyzed Arabidopsis salicylic acid-synthesis or catabolism and ethylene-signaling mutants exposed for 4 days to permanent darkness. The thermograms displaying whole rosettes of various Arabidopsis genotypes after 30 seconds of high light (4000 µmol photons m^{-2} s^{-1}) (**A**). The plot displays dynamic variations in delta foliar temperature (ΔT_{max}) under varying light levels relative to the white background on which the plants were placed for the measurement. The white surface reflected light, and exposure to intense light only caused a 1.5 °C rise in temperature. The following was the program's setup: 30 s of ambient light (150 µmol photons m^{-2} s^{-1}), followed by 60 s of high blue light (4000 µmol photons m^{-2} s^{-1}), and lastly by 60 s of ambient light (150 µmol photons m^{-2} s^{-1}) (**B**). For temperature analysis, data represent mean values of 3 different leaves from 9 plants (n = 27).

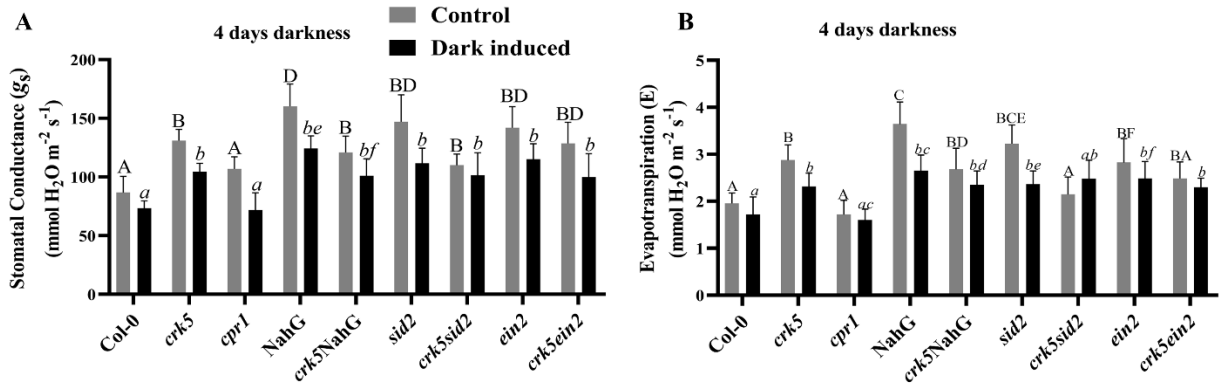


Supplementary Figure S6. Foliar temperature analysis in the analyzed Arabidopsis salicylic acid-synthesis or catabolism and ethylene-signaling mutants after exogenous salicylic acid spray (1mM). The thermograms displaying whole rosettes of various Arabidopsis genotypes after 30 seconds of high light (4000 µmol photons m^{-2} s^{-1}) (**A**). The plot displays dynamic variations in delta foliar temperature (ΔT_{max}) under varying light levels relative to the white background on which the plants were placed for the measurement. The white surface reflected light, and exposure to intense light only caused a 1.5 °C rise in temperature. The following was the program's setup: 30 s of ambient light (150 µmol photons m^{-2} s^{-1}), followed by 60 s of high blue light (4000 µmol photons m^{-2} s^{-1}), and lastly by 60 s of ambient light (150 µmol photons m^{-2} s^{-1}) (**B**). For temperature analysis, data represent mean values of 3 different leaves from 9 plants (n = 27).

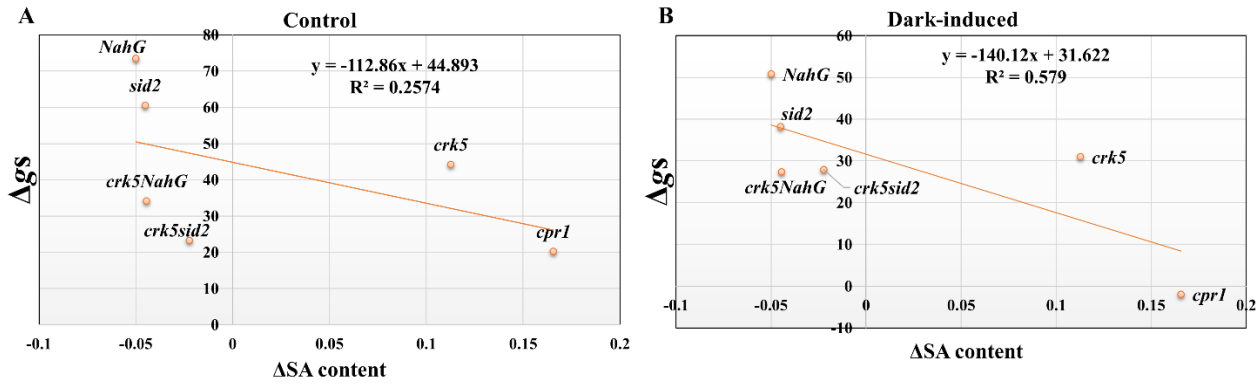


Supplementary Figure S7. Pearson's correlation analysis between salicylic acid levels and foliar ΔT in Arabidopsis salicylic acid-synthesis or catabolism mutants in control and after exposure for 4 days of permanent darkness. Correlations between changes in ΔT_{max} ($\Delta\Delta T_{max}$) and

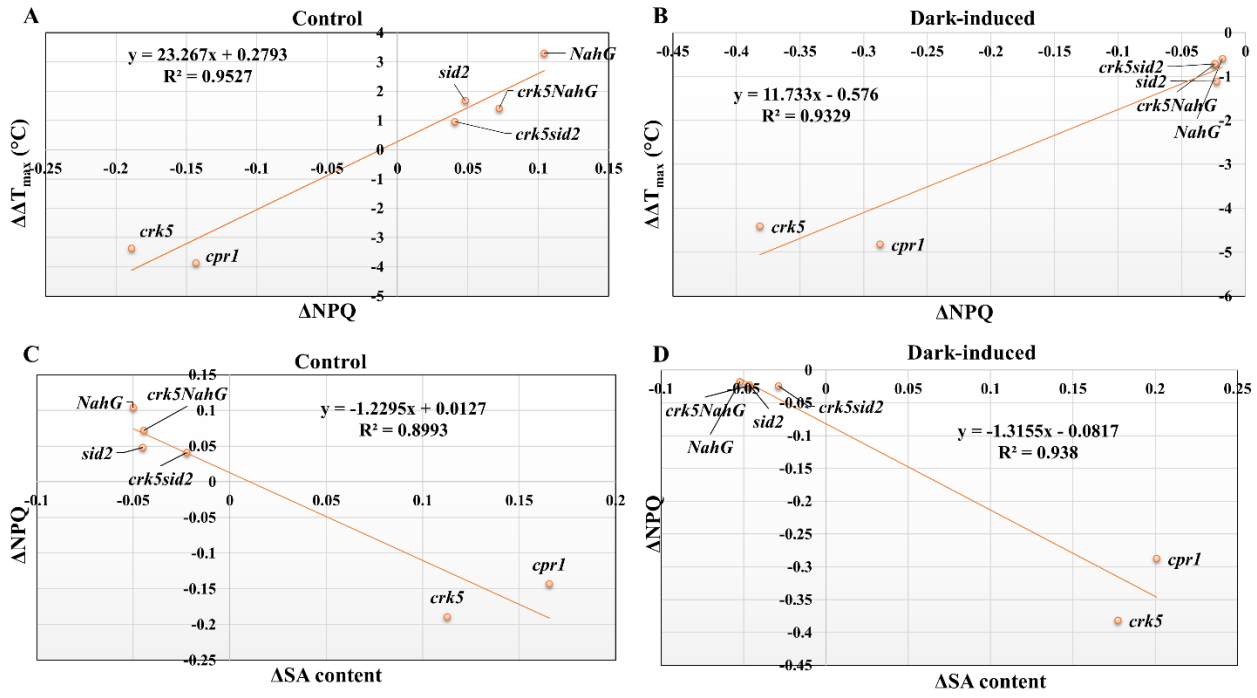
ΔSA content in control conditions (**A**) and in the prolonged darkness (**B**). Pearson's correlation coefficient (R).



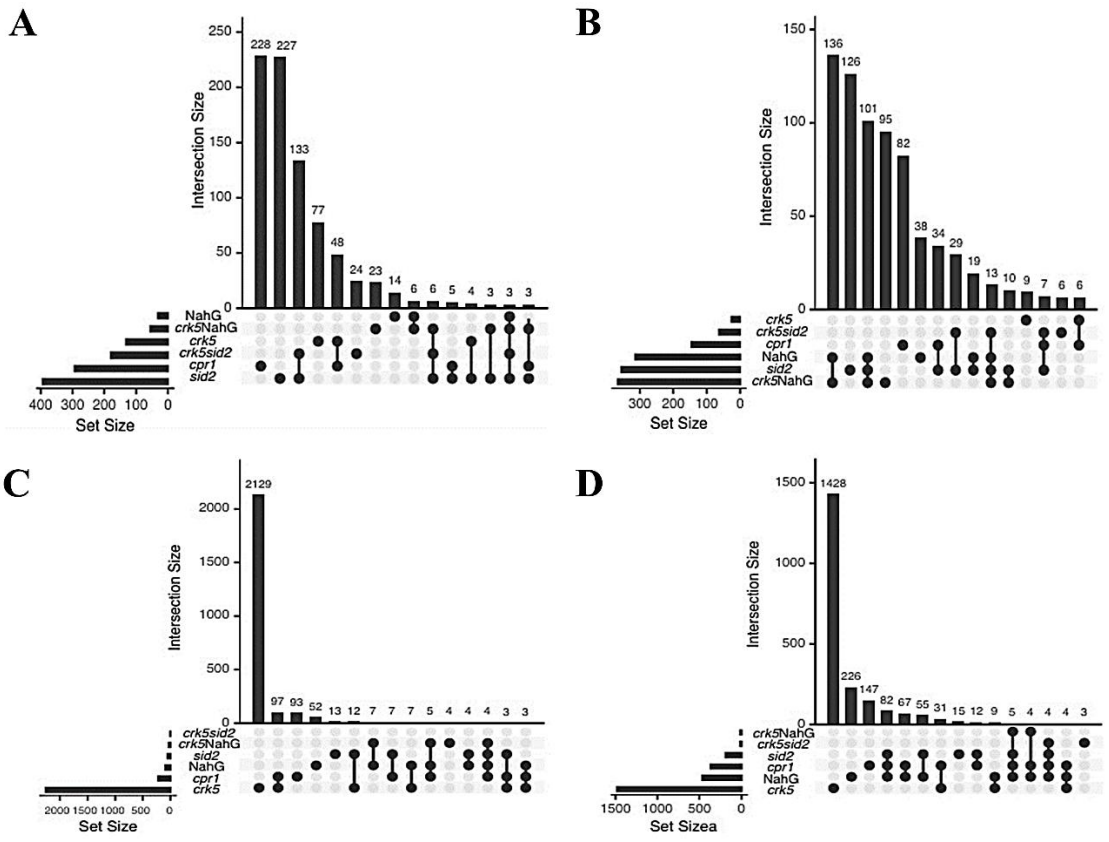
Supplementary Figure S8. Foliar stomatal conductance and evapotranspiration parameters analysis of the Arabidopsis salicylic acid-synthesis or catabolism and ethylene-signaling mutants in control and after exposure for 4 days to permanent darkness. Foliar stomatal conductance (**A**) and evapotranspiration (**B**). Data represent mean values of 9 plants ± SD (n = 9). Statistical analysis was performed according to a *t*-test at a level of $P < 0.05$. Letters A, B, C, D, E, F, and a, b, c, d, e, f above the bars indicate homogenous groups, and values sharing common labels (letters) are not significantly different from each other.



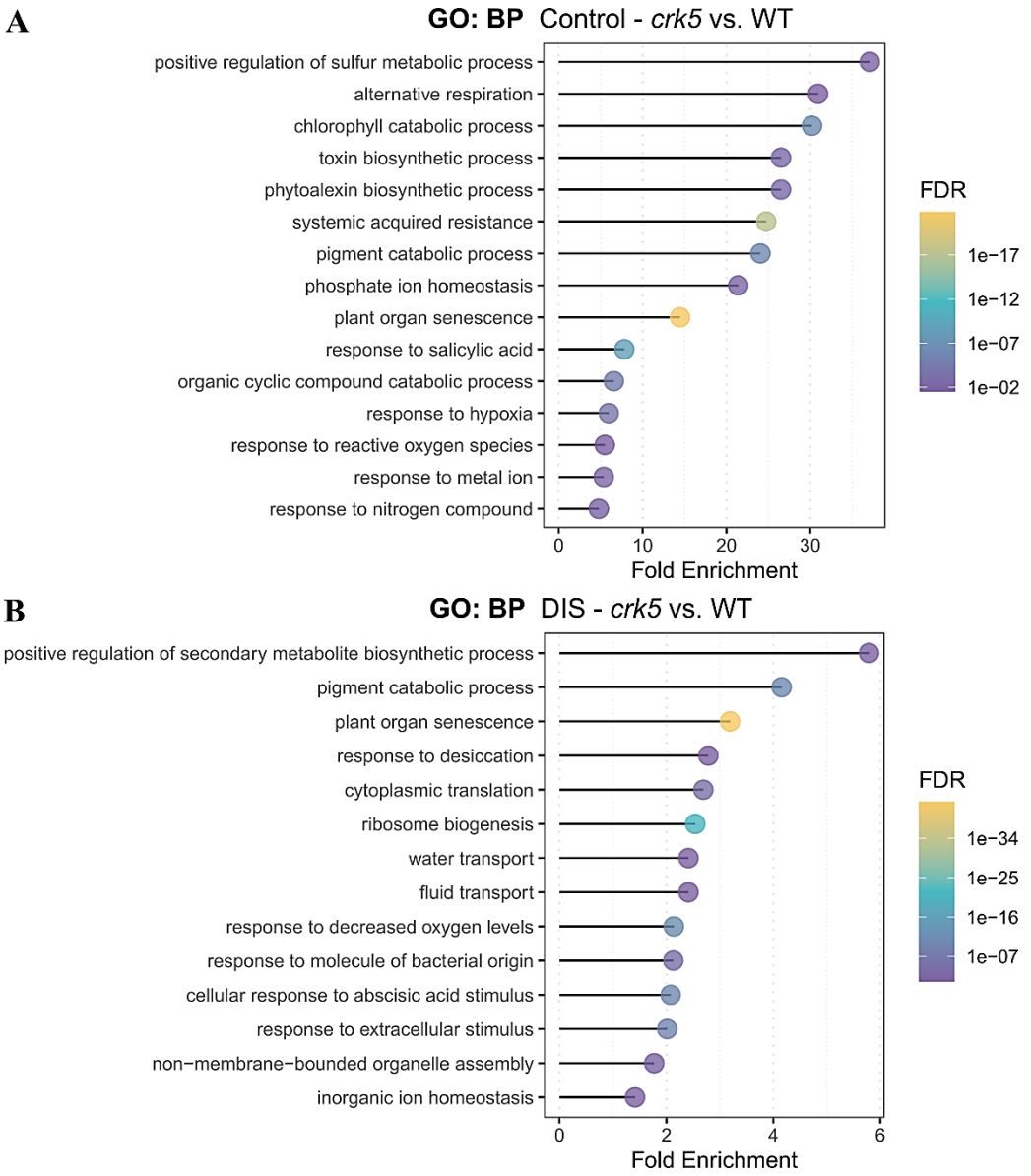
Supplementary Figure S9. Pearson's correlation analysis between salicylic acid levels and stomatal conductance in Arabidopsis salicylic acid-synthesis or catabolism mutants in control and after exposure for 4 days to permanent darkness. Correlations between changes in Δ*g*s and ΔSA content in control conditions (**A**) and in the prolonged darkness (**B**). Pearson's correlation coefficient (R).



Supplementary Figure S10. Pearson's correlation analysis between NPQ, foliar ΔT, and salicylic acid levels in Arabidopsis salicylic acid-synthesis or catabolism mutants in control and after exposure for 4 days to permanent darkness. Correlations between changes in $\Delta\Delta T_{max}$ and ΔNPQ in control conditions (**A**) and in the prolonged darkness (**B**). Correlations between changes in ΔNPQ and ΔSA in control (**C**) and in the prolonged darkness (**D**). Pearson's correlation coefficient (R).

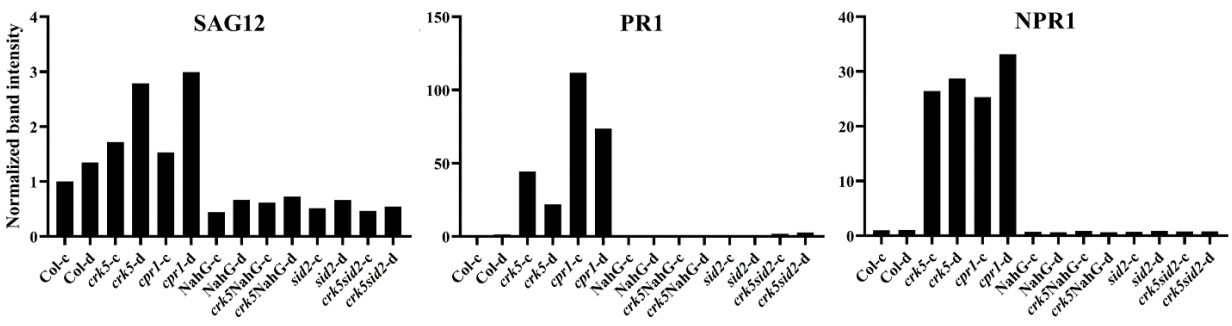


Supplementary Figure S11. Upset plots showing the number of differentially expressed genes (DEGs) in control and dark-induced conditions. Plot (**A**) shows upregulated genes in the control condition, while plot (**B**) shows downregulated genes in the control condition, while plots (**C**) and (**D**) show upregulated and downregulated genes in dark-induced conditions, respectively. The total number of DEGs identified for each genotype is listed on the side bar plot, and the number of unique and common (non-overlapping and overlapping) genes is displayed in the main histogram.



92

93 **Supplementary Figure S12.** Gene ontology (GO) analysis of Arabidopsis wild type and
94 *crk5* mutant in control and dark-induced conditions. Genes significantly enriched in *crk5*
95 compared to Col-0 in the RNA-seq experiment in control (**A**) and dark-induced (**B**) conditions.



96

Supplementary Figure S13. The graphs show the relative intensities, normalized to the Col-0 control, of the bands detected in the WB analysis of the gels shown in Figure 5 for each tested protein.

Supplementary Table S1. Table for Figure 3C, D, and E statistics.

Yield of PSII-Control	
Alpha at 0.05	P < 0.05 (*)
Tukey's multiple comparisons test	P < 0.005 (**)
	P < 0.001 (***)
PAR (µmol. m^{-2}. s^{-1})	Significance (*)/Not Significant (ns)
0	
Col-0 vs. crk5	ns
Col-0 vs. cpr1	**
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	ns
11	
Col-0 vs. crk5	**
Col-0 vs. cpr1	***
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	ns
18	
Col-0 vs. crk5	ns
Col-0 vs. cpr1	ns

Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	ns
27	
Col-0 vs. crk5	***
Col-0 vs. cpr1	***
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	***
58	
Col-0 vs. crk5	***
Col-0 vs. cpr1	**
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	***
100	
Col-0 vs. crk5	***
Col-0 vs. cpr1	**
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	*
Col-0 vs. sid2	**
Col-0 vs. crk5/sid2	***
Col-0 vs. ein2	**
Col-0 vs. crk5/ein2	***

131	
Col-0 vs. crk5	***
Col-0 vs. cpr1	***
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	***
Col-0 vs. sid2	***
Col-0 vs. crk5/sid2	***
Col-0 vs. ein2	***
Col-0 vs. crk5/ein2	***
221	
Col-0 vs. crk5	*
Col-0 vs. cpr1	***
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	*
Col-0 vs. crk5/sid2	***
Col-0 vs. ein2	***
Col-0 vs. crk5/ein2	**
344	
Col-0 vs. crk5	ns
Col-0 vs. cpr1	*
Col-0 vs. NahG	**
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	ns
536	
Col-0 vs. crk5	ns
Col-0 vs. cpr1	ns
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns

Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	ns
830	
Col-0 vs. crk5	ns
Col-0 vs. cpr1	ns
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	ns
Yield of PSII-Dark condition	
PAR (µmol. m^{-2}. s^{-1})	Significance (*)/Not Significant (ns)
0	
Col-0 vs. crk5	ns
Col-0 vs. cpr1	***
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	ns
11	
Col-0 vs. crk5	ns
Col-0 vs. cpr1	ns
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns

Col-0 vs. crk5/ein2	ns
18	
Col-0 vs. crk5	ns
Col-0 vs. cpr1	ns
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	ns
27	
Col-0 vs. crk5	ns
Col-0 vs. cpr1	***
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	ns
58	
Col-0 vs. crk5	***
Col-0 vs. cpr1	***
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	ns
100	
Col-0 vs. crk5	***
Col-0 vs. cpr1	*
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns

Col-0 vs. sid2	*
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	ns
131	
Col-0 vs. crk5	***
Col-0 vs. cpr1	ns
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	ns
221	
Col-0 vs. crk5	**
Col-0 vs. cpr1	ns
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	ns
344	
Col-0 vs. crk5	ns
Col-0 vs. cpr1	ns
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	ns
536	
Col-0 vs. crk5	ns

Col-0 vs. cpr1	ns
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	ns
830	
Col-0 vs. crk5	ns
Col-0 vs. cpr1	ns
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	ns
ETRII at control condition	
PAR (µmol. m^{-2}. s^{-1})	Significance (*)/Not Significant (ns)
0	
Col-0 vs. crk5	ns
Col-0 vs. cpr1	ns
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	ns
11	
Col-0 vs. crk5	ns
Col-0 vs. cpr1	ns
Col-0 vs. NahG	ns

Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	ns
18	
Col-0 vs. crk5	ns
Col-0 vs. cpr1	ns
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	ns
27	
Col-0 vs. crk5	ns
Col-0 vs. cpr1	ns
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	ns
58	
Col-0 vs. crk5	ns
Col-0 vs. cpr1	ns
Col-0 vs. NahG	***
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	ns
100	

Col-0 vs. crk5	***
Col-0 vs. cpr1	*
Col-0 vs. NahG	***
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	***
131	
Col-0 vs. crk5	***
Col-0 vs. cpr1	***
Col-0 vs. NahG	*
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	***
221	
Col-0 vs. crk5	***
Col-0 vs. cpr1	***
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	***
344	
Col-0 vs. crk5	***
Col-0 vs. cpr1	***
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns

Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	*
536	
Col-0 vs. crk5	**
Col-0 vs. cpr1	***
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	*
830	
Col-0 vs. crk5	***
Col-0 vs. cpr1	***
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	*
ETRII at dark condition	
PAR (µmol. m^{-2}. s^{-1})	Significance (*)/Not Significant (ns)
0	
Col-0 vs. crk5	ns
Col-0 vs. cpr1	ns
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	ns

11	
Col-0 vs. crk5	ns
Col-0 vs. cpr1	ns
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	ns
18	
Col-0 vs. crk5	ns
Col-0 vs. cpr1	ns
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	ns
27	
Col-0 vs. crk5	ns
Col-0 vs. cpr1	*
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	ns
58	
Col-0 vs. crk5	**
Col-0 vs. cpr1	***
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns

Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	***
100	
Col-0 vs. crk5	***
Col-0 vs. cpr1	***
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	***
131	
Col-0 vs. crk5	***
Col-0 vs. cpr1	***
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	**
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	***
221	
Col-0 vs. crk5	***
Col-0 vs. cpr1	***
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	***
Col-0 vs. sid2	***
Col-0 vs. crk5/sid2	***
Col-0 vs. ein2	***
Col-0 vs. crk5/ein2	***
344	
Col-0 vs. crk5	***
Col-0 vs. cpr1	***

Col-0 vs. NahG	*
Col-0 vs. crk5/NahG	***
Col-0 vs. sid2	***
Col-0 vs. crk5/sid2	***
Col-0 vs. ein2	***
Col-0 vs. crk5/ein2	*
536	
Col-0 vs. crk5	***
Col-0 vs. cpr1	***
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	***
Col-0 vs. crk5/sid2	***
Col-0 vs. ein2	*
Col-0 vs. crk5/ein2	ns
830	
Col-0 vs. crk5	***
Col-0 vs. cpr1	ns
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	***
Col-0 vs. crk5/sid2	***
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	**
CO2 assimilation at control condition	
CO2 concentration (ppm)	Significance (*)/Not Significant (ns)
50	
Col-0 vs. crk5	ns
Col-0 vs. cpr1	*
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns

Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	ns
150	
Col-0 vs. crk5	ns
Col-0 vs. cpr1	***
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	ns
250	
Col-0 vs. crk5	**
Col-0 vs. cpr1	***
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	*
350	
Col-0 vs. crk5	***
Col-0 vs. cpr1	***
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	**
450	
Col-0 vs. crk5	***

Col-0 vs. cpr1	***
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	*
550	
Col-0 vs. crk5	***
Col-0 vs. cpr1	***
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	***
650	
Col-0 vs. crk5	***
Col-0 vs. cpr1	***
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	***
750	
Col-0 vs. crk5	***
Col-0 vs. cpr1	***
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns

Col-0 vs. crk5/ein2	***
850	
Col-0 vs. crk5	***
Col-0 vs. cpr1	***
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	***
CO2 assimilation at dark condition	
CO2 concentration (ppm)	
50	Significance (*)/Not Significant (ns)
Col-0 vs. crk5	**
Col-0 vs. cpr1	*
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	ns
150	
Col-0 vs. crk5	**
Col-0 vs. cpr1	***
Col-0 vs. NahG	*
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	ns
250	

Col-0 vs. crk5	***
Col-0 vs. cpr1	***
Col-0 vs. NahG	***
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	*
350	
Col-0 vs. crk5	***
Col-0 vs. cpr1	***
Col-0 vs. NahG	***
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	**
450	
Col-0 vs. crk5	***
Col-0 vs. cpr1	***
Col-0 vs. NahG	***
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	*
550	
Col-0 vs. crk5	***
Col-0 vs. cpr1	***
Col-0 vs. NahG	**
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns

Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	***
650	
Col-0 vs. crk5	***
Col-0 vs. cpr1	***
Col-0 vs. NahG	*
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	***
750	
Col-0 vs. crk5	***
Col-0 vs. cpr1	***
Col-0 vs. NahG	ns
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	***
850	
Col-0 vs. crk5	***
Col-0 vs. cpr1	***
Col-0 vs. NahG	*
Col-0 vs. crk5/NahG	ns
Col-0 vs. sid2	ns
Col-0 vs. crk5/sid2	ns
Col-0 vs. ein2	ns
Col-0 vs. crk5/ein2	***

Supplementary Table S2. Primers used in this study.

Gene	AGI Code	Sequence LP Primer	Sequence RP Primer
CRK5	AT4G23130	AGGAGATCTCTCGCCAGAATC	CGATAGTCTCTTCACGGCAAC
NahG	Transgene line (M60055)	ACTCTGCCGCTACTCCCATA	CGAGCCCTAGGTACATCTGC
SID2	AT1G74710	AATTGGCAGGGAGACTTACGAA	TCCTCTATCGAATGATTCTATCTCCTT
EIN2	AT5G03280	TAAAATTGGTTTCAGCATCGG	CCTGAGCTTTGGGGAAAGTAC

Supplementary Table S3. *Accession Numbers* for Figure 4A and B.

Senescence Marker Genes	AGI Code	SA Signaling Pathway Genes	AGI Code
SAG12	AT5G45890	ICS1	AT1G74710
SAG29	AT5G13170	ICS2	AT1G18870
BFN1	AT1G11190	PBS3	AT5G13320
ATG5	AT5G17290	PR1	AT2G14610
ATG7	AT5G45900	PR5	AT1G75040
ATG8a	AT4G21980	WRKY38	AT5G22570
ATG9	AT2G31260	NPR1	AT1G64280
CAT1	AT1G20630	NPR2	AT4G26120
BAP1	AT3G61190	NPR3	AT5G45110
ANAC016	AT1G34180	TGA1	AT5G65210
ANAC055	AT3G15500	EDS5	AT4G39030
NAP	AT1G69490	PAD4	AT3G52430
SGR1	AT4G22920	CBP60g	AT5G26920
RNS3	AT1G26820	SARD1	AT1G73805
NYC1	AT4G13250	EBF1	AT2G25490
SDG8	AT1G77300	EBF2	AT5G25350
DIN1	AT1G28130		
PRX34	AT3G49120		
PAO	AT3G44880		
NBR1	AT4G24690		

Supplementary Table S4. *Accession Numbers* for Figure 4C and D.

WRKY Transcription Factors	AGI Code	NPQ and Photosynthesis Genes	AGI Code
WRKY6	AT1G62300	VDE	AT1G08550
WRKY8	AT5G46350	ZEP	AT5G67030
WRKY15	AT2G23320	PHYB	AT2G18790
WRKY30	AT5G24110	Lhcb2.1	AT2G05100
WRKY31	AT5G01900	Lhcb2.2	AT2G05070
WRKY33	AT2G38470	Lhcb4.1	AT5G01530
WRKY36	AT1G69810	Lhcb4.2	AT3G08940
WRKY40	AT1G80840	Lhcb3	AT5G54270
WRKY41	AT4G11070	Lhcb6	AT1G15820
WRKY45	AT3G01970	STN7	AT1G68830
WRKY46	AT2G46400	STN8	AT5G01920
WRKY47	AT4G39780	NDH-M	AT4G37925
WRKY50	AT5G26170	NDH-N	AT1G15980
WRKY51	AT5G64810	TRX-F1	AT3G02730
WRKY53	AT4G23810	TRX-M4	AT3G15360
WRKY55	AT2G40740	PETE1	AT1G20340
WRKY59	AT2G21900	PAM68	AT1G14150
WRKY60	AT2G25000		
WRKY61	AT1G18860		
WRKY63	AT1G66600		
WRKY71	AT1G29860		
WRKY75	AT5G13080		

Warsaw, 12.03.2026

Muhammad Kamran

muhammad_kamran@sggw.edu.pl

Department of Plant Genetics, Breeding and Biotechnology, Institute of Biology

Warsaw University of Life Sciences

Biological Sciences Discipline Counsel

of the Warsaw University

of Life Sciences

Statement

I hereby declare that in the publication:

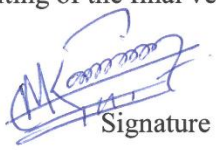
Kamran, M., Burdiak, P., Zarrin Ghalami, R., Rusaczonek, A., Duszyn, M., Gołębiewska, K., Gawroński, P., Karpiński, SM (2026a). The kinase CRK5 regulates dark-induced senescence and dissipation of energy as heat by inhibiting salicylic acid signaling, *Plant Physiology*, Volume 200, Issue 2, February 2026, kiag046, https://doi.org/10.1093/plphys/kiag046. **(Publication 2)**

My participation constitutes:

- participation in the experimental design of the study
- generation of double mutant plants through genetic crossing and PCR validations
- plant cultivation
- preparations for the stress treatment conditions (dark-induced senescence)
- collection of material for analysis
- preparation and implementation of experiments involving:
    - phenotypic analysis of the dark-induced plants
    - measurements of ethylene content using gas chromatography
    - measurements of foliar temperature under variable light conditions using FLIR A600 thermal imaging infrared camera in control, dark-induced, and salicylic acid-sprayed plants
    - measurements of chlorophyll *a* fluorescence parameters (maximum PSII quantum yield (*Fv/Fm*), non-photochemical quenching (NPQ), effective PSII quantum yield Y(II), and electron transport chain (ETRII)) using FlourCam 800 MF and Dual Pam-100

    - measurements of gas exchange analysis (stomatal conductance (*gs*), evapotranspiration (E), and net CO_2 assimilation
    - protein extraction and western blot analysis using Laemmli buffer for extraction of protein, RC-DC protein assay for concentration, and SDS-PAGE system
- participation in preparing samples and analysis concerning confocal microscopy, pigment content, determination of salicylic acid content, and RNA-seq library preparation
- performed the statistical analysis for chlorophyll *a* and *b* fluorescence, non-photochemical quenching, gas exchange, pigment content, SA and ET content, and foliar temperature
- resource collection for the article
- visualization of the data (preparing all the figures and tables)
- drafting of the original manuscript, and the review and editing of the final version.

I estimate my contribution at: 60%



Signature

Warsaw, 12.03.2026

Paweł Burdiak

pawel_burdiak@sggw.edu.pl

Department of Plant Genetics, Breeding and Biotechnology, Institute of Biology

Warsaw University of Life Sciences

Biological Sciences Discipline Counsel

of the Warsaw University

of Life Sciences

Statement

regarding co-authorship in the publication with Muhammad Kamran, which is the basis of his doctoral thesis

I hereby declare that I am aware that the publication:

Kamran, M., Burdiak, P., Zarrin Ghalami, R., Rusaczonek, A., Duszyn, M., Gołębiewska, K., Gawroński, P., Karpiński, SM (2026a). The kinase CRK5 regulates dark-induced senescence and dissipation of energy as heat by inhibiting salicylic acid signaling, *Plant Physiology*, Volume 200, Issue 2, February 2026, kiag046, https://doi.org/10.1093/plphys/kiag046 **(Publication 2),** of which I am a co-author, has been included in Muhammad Kamran's doctoral thesis.

I contributed to:

- participated in the conceptualization of the study
- RNA-seq library preparation
- holistically analyzed the data
- review and editing of the final version of the manuscript.

I estimate my contribution at: 7.5%

Signature

Paweł Burdiak

Warsaw, 12.03.2026

Roshanak Zarrin Ghalami

roshanak_zarrin_ghalami@sggw.edu.pl

Department of Plant Genetics, Breeding and Biotechnology, Institute of Biology

Warsaw University of Life Sciences

Biological Sciences Discipline Counsel

of the Warsaw University

of Life Sciences

Statement

regarding co-authorship in the publication with Muhammad Kamran, which is the basis of his doctoral thesis

I hereby declare that I am aware that the publication:

Kamran, M., Burdiak, P., Zarrin Ghalami, R., Rusaczonek, A., Duszyn, M., Gołębiewska, K., Gawroński, P., Karpiński, SM (2026a). The kinase CRK5 regulates dark-induced senescence and dissipation of energy as heat by inhibiting salicylic acid signaling, *Plant Physiology*, Volume 200, Issue 2, February 2026, kiag046, https://doi.org/10.1093/plphys/kiag046 **(Publication 2),** of which I am a co-author, has been included in Muhammad Kamran's doctoral thesis.

I contributed to:

- participation in the generation of double mutant lines
- participation in the growing of plant materials for experiments

I estimate my contribution at: 4%

Signature

Warsaw, 12.03.2026

Anna Rusaczonek

anna_rusaczonek@sggw.edu.pl

Department of Botany and Plant Physiology, Institute of Biology

Warsaw University of Life Sciences

Biological Sciences Discipline Counsel

of the Warsaw University

of Life Sciences

Statement

regarding co-authorship in the publication with Muhammad Kamran, which is the basis of his doctoral thesis

I hereby declare that I am aware that the publication:

Kamran, M., Burdiak, P., Zarrin Ghalami, R., Rusaczonek, A., Duszyn, M., Gołębiewska, K., Gawroński, P., Karpiński, SM (2026a). The kinase CRK5 regulates dark-induced senescence and dissipation of energy as heat by inhibiting salicylic acid signaling, *Plant Physiology*, Volume 200, Issue 2, February 2026, kiag046, https://doi.org/10.1093/plphys/kiag046 **(Publication 2),** of which I am a co-author, has been included in Muhammad Kamran's doctoral thesis.

I contributed to:

- participation in the measurements and analysis of salicylic acid content
- participation in the measurements and analysis of chlorophyll content

I estimate my contribution at: 5%

Signature

Warsaw, 12.03.2026

Maria Duszyn

maria_duszyn@sggw.edu.pl

Department of Plant Genetics, Breeding and Biotechnology, Institute of Biology

Warsaw University of Life Sciences

Biological Sciences Discipline Counsel

of the Warsaw University

of Life Sciences

Statement

regarding co-authorship in the publication with Muhammad Kamran, which is the basis of his doctoral thesis

I hereby declare that I am aware that the publication:

Kamran, M., Burdiak, P., Zarrin Ghalami, R., Rusaczonek, A., Duszyn, M., Gołębiewska, K., Gawroński, P., Karpiński, SM (2026a). The kinase CRK5 regulates dark-induced senescence and dissipation of energy as heat by inhibiting salicylic acid signaling, *Plant Physiology*, Volume 200, Issue 2, February 2026, kiag046, https://doi.org/10.1093/plphys/kiag046 **(Publication 2),** of which I am a co-author, has been included in Muhammad Kamran's doctoral thesis.

I contributed to:

- participation in the analysis of confocal microscopy and Figure S2 generation

I estimate my contribution at: 4%

Maria Duszyn

Signature

Warsaw, 12.03.2026

Kinga Gołębiewska

kinga_golebiewska@sggw.edu.pl

Department of Plant Genetics, Breeding and Biotechnology, Institute of Biology

Warsaw University of Life Sciences

Biological Sciences Discipline Counsel

of the Warsaw University

of Life Sciences

Statement

regarding co-authorship in the publication with Muhammad Kamran, which is the basis of his doctoral thesis

I hereby declare that I am aware that the publication:

Kamran, M., Burdiak, P., Zarrin Ghalami, R., Rusaczonek, A., Duszyn, M., Gołębiewska, K., Gawroński, P., Karpiński, SM (2026a). The kinase CRK5 regulates dark-induced senescence and dissipation of energy as heat by inhibiting salicylic acid signaling, *Plant Physiology*, Volume 200, Issue 2, February 2026, kiag046, https://doi.org/10.1093/plphys/kiag046 **(Publication 2),** of which I am a co-author, has been included in Muhammad Kamran's doctoral thesis.

I contributed to:

- participation in the RNA-seq analysis
- participation in the generation of Figure 4 for the manuscript

I estimate my contribution at: 4%

Signature

Gołębiewska Kinga

Warsaw, 12.03.2026

Piotr Gawroński

piotr_gawronski@sggw.edu.pl

Department of Plant Genetics, Breeding and Biotechnology, Institute of Biology

Warsaw University of Life Sciences

Biological Sciences Discipline Counsel

of the Warsaw University

of Life Sciences

Statement

regarding co-authorship in the publication with Muhammad Kamran, which is the basis of his doctoral thesis

I hereby declare that I am aware that the publication:

Kamran, M., Burdiak, P., Zarrin Ghalami, R., Rusaczonek, A., Duszyn, M., Gołębiewska, K., Gawroński, P., Karpiński, SM (2026a). The kinase CRK5 regulates dark-induced senescence and dissipation of energy as heat by inhibiting salicylic acid signaling, *Plant Physiology*, Volume 200, Issue 2, February 2026, kiag046, https://doi.org/10.1093/plphys/kiag046 **(Publication 2),** of which I am a co-author, has been included in Muhammad Kamran's doctoral thesis.

I contributed to:

- RNA-seq data analysis

I estimate my contribution at: 5%

Signature

Piotr Gawroński

Warsaw, 12.03.2026

Stanisław Karpinski

stanislaw_karpinski@sggw.edu.pl

Department of Plant Genetics, Breeding and Biotechnology, Institute of Biology

Warsaw University of Life Sciences

Biological Sciences Discipline Counsel

of the Warsaw University

of Life Sciences

Statement

regarding co-authorship in the publication with Muhammad Kamran, which is the basis of his doctoral thesis

I hereby declare that I am aware that the publication:

Kamran, M., Burdiak, P., Zarrin Ghalami, R., Rusaczonek, A., Duszyn, M., Gołębiewska, K., Gawroński, P., Karpiński, SM (2026a). The kinase CRK5 regulates dark-induced senescence and dissipation of energy as heat by inhibiting salicylic acid signaling, *Plant Physiology*, Volume 200, Issue 2, February 2026, kiag046, https://doi.org/10.1093/plphys/kiag046, **(Publication 2),** of which I am a co-author, has been included in Muhammad Kamran's doctoral thesis.

I contributed to:

- securing research funding
- conceptualization of the study
- holistically analyzed the data
- review and editing of the final version.

I estimate my contribution at: 10.5%

S. Karpiński

Signature

Article 3: <u>Kamran, M</u>., Burdiak, P., Rusaczonek A., Zarrin Ghalami R., & Karpiński, S. (2026b). CRK5 preserves antioxidant homeostasis and prevents cell death during dark-induced senescence through inhibiting the salicylic acid signaling pathway. Under peer review in BMC Plant Biology, Springer Nature.

(Ministerial score: 140; IF: 4.8)

CRK5 preserves antioxidant homeostasis and prevents cell death during dark- induced senescence thr...

CURRENT STATUS

Your submission is in peer review

News about your peer review process

- The editor has invited 2 reviewer(s)
- There is 1 reviewer(s) that has accepted to review your manuscript

After the editor has collated and reviewed all the reports they need, which may involve seeking additional reviews, you'll be notified about their decision.

The editor has decided that your submission is suitable for peer review and is now inviting reviewers to evaluate your manuscript. The process of finding, inviting, and securing reviewers can take a few weeks.

We'll let you know if you need to make any revisions.

Need help?

If you have any questions about this submission, you can email the Editorial Office (bmcplantbiology@biomedcentral.com).

For general enquiries, please look at our support information.

How was your experience today?

Awful Bad OK Good Great

Send feedback

Progress so far

Show history

- Submission received
- Technical check
- Editorial assignment
- With editor
- Peer review

Learn about our submission process

Your submission

Title
CRK5 preserves antioxidant homeostasis and prevents cell death during dark- induced senescence through inhibiting the salicylic acid signaling pathway

Type
Research

Journal
BMC Plant Biology

Collection
Salicylic acid in plant growth, development and immunity

Submission ID
1ee28317-9192-40e2-b9b4-3569a2669de1

Submission version
v.1.0

Manuscript File
BMC PB Manuscript.docx

WARSAW
UNIVERSITY
OF LIFE SCIENCES

Warsaw, 12.01.2026

Dear Editor,

I would like to enclose a manuscript entitled “CRK5 preserves antioxidant homeostasis and prevents cell death during dark-induced senescence through inhibiting the salicylic acid signaling pathway” to be considered for publication in the BMC Plant Biology journal under the collection entitled ‘‘Salicylic acid in plant growth, development and immunity”.

Dark-induced senescence (DIS) is a good tool for investigating the molecular mechanisms underlying leaf aging, redox imbalance, and cell death in plants. Although salicylic acid (SA) is well recognized as a central regulator of these processes, the upstream signaling components that integrate SA-dependent pathways with antioxidant homeostasis during DIS remain poorly understood. In this study, we identify CYSTEINE-RICH RECEPTOR-LIKE KINASE 5 (CRK5) as a key regulator that links SA-signaling to redox control and cell death during senescence.

Using a combination of genetic, physiological, biochemical, and transcriptomic approaches, we demonstrate that loss of CRK5 function results in elevated SA-accumulation, accelerated senescence, increased reactive oxygen species production, and enhanced cell death under both control and dark conditions, accompanied by pronounced alterations in antioxidant systems. Importantly, genetic suppression of SA-synthesis or -catabolism fully restores the wild-type phenotype in the *crk5* background, establishing that the observed effects are SA-dependent. Transcriptome profiling further reveals extensive deregulation of senescence-, redox-, and cell death-related gene networks in *crk5*, positioning CRK5 as an upstream negative regulator of SA-mediated cell death and positive regulator of antioxidant homeostasis. Our findings provide new insights into how plants balance hormonal signaling, redox dynamics, and cellular viability under stress conditions, and offer potential strategies for enhancing stress tolerance and longevity in plants.

The manuscript has been seen and approved by all authors and has not been submitted for publication elsewhere.

We hope that this manuscript will meet the requirements of the BMC Plant Biology journal and make a meaningful contribution to the field of plant development and acclimation.

We thank you for your time and consideration, and we look forward to your response.

WARSAW
UNIVERSITY
OF LIFE SCIENCES

With best regards,

Prof. Stanisław Karpiński
(on behalf of all authors)

Prof Stanisław Karpiński

Warsaw University
of Life Sciences

Institute of Biology
Department of Plant Genetics, Breeding and Biotechnology

ul. Nowoursynowska 159
02-776 Warszawa
+48 22 59 32 172
stanislaw_karpinski@sggw.edu.pl

CRK5 preserves antioxidant homeostasis and prevents cell death during dark-induced senescence through inhibiting the salicylic acid signaling pathway

Muhammad Kamran[1], Paweł Burdiak[1], Anna Rusaczonek[2], Roshanak Zarrin Ghalami[1], Stanisław M. Karpiński[1*]

[1]Department of Plant Genetics, Breeding and Biotechnology, Institute of Biology, Warsaw University of Life Sciences, Nowoursynowska Street 159, 02-776 Warsaw, Poland

[2]Department of Botany and Plant Physiology, Institute of Biology, Warsaw University of Life Sciences, Nowoursynowska 159, 02-776 Warsaw, Poland

*__Corresponding author__: Stanislaw_Karpinski@sggw.edu.pl

Abstract

Background

Dark-induced senescence (DIS) is a widely used model for dissecting the regulatory mechanisms that manage leaf aging, redox imbalance, and cell death (CD) in plants. Salicylic acid (SA) is a central hormonal regulator of these processes. However, the mechanism involving upstream components, which integrate SA-dependent pathways with antioxidant homeostasis during DIS, remains unresolved. CYSTEINE-RICH RECEPTOR-LIKE KINASE 5 (CRK5) is a membrane-localized protein that plays a role in developmental and stress-responsive pathways. Its promoter contains multiple W-box *cis*-elements, indicating regulation by WRKY factors in SA-mediated pathways. This study investigates how CRK5 modulates SA-dependent CD and antioxidant dynamics during DIS.

Results

In this study, SA-accumulating mutant *crk5* exhibited accelerated senescence, elevated electrolyte leakage, enhanced micro-lesion formation, and markedly increased reactive oxygen species (ROS) accumulation under both control and dark conditions. These phenotypes were accompanied by a substantial reduction in carotenoid and xanthophyll pools, enhanced accumulation of phenolic compounds, and increased free radical scavenging capacity, including ascorbate peroxidase, catalase, and superoxide dismutase activities. Importantly, *crk5* phenotype was fully reverted in

crk5sid2 and *crk5*NahG double mutants, confirming that *crk5* DIS phenotype is induced by activation of the SA-signaling pathway.

Transcriptome profiling revealed extensive deregulation of senescence-, CD-, and redox-associated genes in *crk5* during darkness, including strong induction of SAGs, metacaspases, autophagy, and antioxidant-related transcripts. The line with constitutively enhanced SA level (*cpr1*), used as a control, showed similar phenotypes to *crk5*, although transcriptional reprogramming was largely absent in *cpr1* after darkness, highlighting CRK5 as a key upstream negative regulator of SA-mediated CD and positive regulator of antioxidant homeostasis.

Conclusion

Our work presents CRK5 as a central regulatory hub that inhibits the SA-signaling, ROS burst, and CD activation during DIS. Loss of CRK5 function is associated with the activation of SA-signaling, altered antioxidant systems, increased ROS burden, and ROS-driven CD acceleration, resulting in accelerated senescence. Conversely, suppression of SA-biosynthesis or -catabolism in a *crk5* background restores the wild-type phenotype. These findings position this receptor kinase as a key mediator that coordinates hormonal, metabolic, and oxidative pathways to maintain leaf viability, providing mechanistic insight into the control of stress-induced senescence and CD in Arabidopsis.

Keywords: dark-induced senescence, reactive oxygen species, cell death, antioxidant system, salicylic acid, receptor-like kinase

Background

Leaf senescence represents the final, essential stage of foliar development, characterized by a fundamental functional transition from active nutrient assimilation to systematic nutrient remobilization, which is critical for overall plant fitness and survival. This process is highly regulated, following a predetermined program of events often classified as cell death (CD) [1, 2]. In plant biology, dark-induced senescence (DIS) is frequently utilized as a critical experimental model system to study natural age-related senescence. This artificial induction effectively promotes many typical senescence symptoms, including extensive chlorophyll degradation, altered photosystem II performance, and organized protein catabolism [2, 3].

Understanding the manner in which DIS triggers the controlled collapse of antioxidant defenses is vital to defining the regulatory frontiers governing plant longevity [4]. Reactive Oxygen Species (ROS) are unavoidable byproducts of aerobic metabolism [5]. They include highly reactive molecules such as hydrogen peroxide (H_2O_2), superoxide (O^{2-}), and the hydroxyl radical (OH^-). In the context of senescence, ROS exhibit a dual function. At low, controlled concentrations, they act as indispensable signaling molecules, actively initiating the senescence program, whereas at high levels they trigger oxidative damage to nucleic acids, lipids, and proteins, leading to irreversible injury and CD [5]. Consequently, leaves undergoing senescence are typically characterized by an acute imbalance: excessive ROS accumulation coupled with a significant reduction in the cellular capacity for antioxidant scavenging [6]. Maintaining cellular redox homeostasis relies on balancing ROS production with immediate scavenging by antioxidant systems [7]. When scavenging systems become overwhelmed, the delicate redox balance is permanently disturbed, pushing the cell toward self-destruction [8]. Plants under stress conditions significantly aggravate the generation of ROS, creating a persistent challenge to cellular redox homeostasis that ultimately dictates the transition from stress tolerance to CD [9].

CD is an active, conserved mechanism of cellular suicide that is central to both normal plant development and environmental stress management, commonly preceded by morphological changes such as nuclear condensation [10]. The activation of the CD process requires the creation of a specific, oxidizing redox environment in various cellular compartments. Research has focused on understanding the interplay between ROS and the complex network of antioxidant molecules and enzymes necessary to maintain the redox environment required for cell survival [11]. Studies

on Arabidopsis have demonstrated that H_2O_2 directly induces CD, confirming the central role of antioxidant capacity in determining whether ROS serves as an adaptive signal or a death trigger [12].

This critical life-or-death decision, which depends on chloroplasts and mitochondria membrane stability, is regulated by the efficiency and coordination of both enzymatic and non-enzymatic defenses [13–15]. Key enzymatic antioxidant, such as Superoxide Dismutase (SOD), serves as the primary defensive line by converting highly reactive superoxide (O^{2-}) into the more stable signaling molecule, hydrogen peroxide (H_2O_2) [12]. Downstream H_2O_2 scavengers, including Catalase (CAT) and Ascorbate Peroxidase (APX), function to maintain H_2O_2 at basal signaling concentrations, essential for acclimation [9, 16]. Concurrently, non-enzymatic antioxidants, encompassing potent radical scavengers such as phenolic compounds and critical photo-protective agents like carotenoids, establish a vital constitutive defense buffer [17]. Two main classes of carotenoids are carotene and xanthophyll, which have been described in their roles as oxidative protectants [18, 19]. Genetic evidence from Arabidopsis, notably the *radical-induced cell death1* (*rcd1*) mutant, reveals that the constitutive upregulation and enhanced redox cycling of phenolic compounds confer remarkable resistance to oxidative stress and subsequent CD, thereby actively blocking the CD cascade [20, 21]. The regulatory role of signaling molecules, particularly salicylic acid (SA), in modulating the timing of the shift in redox balance is crucial in determining cell fate during DIS. The central question addressed by detailed mechanistic studies is how DIS specifically manipulates the antioxidant machinery to manage this shift, from maintaining a highly regulated, low-level signaling ROS pool to permitting the accumulation of damaging ROS necessary for the final execution phase of CD [22, 23].

Salicylic acid (SA) serves as a pivotal phytohormone, coordinating numerous physiological and biochemical processes throughout the entire plant's lifespan. Its regulatory influence spans vegetative growth, photosynthesis, respiration, thermogenesis, and, critically, both senescence and a specific type of CD that is independent of the canonical hypersensitive response [24–26]. An important aspect of SA's activity is its contribution to maintaining cellular redox homeostasis, primarily achieved through the modulation of antioxidant enzyme activity [27]. Studies investigating SA's influence on plant physiology, such as those conducted during acclimation to high light stress, reveal a tight regulatory relationship between SA levels, H_2O_2 content, and major

non-enzymatic antioxidant pools. Specifically, high endogenous foliar SA concentrations in Arabidopsis mutants are found to correlate strictly with elevated levels of H_2O_2. Crucially, this elevation in the oxidative signal is paralleled by a higher content of reduced glutathione (GSH) [28]. This synchronized increase in both the primary H_2O_2 and the major non-enzymatic protective buffer (GSH) suggests that SA is not merely inducing stress but is actively coordinating a state of high-flux redox signaling. By increasing the capacity of GSH pool, which is essential for recycling ascorbic acid (AsA) and supporting H_2O_2 detoxification, the H_2O_2 signal is received without causing immediate, widespread catastrophic oxidative failure [28, 29].

It has been shown that mutants with lower SA levels exhibit better fitness, produce more seeds, and accumulate more biomass, whereas mutants with higher SA content often exhibit a dwarf phenotype [30–33]. Interestingly, some mutants with decreased SA biosynthesis are more susceptible to biotrophic pathogen infection (i.e., *eds5* and *sid2*), while others are more resistant to abiotic stresses (i.e., *eds1* or *pad4*) [30, 34, 35]. In response to stress, the *lsd1* mutant exhibits elevated SA-accumulation and increased CD due to activation of EDS1 and PAD4, because these phenotypes are suppressed in the *lsd1/eds1* and *lsd1/pad4* double mutants, which also show markedly reduced SA and ROS levels [30, 36–39]. These findings suggest that the role of SA in response to biotic and abiotic stresses differs, and it has been postulated that SA acts as a double-edged sword for CD in plants [40]. SA-biosynthesis and -signaling pathways are tightly regulated and integrate multiple upstream signals, including those perceived by receptor-like kinases (RLKs) at the cell surface [41, 42].

Cysteine-rich Receptor-Like Kinases (CRKs) constitute a distinctive subfamily of Receptor-Like Kinases (RLKs) that contain multiple cysteine residues in the extracellular domain (DUF26) organized in conserved motifs [43]. The structural configuration of CRKs provides a powerful conceptual framework for their role in environmental sensing. Due to the high abundance and reactivity of nucleophilic thiol groups within the cysteine residues, CRKs are considered excellent candidates for functioning as extracellular ROS sensors, regulating signal transduction pathways related to plant defense and acclimation, stomatal closure, CD, and developmental senescence [42, 44, 45]. By directly linking extracellular oxidative signals to intracellular kinase activity, CRKs serve as fundamental convergence points for converting stress-induced redox perturbations into regulated defense and survival responses, thereby directly influencing the

maintenance of cellular redox homeostasis and the status of the antioxidant system [42]. CYSTEINE-RICH RECEPTOR-LIKE KINASE 5 (CRK5) in Arabidopsis has established its critical role in regulating growth, senescence, thermoregulation, and acclimatory responses under basal and abiotic stress conditions [43, 46]. CRK5 plays an essential function in plant defense against biotic stimuli, specifically by regulating the SA-signaling pathway, via the MPK3/6–WRKY70–TGA2/6 cascade, positioning CRK5 upstream of the MPK module in response to *Vd*-toxins [47].

The objective of this study was to check the relationship between SA-mediated antioxidant homeostasis during CD through CRK5-mediated negative regulation of dark-induced senescence. We hypothesized that during dark-induced senescence, *crk5* plants would exhibit enhanced CD and more pronounced alterations in antioxidant activity, driven by their elevated SA-accumulation relative to control conditions. To prove this, we used single and double mutant lines impaired in CRK5 activity and deficient in the SA-synthesis or -catabolism (*sid2* and NahG). The *cpr1* (constitutive expresser of pathogenesis-related genes 1) mutant was used as a control, since it has increased levels of SA and elevated expression of pathogenesis-related (PR) genes. The CPR1 is a part of the ubiquitin ligase complex and acts upstream of SA, a negative regulator of plant defense and immune responses [48, 49]. Moreover, our data showed a higher CD rate, reduced pigment content, altered enzymatic and non-enzymatic antioxidant activity, and higher ROS accumulation in SA-accumulating lines (*crk5* and *cpr1*) compared to the wild-type. The RNA-seq data supported our hypothesis regarding the proposed inhibitory role of CRK5 in these processes. Taken together, our data indicate that CRK5 acts as a negative regulator of the SA-signaling pathway, inhibiting accelerated DIS and CD, and altering the antioxidant and redox homeostasis.

Materials and methods

Plant Material and Growing Conditions

All Arabidopsis lines used in this study were in the Columbia-0 (Col-0) genetic background. Double mutant lines were generated through genetic crosses, and primers employed for genotyping are listed in Table S1. Seeds were stratified at 4 °C for three days prior to germination. Plants were then cultivated in a controlled growth chamber on Jiffy Pots under long-day conditions (16 h light/8 h dark) with a constant light intensity of 120 µmol photons m^{-2} s^{-1}, at 22 °C and 70 ± 5% relative humidity, for 4–5 weeks. To induce dark-associated senescence, four-week-old plants were transferred to continuous darkness for a period of four days.

Relative electrolyte leakage

Leaves were detached and placed into 50 mL Falcon tubes containing 35 mL of Milli-Q water (Merck Millipore, Darmstadt, Germany). Electrolyte leakage was determined using a conductivity meter (WTW INOLAB Cond Level 1, Weilheim, Germany). Relative electrolyte leakage was calculated as the ratio of conductivity measured after 1 h of incubation to the total leakage obtained following autoclaving of the samples.

Trypan blue staining

A trypan blue (TB) stock solution (30 µmol; Sigma-Aldrich, St. Louis, MO, USA) was prepared in a mixture of lactic acid, glycerol, and water (10:10:20, v/v/v) and diluted 1:2 with 96% ethanol to obtain the working solution. Leaves from control and dark-treated plants were harvested and immediately immersed in the TB working solution in 50 mL Falcon tubes. Samples were incubated for 30 min at room temperature with gentle agitation. Following staining, the TB solution was discarded and replaced with methanol. Leaves were destained in methanol for 24 h, with the methanol refreshed several times to ensure complete removal of chlorophyll. Chlorophyll-depleted leaves were imaged using a Nikon SMZ18 stereomicroscope equipped with a Nikon d5100 camera (Nikon Inc., Melville, NY, USA). Images were analyzed using ImageJ software (version 1.8.0), and micro-lesions (trypan blue–positive spots) were quantified per mm^2 of leaf area.

Pigment content analysis

Frozen leaf material (20–50 mg) was ground using a Mixer Mill MM 400 (Retsch, Düsseldorf, Germany) for 5 min at 30 Hz and 4 °C in the presence of 1 mL pre-chilled acetone (−20 °C). The resulting extract was evaporated to dryness using a Savant DNA120 SpeedVac concentrator (Thermo Scientific, Waltham, MA, USA). The residue was subsequently resuspended in cold solvent A (acetonitrile:methanol, 90:10, v/v) and briefly homogenized for 1 min. After filtration through a 0.2 μm nylon membrane filter (Whatman, Marlborough, MA, USA), samples were transferred to autosampler vials, sealed, and stored at −80 °C in darkness until HPLC analysis.

Pigment separation was performed using a Shimadzu HPLC system (Kyoto, Japan) equipped with a Synergi™ MAX-RP column (4 μm, 80 Å, 250 × 4.6 mm; Phenomenex, Torrance, CA, USA) maintained at 30 °C. Xanthophylls were eluted using solvent A for 10 min, followed by elution of carotenes with solvent B (methanol:ethyl acetate, 68:32, v/v) for an additional 10 min at a constant flow rate of 1 mL min^{-1}. Individual pigments were identified by comparison with authentic standards (Sigma-Aldrich, St. Louis, MO, USA; Hoffmann-La Roche, Basel, Switzerland; Fluka, Buchs, Switzerland). Total carotenoid content was calculated as the sum of quantified xanthophylls and carotenes and expressed as peak area per μg of DW, following previously established protocols [50, 51].

ABTS and DPPH scavenging activity

The antioxidant capacity of methanol extracts was assessed by evaluating their ability to scavenge ABTS and DPPH free radicals, as well as their ferric reducing power using the FRAP assay. Freeze-dried tissue was finely powdered, extracted with methanol, and centrifuged at 13,000 rpm for 15 min at room temperature. The resulting supernatant was collected and used for antioxidant activity measurements.

ABTS radical scavenging activity was determined according to the method originally described by [52]. The $ABTS^{\bullet+}$ working solution was generated by incubating an aqueous mixture of 7 mM ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) and 2.45 mM potassium persulfate in darkness at room temperature for 16 h. Prior to analysis, the solution was diluted with ethanol to obtain an absorbance of 0.70 ± 0.02 at 734 nm. Sample extracts were mixed with the $ABTS^{\bullet+}$

solution, and absorbance was recorded at 734 nm after 6 min using a microplate reader (Multiskan GO, Thermo Scientific, Waltham, MA, USA).

DPPH radical scavenging activity was measured following the established procedure of [53]. The extracts of the prepared samples were incubated with an ethanol solution of DPPH• (2,2-diphenyl-1-picrylhydrazyl), and absorbance was measured at 517 nm after 20 min of reaction. Radical scavenging capacity was calculated as the percentage inhibition relative to the control.

Antioxidant activity for both ABTS and DPPH assays was calculated using the equation: (%) inhibition = $[(A_0 - A_1)/A_0] \times 100$, where A_0 and A_1 represent the absorbance of the control and sample, respectively. Results were expressed as μmol Trolox equivalents (TE) per gram of DW.

Ferric reducing antioxidant power (FRAP) was determined by measuring the reduction of Fe^{3+}–TPTZ to Fe^{2+}–TPTZ according to the method described by [52].

Phenolic Profile

Total phenolic compounds were quantified following the extraction of 100 mg of plant tissue with 80% (v/v) methanol. Phenolic content was determined using the Folin–Ciocalteu colorimetric assay, according to [54]. After centrifugation, aliquots of the extracts were combined with deionized water, Folin–Ciocalteu reagent, and saturated sodium carbonate (Na_2CO_3). The reaction mixtures were incubated at 40 °C for 30 min, cooled to room temperature, and the absorbance was measured at 740 nm. Total polyphenol content was expressed as milligrams of gallic acid equivalents (GAE) per 100 g DW.

Phenylpropanoids, flavonols, and anthocyanins were quantified using the spectrophotometric method described by [55]. Methanol extracts were mixed with acidified ethanol (0.1% HCl in 96% ethanol) and aqueous HCl (2% HCl in water). After a 15 min incubation, absorbance was recorded at 320 nm, 360 nm, and 520 nm using a BioSpectrometer kinetic system. Caffeic acid, quercetin, and cyanidin were used as calibration standards for phenylpropanoids, flavonols, and anthocyanins, respectively. The concentrations of individual phenolic groups were expressed as milligrams per 100 g DW.

Hydrogen Peroxide Levels Determination

Hydrogen peroxide (H_2O_2) content was quantified following the method of [56] with minor modifications. Approximately 50 mg of leaf tissue was homogenized in a TissueLyser LT (Qiagen, The Netherlands) for 5 min at 50 Hz and 4 °C in 300 µL of ice-cold 0.1% (w/v) trichloroacetic acid. The homogenate was centrifuged at 13,000 rpm for 15 min, and the resulting supernatant was collected for analysis. An aliquot of the extract was combined with 10 mM potassium phosphate buffer (pH 7.0) and 1 M potassium iodide (KI) in a volumetric ratio of 1:1:2. Absorbance was recorded at 390 nm using a Multiscan GO microplate reader (Thermo Scientific, USA). Hydrogen peroxide concentration was calculated from a standard calibration curve and expressed as µmol H_2O_2 per 100 mg DW.

Histochemical detection of H_2O_2 was performed using 3,3′-diaminobenzidine (DAB) staining as described by [57]. Excised leaves were immersed in a staining solution containing 0.1% (w/v) DAB, 2 mM dimethyl sulfoxide (DMSO), and 0.05% (v/v) Tween-20 prepared in Milli-Q water (pH 3.8) and incubated for 4 h at room temperature. Following staining, chlorophyll was removed by incubation in 0.25% (w/v) chloral hydrate for 24 h. Stained leaves were subsequently examined using a Leica M165 FC stereomicroscope.

Protein extraction

Leaf samples were detached, immediately frozen in liquid nitrogen, and 50–100 mg portions homogenized using a TissueLyser LT (Qiagen) at 50 s^{-1} for 5 min at 4°C in 1 ml extraction buffer (100 mM Tricine-Tris, pH 7.5, 3 mM $MgSO_4$, 3 mM EGTA, 1 mM DTT). Samples were incubated on ice for 15 min, then centrifuged at 18,000 × g for 20 min. The resulting supernatant served as the enzyme extract and was stored at −80°C for subsequent assays. For ascorbate peroxidase activity measurements, the extract was supplemented with 5 mM L-ascorbate. Protein concentrations were quantified via the Bradford (1976) method [58] using a commercial protein assay kit (Fermentas) standardized against bovine serum albumin (Fermentas).

Determination of antioxidant enzyme activities

APX activity was determined following [59] with modifications. Enzyme extract was combined with assay buffer (50 mM phosphate, pH 7.0, 1 mM EDTA, 15 mM L-ascorbate, 40 mM H_2O_2) at

ratios ensuring 20–80% absorbance decline. Activity was calculated from the ascorbate oxidation rate over 2 min, using its extinction coefficient at 290 nm (ε =2.8 $mM^{-1}cm^{-1}$). Values are reported as μmol L-AsA mg^{-1} protein min^{-1}.

CAT activity was performed according to [60] with modifications. A 30% (v/v) H_2O_2 solution was diluted in 50 mM phosphate buffer (pH 7.0) to an absorbance of 0.5 (±0.02) at 240 nm (initial concentration of H_2O_2 ca. 13 mM). Enzyme extract was added at ratios yielding 20–80% absorbance decrease. Activity was measured by H_2O_2 decomposition rate over 2 min, using its extinction coefficient of H_2O_2 at 240 nm (ε =43.6 $mM^{-1}cm^{-1}$). Results are expressed as μmol H_2O_2 decomposed mg^{-1} protein min^{-1}.

SOD activity was assayed spectrophotometrically per [61]. Freshly prepared enzyme assay mixture contained 0.1 M phosphate buffer (pH 7.5), 2.4 μM riboflavin, 840 μM nitroblue tetrazolium (NBT) salt, 150 mM methionine, 12 mM Na_2EDTA in the proportion 8:1:1:1:1 by vol., respectively. Enzyme extract was added to achieve 20–80% inhibition of NBT reduction. After 15 min illumination (500 μmol m^{-2} s^{-1} white LED; Photon System Instruments SL 3500-W-D) or dark incubation (blank), absorbance was measured at 560 nm. Results were expressed in units (U) defined as the amount of enzyme that inhibits NBT photo-reduction to blue formazan by 50% *per* 1 mg of protein.

All assays were performed using a UV-Vis Multiskan GO Microplate Spectrophotometer (Thermo Scientific, Waltham, MA, USA).

Library preparation and RNA-seq analysis

For RNA isolation, 4- or 4.5-week-old plants were used. The experiment was performed on three biological replicates. Each replicate consisted of several pooled detached leaves from at least three individual plants. Frozen leaves were ground using a mortar and pestle in liquid nitrogen, and RNA was isolated using the Spectrum™ Plant Total RNA Kit (Sigma, St. Louis, MO, USA). mRNA fraction was isolated using Oligo d(T)25 Magnetic Beads (NEB, S1419S) from 20 ug of total RNA. RNA-seq libraries were self-prepared according to [62]. RNA-seq libraries were sequenced in 150-bp paired-end reads using the Aviti High flowcell platform (Element Biosciences), conducted by Genomed (Warsaw, Poland). Unique Molecular Identifiers (UMIs) were extracted from R2 reads using UMI-tools extract [63]. Subsequently, R1 reads underwent adapter trimming and quality

filtering to remove low-quality sequences using Trim Galore (https://github.com/FelixKrueger/TrimGalore). The cleaned reads were then aligned using STAR to the *Arabidopsis thaliana* reference genome (Ensembl Plants release 60) [64]. To eliminate PCR duplicates, UMI-tools dedup was employed, leveraging both UMI sequences and mapping positions. Gene-level read counts were generated using htseq-count [65]. The resulting count data were imported into R, where a count matrix was constructed. Differential expression analysis was performed using the DESeq2 package [66]. Significantly enriched GO terms were identified in up-/downregulated gene sets using the package (v4.10.1) [67].

Statistical analysis

The statistical analysis for ion leakage, micro-lesions, pigment content, scavenging activities (DPPH and ABTS), Phenolic content, H_2O_2 content, and antioxidant activities (APX, CAT, and SOD) was performed using GraphPad Prism 8 software.

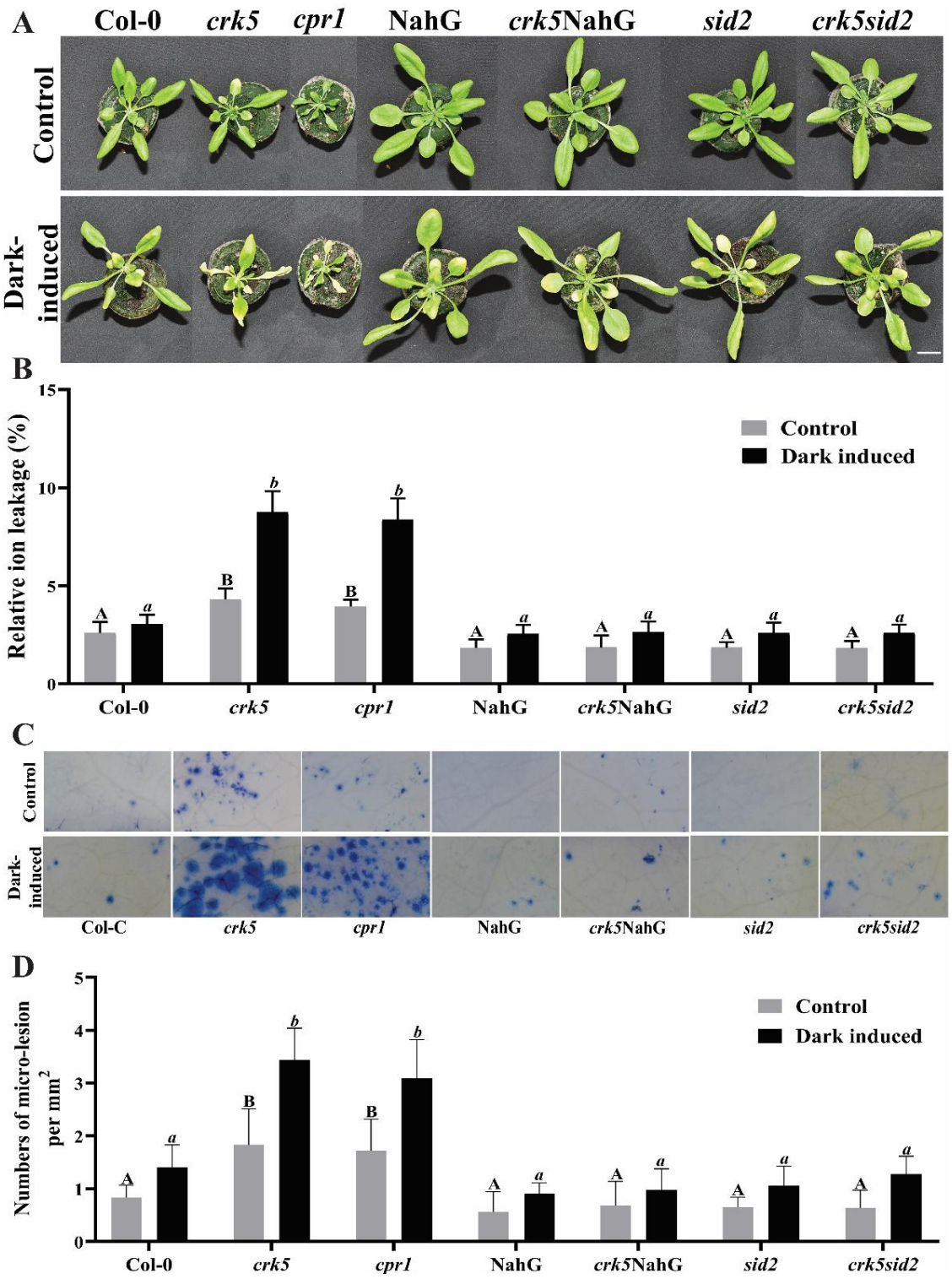
Accession numbers

Accession numbers of all genes in Fig. 5C and D are given in Table S2.

Results

CRK5, through the prevention of SA-signaling, accelerates dark-induced senescence and cell death

To clarify the role of salicylic acid signaling in CRK5-mediated inhibition of dark-induced senescence and cell death, we analyzed mutants with elevated SA levels (*crk5* and *cpr1*), SA-synthesis or -catabolism deficient lines (*sid2* and NahG), and the corresponding double mutants (*crk5sid2* and *crk5*NahG) (Fig. S1). We observed induced accelerated senescence in plants with elevated SA content under dark conditions (Fig. 1A). Subsequently, we measured electrolyte leakage to assess CD in these genotypes. We found higher ion leakage levels in mutants with elevated SA content (*crk5* and *cpr1*) even in control conditions, compared to the wild-type (Col-0). In contrast, the CD level was not significantly different in SA-synthesis or -catabolism deficient mutants (*sid2* and NahG) and double mutants (*crk5sid2* and *crk5*NahG). In the dark-treated plants, these changes were even more pronounced in the *crk5* and *cpr1* mutants. (Fig. 1B). Moreover, micro-lesion formation was assessed using TB staining. Micro-lesions constitute small lesion areas within the leaf tissue, comprising one or a couple of dead cells [68, 69]. In control conditions, micro-lesions were consistently higher in *crk5* and *cpr1*. Under dark-induced senescence, these differences became even more pronounced compared with Col-0 and SA-synthesis or -catabolism deficient lines (Fig. 1C, D). These results indicate that SA-signaling during DIS enhances senescence and promote CD.



333

334 **Figure 1**. CRK5-dependent blockade of salicylic acid accumulation and signaling during
335 dark-induced senescence and cell death. Morphology (**A**), relative ion leakage (**B**), trypan blue
336 staining for the detection of cell death (**C**), and cell death quantified as micro-lesion number per

mm^2 (**D**) of wild-type, *crk5*, c*pr1*, and salicylic acid-synthesis dysfunction *sid2* mutant or salicylic acid-catabolism transgenic NahG line after exposure for 4 days to permanent darkness. The picture shows a phenotype of 4-week-old plants growing under ambient light (120 μmol m^{-2} s^{-1}) conditions and exposed to four days of permanent darkness. White scale bars indicate 1 cm. Mean values (±SD) were derived from 9 plants (n = 9). Statistical analysis was performed using a t-test at a significance level of $p < 0.05$. Letters A, B, and a, b above the bars indicate homogenous groups, and values sharing common labels (letters) are not significantly different from each other.

Carotenoid degradation during dark-induced senescence is suppressed by CRK5 and its inhibition of SA-signaling

Considering that SA accumulation under prolonged darkness enhances CD and promotes the aging processes, we examined the effect of these changes on carotene and xanthophyll levels. In our study, we observed a significant reduction in total carotenes (including α-carotenes and ß-carotenes) and xanthophylls (including lutein, violaxanthin, antheraxanthin, and zeaxanthin) in SA-accumulating *crk5* and *cpr1* mutants under both control and dark-induced conditions compared to the wild-type, while introduction of deregulation of SA-synthesis mutant or SA-catabolism transgenic line, respectively (*sid2* and NahG) into *crk5* mutant background reverted its phenotype of accelerated carotenoids and xanthophyll dark-induced degradation to the wild-type phenotype (Fig. 2A, B, and Fig. S2). These results support the role of CRK5 in inhibiting the SA-signaling pathway and its role in protecting SA-dependent degradation of carotenoids and xanthophylls during the onset of DIS.

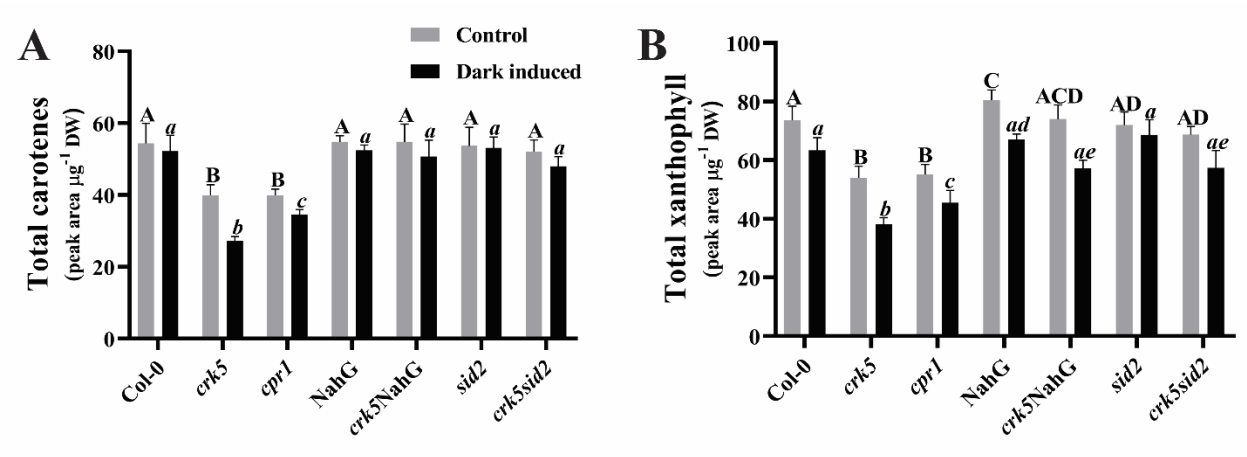


Figure 2. Foliar total carotenes and xanthophyll content of wild-type, *crk5*, *cpr1*, and salicylic acid-synthesis dysfunction *sid2* mutant or salicylic acid-catabolism transgenic NahG line in control conditions and after exposure for 4 days of darkness. Total carotenes content (**A**) and total xanthophyll content (**B**). Mean values (±SD) were derived from 9 plants (n = 9). Statistical analysis was performed using a t-test at a significance level of $p < 0.05$. Letters A, B, C, D, and a, b, c, d, e above the bars indicate homogenous groups, and values sharing common labels (letters) are not significantly different from each other.

ROS formation in dark-induced senescence is suppressed by CRK5 and its inhibition of SA-signaling

Since CRK5 was found to affect carotenoid degradation, we further investigated whether it influences the ROS formation and its prevention systems. We found that DPPH and ABTS antioxidant activities were strongly increased in the SA-accumulating *crk5* and *cpr1* mutant lines compared to wild-type, with a further increase under 4 days of DIS. In contrast, SA-synthesis deficient mutant (*sid2*) and transgenic SA-catabolic (NahG) line showed significantly lower antioxidant activity. Introduction of *sid2* and NahG into the *crk5* background reverted antioxidant activity to wild-type phenotype, indicating that CRK5 and its suppression of SA-signaling prevent ROS formation during DIS (Fig. 3A, B). We also observed significantly elevated level of total phenolic compounds in SA-accumulating plants (Fig. 3C). The phenolic profile showed that the levels of phenylpropanoids, flavonols, and anthocyanins in our study were significantly higher in *crk5* and *cpr1*, while in SA-synthesis deficient mutant (*sid2*) and SA-catabolic transgenic (NahG) line, the levels were considerably lower than in wild-type in both control and dark conditions. In double mutant lines, there was reversion in the levels of these phenolic compounds, and the levels were similar to those of SA-synthesis deficient mutant or SA-catabolic line (Fig. 3D–F). These results also indicate a higher level of non-enzymatic antioxidant activity in the SA-accumulating lines during DIS.

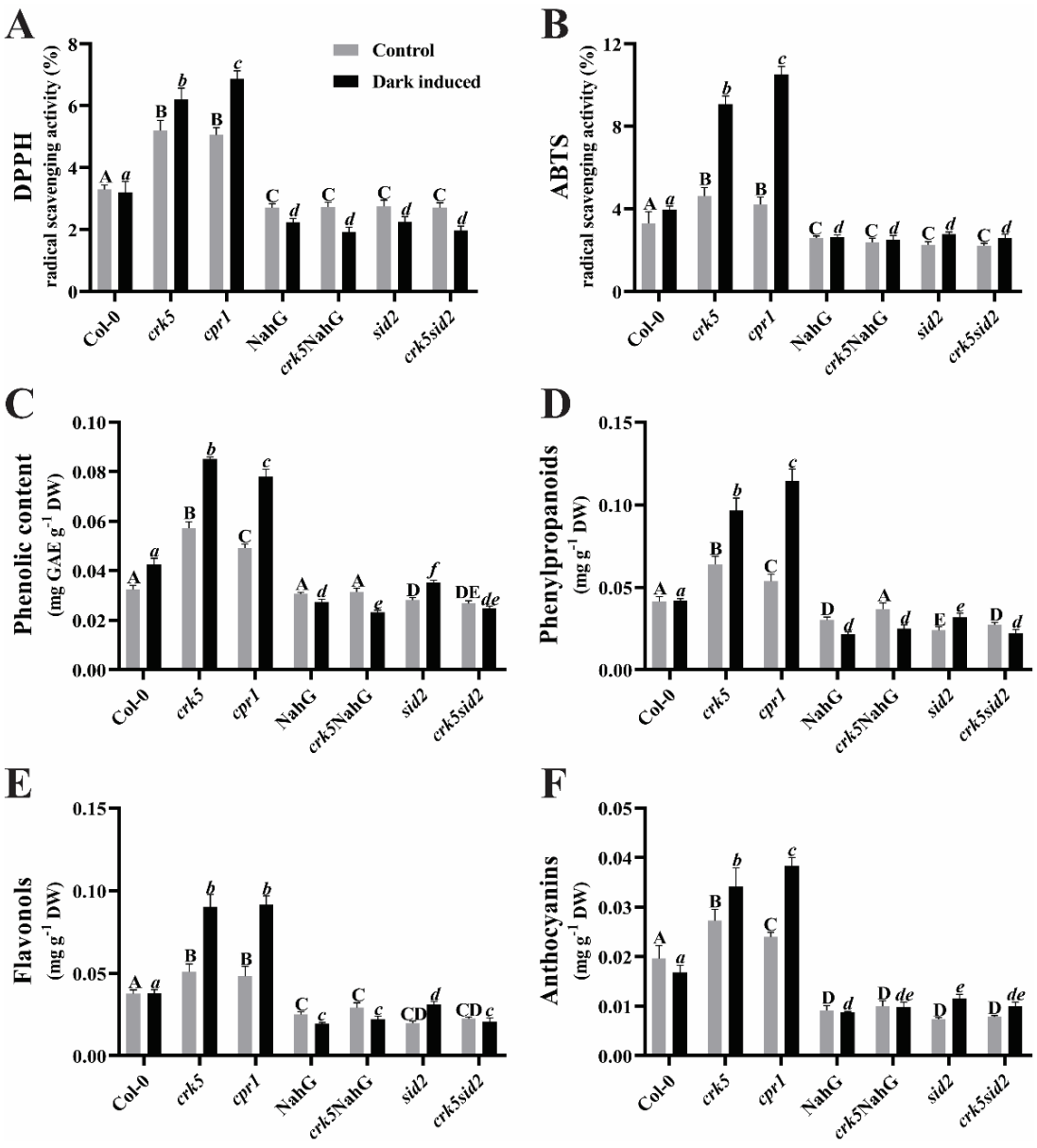
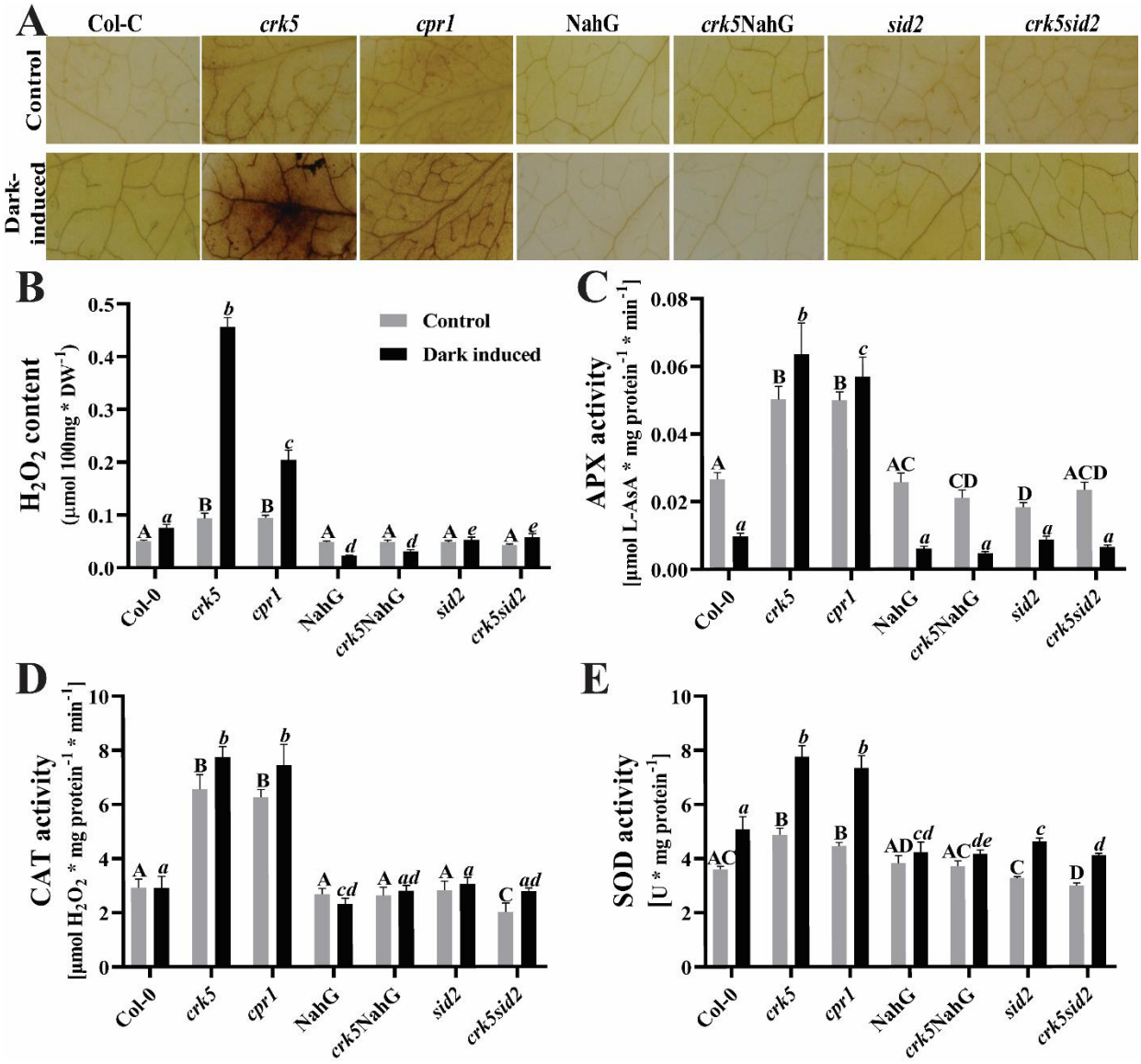


Figure 3. The antioxidant activity of wild-type, *crk5*, *cpr1*, and salicylic acid-synthesis dysfunction *sid2* mutant or salicylic acid-catabolism transgenic NahG line in control and after 4 days exposure to darkness. DPPH (2,2-diphenyl-1-picrylhydrazyl) (**A**), ABTS (2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)) ROS scavenging activity (**B**), Phenolic (**C**), phenylpropanoids (**D**), flavonols (**E**), and anthocyanins foliar content (**F**). Mean values (±SD) were derived from 9 plants (n = 9). Statistical analysis was performed using a t-test at a significance level of $p < 0.05$. Letters A, B, C, D, E, and a, b, c, d, e, f above the bars indicate homogenous groups, and values sharing common labels (letters) are not significantly different from each other.

The ROS level and enzymatic ROS scavengers are dependent on CRK5 and SA-signaling

To assess ROS levels and enzymatic antioxidant system responses to DIS and SA-signaling, we quantified foliar ROS levels and measured the activities of major antioxidant enzymes (APX, CAT, and SOD). Deregulated SA-signaling and -accumulating mutants (*crk5* and *cpr1*) exhibited significantly higher basal and dark-induced H_2O_2 levels than Col-0, whereas SA-synthesis dysfunction mutant (*sid2*) or transgenic SA-catabolic lines (NahG) showed reduced H_2O_2 foliar levels (Fig. 4A, B; Fig. S1). Introduction of *sid2* mutations or NahG activity into the *crk5* background largely suppressed the elevated H_2O_2 levels, demonstrating that CRK5-dependent inhibition of ROS accumulation is mediated by inhibition of SA-signaling. Consistent with ROS patterns, APX, CAT, and SOD enzymatic activities were increased in *crk5* and *cpr1* but remained unchanged in *sid2* and NahG compared with Col-0. These increased activities in *crk5* and *cpr1* were reverted to near wild-type levels in *crk5*NahG and *crk5sid2* double mutants and lines (Fig. 4C–E). Together, these results indicate that CRK5 inhibits the SA-signaling and foliar ROS levels, thus accelerating DIS, characterized by enhanced ROS production and CD.



406

407 **Figure 4.** Foliar ROS levels and enzymatic ROS scavenging activity of wild-type, *crk5*,
408 *cpr1*, and salicylic acid-synthesis dysfunction *sid2* mutant or salicylic acid-catabolism transgenic
409 NahG line in control and after exposure for 4 days to darkness. 3,3'diaminobenzidine (DAB)
410 staining showing H_2O_2 foliar levels (**A**), H_2O_2 foliar content (**B**), APX (**C**), CAT (**D**), and SOD (**E**)
411 foliar enzymatic activity. Mean values (±SD) were derived from 9 plants (n = 9). Statistical
412 analysis was performed using a t-test at a significance level of $p < 0.05$. Letters A, B, C, D, and a,
413 b, c, d, e above the bars indicate homogenous groups, and values sharing common labels (letters)
414 are not significantly different from each other.

415 **CRK5 prevents the transcriptomic changes by inhibiting the SA-signaling**

To gain a deeper insight into CRK5's negative regulation of DIS and CD, as well as cellular redox homeostasis, we performed whole-transcriptome sequencing of the analyzed genotypes under ambient light and after four days of continuous darkness. Under control conditions, the SA-synthesis deficient mutant *sid2* and the SA-accumulating mutant *cpr1* exhibited the most pronounced transcriptional changes, with a total of 93 differentially expressed genes (DEGs), underscoring the importance of SA-signaling in senescence- and CD-related processes (Fig. 5A). As expected, senescence- and CD-associated genes showed limited transcriptional regulation under ambient light and photoperiod, with the notable exception of *crk5*, which displayed elevated expression of the senescence markers *SAG12* and *SAG13*, as well as the CD-related genes *MC2* and *MC8*, that is consistent with its accelerated DIS phenotype (Fig. 5C). Gene ontology (GO) enrichment analysis further revealed significant overrepresentation of terms related to senescence, CD, and SA response among genes induced in *crk5* (Fig. S3). Dark-treatment strongly induced senescence- and CD-related transcriptional responses across all genotypes (Fig. 5C). Notably, *crk5* exhibited the most extensive transcriptomic reprogramming, with more than 7,187 DEGs compared with wild-type plants (Fig. 5B). GO analysis of induced genes in *crk5* showed significant enrichment of processes related to pigment catabolism, organ senescence, and responses to light intensity (Fig. S3). Consistent with these findings, *crk5* displayed marked up-deregulation of key senescence regulators (*SAG12*, *SAG29*, *ANAC046*, *NAP*, *WRKY75*, *NYE1*), autophagy-related genes (*ATG5*, *ATG7*, *ATG8a*), and CD-associated genes (*MC2*, *MC8*, *LSD1*, *HRD1A*) (Fig. 5C). In addition, dark-exposed *crk5* plants showed increased expression of genes involved in metabolic and redox-related pathways, including *PALs*, *RBOHs*, *PRXs*, *MPKs*, *GDH2*, *CAT3*, *GPX2*, *GUN1*, *NPR1*, and *CKX2*, suggesting a broad role for SA-signaling in metabolic regulation during accelerated DIS (Fig. 5D). Although the *cpr1* mutant exhibited clear senescence symptoms under darkness, its transcriptional changes were substantially less pronounced than those observed in *crk5*, indicating that CRK5 functions as a major upstream regulator of SA-dependent transcriptional responses during accelerated DIS.

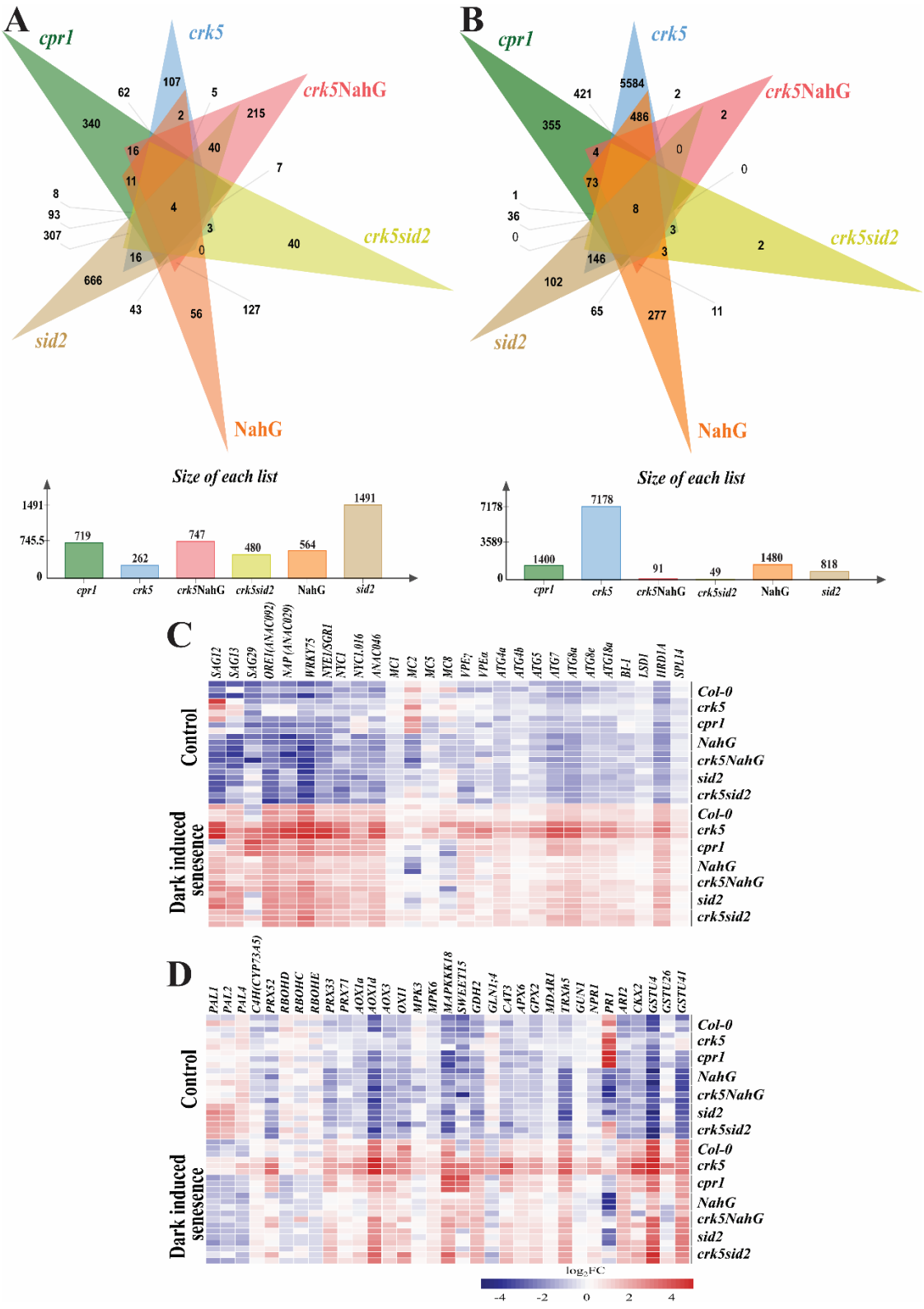


Figure 5. Venn diagram showing commonly and differentially regulated DEGs, and heat maps showing the expression patterns of selected DEGs in wild-type, *crk5*, c*pr1*, and salicylic

acid-synthesis dysfunction *sid2* mutant or salicylic acid-catabolism transgenic NahG line under control and after exposure to permanent darkness for 4 days. Number of deregulated transcripts in control (**A**) and number of deregulated transcripts in dark-induced plants (**B**), Selected senescence and CD pathway-related genes (**C**), and metabolic pathways-related genes (**D**).

Discussion

In the present study, we provided genetic, molecular, and physiological evidence that CRK5 acts as a negative regulator of CD during accelerated DIS through the inhibition of SA-signaling pathway, ROS, and antioxidant homeostasis (Fig. 6). Accelerated senescence and CD in the absence of functional CRK5 in *crk5* mutant was further confirmed by trypan blue staining and quantification of micro-lesions, which revealed a significantly higher number of dead foliar cells (Fig. 1C, D). These observations were consistent with previous studies demonstrating that SA promotes a conditionally dependent hypersensitive disease defense response characterized by spontaneous CD in numerous lesion-mimic mutants [36, 38, 40, 70]. One of our previous studies demonstrated an increased rate of CD in the *lsd1* mutant, characterized by elevated SA levels, under abiotic stress conditions (UV-A+B), further supporting a strong link between SA-signaling and the regulation of CD [68].

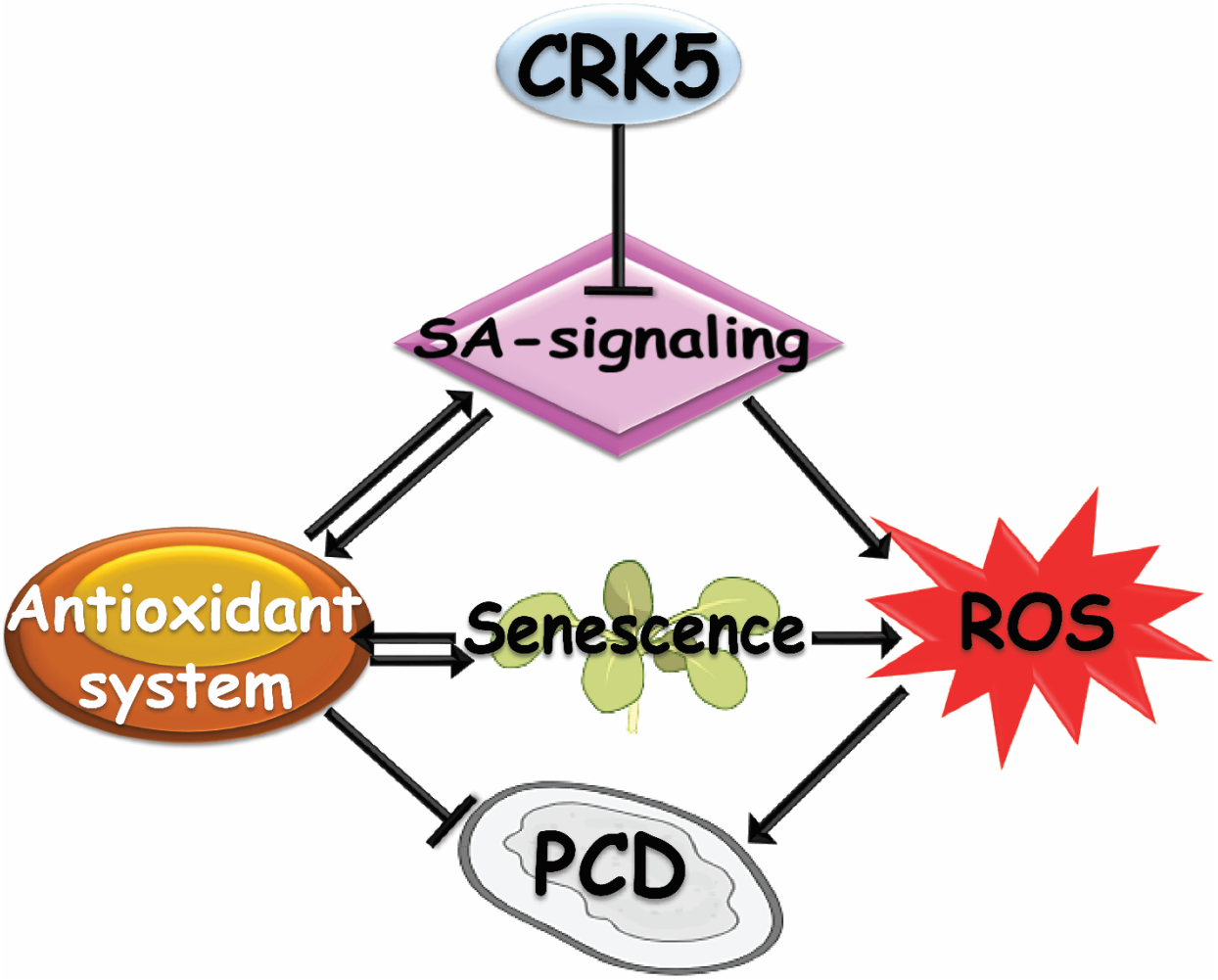


Figure 6. Schematic model illustrating the negative regulatory role of CRK5 in the salicylic acid signaling. The CRK5-driven inhibition of SA-signaling coordinates hormonal, metabolic, and oxidative pathways to maintain leaf viability, providing mechanistic insight into the control of stress-induced senescence and cell death in Arabidopsis.

Previous studies have demonstrated that DIS is accompanied by chlorophyll degradation and major alterations in pigment composition, largely due to impaired photosynthesis and increased photo-oxidative stress. Carotenoids play an essential photo-protective and antioxidant role, and their decline during DIS correlates with enhanced photo-oxidative damage and senescence progression [71, 72]. Consistent with this, we observed a significant reduction in α- and β-carotenes in the SA-accumulating mutants *crk5* and *cpr1* under control conditions, but this reduction was accelerated in dark conditions (Fig. S2A, B). Independent xanthophyll measurements confirmed a similar pattern, with a more pronounced decline during darkness (Fig.

2B and Fig. S2). In contrast, SA-synthesis deficient (*sid2*) and transgenic SA-catabolic (NahG) lines maintained pigment levels comparable to wild-type, and genetic incorporation of these lines into the *crk5* background restored carotenoid content, indicating that CRK5 negatively regulates SA-accumulation and carotenoid depletion during DIS.

SA has been reported to exert concentration- and duration-dependent effects on carotenoid catabolism; while moderate SA levels may stimulate biosynthesis, prolonged or excessive SA-accumulation under stress promotes carotenoid degradation [73, 74]. Accelerated senescence and CD observed in *crk5* and *cpr1* in both conditions likely reflect this imbalance, as carotenoids normally protect against ROS by quenching singlet oxygen, thus reducing free radical formation [19]. Under high SA and oxidative stress, enhanced carotenoid degradation reduces antioxidant capacity, facilitating ROS accumulation and CD progression [75]. SA-mediated regulation of carotenoid cleavage dioxygenases may further link carotenoid turnover to CD signaling through the production of bioactive apo-carotenoids [76].

Consistent with increased oxidative stress, *crk5* and *cpr1* displayed significantly higher DPPH and ABTS antioxidant activities under both control and DIS conditions (Fig. 3A, B). This elevated non-enzymatic antioxidant capacity is characteristic of SA-accumulating and lesion-mimic mutants, where excessive SA induces phenolic biosynthesis as a compensatory response to redox imbalance and CD activation in some cells [28, 38, 68, 70]. Comparable SA-induced enhancements in total antioxidant capacity have been reported under both biotic and abiotic stress conditions, underscoring the dual function of SA as a key signaling molecule and a regulator of cellular redox homeostasis [38, 77]. In agreement with previous reports, *crk5* and *cpr1* accumulated higher levels of total phenolic content and specific subclasses, including phenylpropanoids, flavonols, and anthocyanins, particularly under darkness (Fig. 3C–F). Conversely, SA-synthesis or -catabolic lines showed reduced ROS, CD, and antioxidant capacity, while both parameters were restored to wild-type levels in *crk5sid2* and *crk5*NahG, reinforcing the central role of appropriate SA-signaling in regulating non-enzymatic and enzymatic antioxidant responses during DIS. It is consistent with reports showing that suppression of SA-biosynthesis or -signaling leads to decreased antioxidant potential and reduced CD [40, 78]. Furthermore, the restoration of wild-type antioxidant capacity in *crk5sid2* and *crk5*NahG double mutants supports

the idea that the enhanced antioxidant activity in *crk5* plants is due to deregulation of cellular ROS levels as a secondary effect of SA-dependent signaling and CD activation.

SA is also known to modulate enzymatic antioxidant defenses. Exogenous SA application is related to a significant upregulation of major enzymatic antioxidants, including SOD, APX, and CAT [79–81]. Consistent with this, we observed a significantly higher H_2O_2 level in SA-accumulating mutants and reduced ROS formation in SA-deficient mutants (Fig. 4A and B). The progression of DIS is naturally linked to the loss of cellular redox control, whereas the rapid accumulation of ROS serves as a critical signaling mediator for CD. At lower, protective physiological concentrations, exogenous SA application has been shown to counteract senescence promoting signals, notably by strengthening the overall antioxidant defense system. This protective effect involves enhancing the activity of key scavenging enzymes such as APX, CAT, and SOD, thereby reducing deleterious ROS accumulation [6, 14, 27]. In our study, we also found an elevated activity of antioxidant enzymes APX, CAT, and SOD in SA-accumulating lines (Fig. 4C–E). This upregulation likely represents a compensatory response to excessive ROS accumulation rather than a protective suppression of senescence.

Our transcriptomic analyses further position CRK5 as a pivotal upstream negative regulator of SA-dependent transcriptional reprogramming during DIS. consistent with previous reports showing that SA contributes to developmental and stress-induced leaf aging [24, 82, 83]. Under ambient conditions, SA-synthesis deficient (*sid2*) and SA-accumulating (*cpr1*) mutants exhibited the most pronounced transcriptional divergence, highlighting the importance of SA-signaling in CD and antioxidant regulation. Notably, *crk5* and *cpr1* showed accelerated induction of senescence markers (*SAG12*, *SAG13*) and CD-related genes (*MC2*, *MC8*), consistent with their elevated SA levels. Upon dark treatment, the *crk5* plants displayed the most extensive deregulated transcriptomic remodeling, with over 7,100 DEGs, including strong induction of senescence-, autophagy-, and CD-associated genes (*SAGs*, *ANACs*, *MCs*, *VPEs*, *ATGs*, *LSD1*) (Fig. 5C), which is consistent with previous studies linking CD progression to nutrient remobilization, active catabolic remodeling and senescence [84–86]. At the same time, genes involved in redox regulation and antioxidant catabolism (*PALs*, *PRXs*, *AOXs*, *CAT3*, *APX6*, *GPX2*) were strongly induced, indicating tight coupling between the onset of CD and antioxidant homeostasis.

The GO enrichment analysis corroborated these findings, revealing significant overrepresentation of senescence, ROS response, detoxification, metabolic catabolism, and light-response pathways among *crk5*-induced genes (Figs. 5B, S3), consistent with global transcriptional reprogramming during leaf aging [87]. The comparatively modest transcriptional response of *cpr1* under darkness, despite clear senescence symptoms, suggests that CRK5 functions upstream of CPR1 in orchestrating SA-driven inhibition of CD and redox regulation. Importantly, the near-complete reversion of *crk5* phenotypes and transcriptomic changes in SA-synthesis or -catabolism deficient double mutants confirms that CRK5-mediated inhibitory effects on CD and antioxidant homeostasis are largely SA-signaling dependent (Fig. 6).

Conclusions

In summary, our findings identify CRK5 as a central inhibitor of SA-signaling-dependent CD and antioxidant redox homeostasis during DIS. Loss of CRK5 leads to enhanced SA-accumulation, accelerated CD, and destabilization of carotenoid pools, phenolic metabolism, and enzymatic antioxidant balance, thereby exacerbating oxidative stress and senescence progression. Transcriptomic analyses further position CRK5 upstream of SA-driven CD signaling, coordinating antioxidant defense and catabolic reprogramming during senescence. Collectively, our findings establish CRK5 as a key molecular hub where hormonal signaling, redox regulation, and stress-induced CD integrate to maintain leaf metabolic stability.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The raw RNA-seq (fastq) data are deposited in the NCBI database (BioProject: PRJNA1389866).

Competing interests

The authors declare no competing interests.

Funding

This research was funded by the Polish National Science Centre (Opus 20 UMO 2020/39/B/NZ3/02103), granted to S.K.

Authors' Contributions

Conceptualization and proposed working hypothesis SK and MK; experimental design of the research SK, PB, and MK; resources, performed the research, and RNA-seq data analysis MK, AR, and RZG; Supervision SK and PB; writing the original draft MK and PB; revision of the manuscript SK.

Acknowledgements

Not applicable.

References

1. Guo Y, Ren G, Zhang K, Li Z, Miao Y, Guo H. Leaf senescence: progression, regulation, and application. Molecular Horticulture. 2021;1:5.

2. Yu J, Zhang Y, Di C, Zhang Q, Zhang K, Wang C, et al. JAZ7 negatively regulates dark-induced leaf senescence in Arabidopsis. Journal of experimental botany. 2016;67:751–62.

3. Chaomurilege, Miyagi A, Ishikawa T, Yamaguchi M, Murayama H, Kawai-Yamada M. Metabolic changes associated with dark-induced leaf senescence in Arabidopsis nadk2 mutants. Plant Signaling & Behavior. 2023;18:2215618.

4. Buchanan-Wollaston V, Page T, Harrison E, Breeze E, Lim PO, Nam HG, et al. Comparative transcriptome analysis reveals significant differences in gene expression and signalling pathways between developmental and dark/starvation-induced senescence in Arabidopsis. The Plant Journal. 2005;42:567–85.

5. Rogers H, Munné-Bosch S. Production and scavenging of reactive oxygen species and redox signaling during leaf and flower senescence: similar but different. Plant Physiology. 2016;171:1560–8.

6. Ji Y, Liu J, Xing D. Low concentrations of salicylic acid delay methyl jasmonate-induced leaf senescence by up-regulating nitric oxide synthase activity. Journal of Experimental Botany. 2016;67:5233–45.

7. Mohammadi MA, Cheng Y, Aslam M, Jakada BH, Wai MH, Ye K, et al. ROS and oxidative response systems in plants under biotic and abiotic stresses: revisiting the crucial role of phosphite triggered plants defense response. Frontiers in Microbiology. 2021;12:631318.

8. Van Breusegem F, Dat JF. Reactive oxygen species in plant cell death. Plant physiology. 2006;141:384–90.

9. Fedoreyeva LI. ROS as signaling molecules to initiate the process of plant acclimatization to abiotic stress. International Journal of Molecular Sciences. 2024;25:11820.

10. Mazel A, Levine A. Induction of cell death in Arabidopsis by superoxide in combination with salicylic acid or with protein synthesis inhibitors. Free Radical Biology and Medicine. 2001;30:98–106.

11. DE PINTO MC, Locato V, De Gara L. Redox regulation in plant programmed cell death. Plant, cell & environment. 2012;35:234–44.

12. Desikan R, A.-H.-Mackerness S, Hancock JT, Neill SJ. Regulation of the Arabidopsis transcriptome by oxidative stress. Plant physiology. 2001;127:159–72.

13. Górecka M, Lewandowska M, Dąbrowska-Bronk J, Białasek M, Barczak-Brzyżek A, Kulasek M, et al. Photosystem II 22kDa protein level-a prerequisite for excess light-inducible memory, cross-tolerance to UV-C and regulation of electrical signalling. Plant, Cell & Environment. 2020;43:649–61.

14. Petrov V, Hille J, Mueller-Roeber B, Gechev TS. ROS-mediated abiotic stress-induced programmed cell death in plants. Frontiers in plant science. 2015;6:69.

15. Szechyńska-Hebda M, Lewandowska M, Witoń D, Fichman Y, Mittler R, Karpiński SM. Aboveground plant-to-plant electrical signaling mediates network acquired acclimation. The Plant Cell. 2022;34:3047–65.

16. Czarnocka W, Karpiński S. Friend or foe? Reactive oxygen species production, scavenging and signaling in plant response to environmental stresses. Free Radical Biology and Medicine. 2018;122:4–20.

17. Rao M, Duan M, Zhou C, Jiao J, Cheng P, Yang L, et al. Antioxidant defense system in plants: Reactive oxygen species production, signaling, and scavenging during abiotic stress-induced oxidative damage. Horticulturae 11 (5): 477. 2025.

18. Mordi RC, Ademosun OT, Ajanaku CO, Olanrewaju IO, Walton JC. Free radical mediated oxidative degradation of carotenes and xanthophylls. Molecules. 2020;25:1038.

19. Ramel F, Birtic S, Ginies C, Soubigou-Taconnat L, Triantaphylidès C, Havaux M. Carotenoid oxidation products are stress signals that mediate gene responses to singlet oxygen in plants. Proceedings of the National Academy of Sciences. 2012;109:5535–40.

20. Hiltscher H, Rudnik R, Shaikhali J, Heiber I, Mellenthin M, Meirelles Duarte I, et al. The radical induced cell death protein 1 (RCD1) supports transcriptional activation of genes for chloroplast antioxidant enzymes. Frontiers in Plant Science. 2014;5:475.

21. Overmyer K, Brosché M, Pellinen R, Kuittinen T, Tuominen H, Ahlfors R, et al. Ozone-induced programmed cell death in the Arabidopsis radical-induced cell death1 mutant. Plant Physiology. 2005;137:1092–104.

22. Kamran M, Burdiak P, Karpiński S. Crosstalk Between Abiotic and Biotic Stresses Responses and the Role of Chloroplast Retrograde Signaling in the Cross-Tolerance Phenomena in Plants. Cells. 2025;14:176.

23. Rosenwasser S, Rot I, Sollner E, Meyer AJ, Smith Y, Leviatan N, et al. Organelles contribute differentially to reactive oxygen species-related events during extended darkness. Plant physiology. 2011;156:185–201.

633 24. Morris K, -Mackerness SA, Page T, John CF, Murphy AM, Carr JP, et al. Salicylic acid has a
634 role in regulating gene expression during leaf senescence. The Plant Journal. 2000;23:677–85.

635 25. Hsu C-Y, Chou M-L, Wei W-C, Chung Y-C, Loo X-Y, Lin L-F. Chloroplast protein Tic55
636 involved in dark-induced senescence through AtbHLH/AtWRKY-ANAC003 controlling pathway
637 of Arabidopsis thaliana. Genes. 2022;13:308.

638 26. Liu J, Xu Y, Zhang Z, Wei J. Hydrogen peroxide promotes programmed cell death and salicylic
639 acid accumulation during the induced production of sesquiterpenes in cultured cell suspensions of
640 Aquilaria sinensis. Functional Plant Biology. 2014;42:337–46.

641 27. Rivas-San Vicente M, Plasencia J. Salicylic acid beyond defence: its role in plant growth and
642 development. Journal of experimental botany. 2011;62:3321–38.

643 28. Mateo A, Funck D, Mühlenbock P, Kular B, Mullineaux PM, Karpinski S. Controlled levels
644 of salicylic acid are required for optimal photosynthesis and redox homeostasis. Journal of
645 Experimental Botany. 2006;57:1795–807.

646 29. Mishra N, Jiang C, Chen L, Paul A, Chatterjee A, Shen G. Achieving abiotic stress tolerance
647 in plants through antioxidative defense mechanisms. Frontiers in Plant Science. 2023;14:1110622.

648 30. Bernacki MJ, Czarnocka W, Szechyńska-Hebda M, Mittler R, Karpiński S. Biotechnological
649 Potential of LSD1, EDS1, and PAD4 in the Improvement of Crops and Industrial Plants. Plants.
650 2019;8:290.

651 31. Morse AM, Tschaplinski TJ, Dervinis C, Pijut PM, Schmelz EA, Day W, et al. Salicylate and
652 catechol levels are maintained in nahG transgenic poplar. Phytochemistry. 2007;68:2043–52.

653 32. Miura K, Okamoto H, Okuma E, Shiba H, Kamada H, Hasegawa PM, et al. SIZ1 deficiency
654 causes reduced stomatal aperture and enhanced drought tolerance via controlling salicylic acid-
655 induced accumulation of reactive oxygen species in A rabidopsis. The Plant Journal. 2013;73:91–
656 104.

657 33. Gawroński P, Witoń D, Vashutina K, Bederska M, Betliński B, Rusaczonek A, et al. Mitogen-
658 activated protein kinase 4 is a salicylic acid-independent regulator of growth but not of
659 photosynthesis in Arabidopsis. Molecular Plant. 2014;7:1151–66.

660 34. Devadas SK, Raina R. Preexisting systemic acquired resistance suppresses hypersensitive
661 response-associated cell death in Arabidopsis hrl1 mutant. Plant physiology. 2002;128:1234–44.

662 35. Nawrath C, Heck S, Parinthawong N, Métraux J-P. EDS5, an essential component of salicylic
663 acid–dependent signaling for disease resistance in Arabidopsis, is a member of the MATE
664 transporter family. The Plant Cell. 2002;14:275–86.

665 36. Wituszyńska W, Ślesak I, Vanderauwera S, Szechyńska-Hebda M, Kornaś A, Van Der Kelen
666 K, et al. Lesion simulating disease1, enhanced disease susceptibility1, and phytoalexin deficient4
667 conditionally regulate cellular signaling homeostasis, photosynthesis, water use efficiency, and
668 seed yield in Arabidopsis. Plant Physiology. 2013;161:1795–805.

669 37. Wituszyńska W, SZECHYŃSKA-HEBDA M, Sobczak M, Rusaczonek A, KOZŁOWSKA-
670 MAKULSKA A, Witoń D, et al. LESION SIMULATING DISEASE 1 and ENHANCED
671 DISEASE SUSCEPTIBILITY 1 differentially regulate UV-C-induced photooxidative stress
672 signalling and programmed cell death in A rabidopsis thaliana. Plant, cell & environment.
673 2015;38:315–30.

674 38. Muhlenbock P, Szechynska-Hebda M, Płaszczyca M, Baudo M, Mateo A, Mullineaux PM, et
675 al. Chloroplast signaling and LESION SIMULATING DISEASE1 regulate crosstalk between light
676 acclimation and immunity in Arabidopsis. The Plant Cell. 2008;20:2339–56.

677 39. Mateo A, Mühlenbock P, Rustérucci C, Chang CC-C, Miszalski Z, Karpinska B, et al. LESION
678 SIMULATING DISEASE 1 is required for acclimation to conditions that promote excess
679 excitation energy. Plant Physiology. 2004;136:2818–30.

680 40. Radojičić A, Li X, Zhang Y. Salicylic acid: a double-edged sword for programed cell death in
681 plants. Frontiers in plant science. 2018;9:1133.

682 41. Bourdais G, Burdiak P, Gauthier A, Nitsch L, Salojärvi J, Rayapuram C, et al. Large-scale
683 phenomics identifies primary and fine-tuning roles for CRKs in responses related to oxidative
684 stress. PLoS Genetics. 2015;11:e1005373.

685 42. Wrzaczek M, Brosché M, Salojärvi J, Kangasjärvi S, Idänheimo N, Mersmann S, et al.
686 Transcriptional regulation of the CRK/DUF26 group of receptor-like protein kinases by ozone and
687 plant hormones in Arabidopsis. BMC plant biology. 2010;10:1–19.

688 43. Burdiak P, Rusaczonek A, Witoń D, Głów D, Karpiński S. Cysteine-rich receptor-like kinase
689 CRK5 as a regulator of growth, development, and ultraviolet radiation responses in Arabidopsis
690 thaliana. Journal of Experimental Botany. 2015;66:3325–37.

691 44. Kimura S, Waszczak C, Hunter K, Wrzaczek M. Bound by fate: the role of reactive oxygen
692 species in receptor-like kinase signaling. The Plant Cell. 2017;29:638–54.

693 45. Martin-Ramirez S, Stouthamer J, Smakowska-Luzan E. More questions than answers: insights
694 into potential cysteine-rich receptor-like kinases redox signalling in Arabidopsis. The Plant Journal.
695 2025;122:e70176.

696 46. Burdiak P, Mielecki J, Gawroński P, Karpiński S. The CRK5 and WRKY53 are conditional
697 regulators of senescence and stomatal conductance in Arabidopsis. Cells. 2022;11:3558.

698 47. Zhao J, Sun Y, Li X, Li Y. CYSTEINE-RICH RECEPTOR-LIKE KINASE5 (CRK5) and
699 CRK22 regulate the response to Verticillium dahliae toxins. Plant Physiology. 2022;190:714–31.

700 48. Clarke JD, Volko SM, Ledford H, Ausubel FM, Dong X. Roles of salicylic acid, jasmonic acid,
701 and ethylene in cpr-induced resistance in Arabidopsis. The Plant Cell. 2000;12:2175–90.

702 49. Furlan G, Klinkenberg J, Trujillo M. Regulation of plant immune receptors by ubiquitination.
703 Frontiers in plant science. 2012;3:238.

704 50. Rusaczonek A, Czarnocka W, Kacprzak S, Witoń D, Ślesak I, Szechyńska-Hebda M, et al.
705 Role of phytochromes A and B in the regulation of cell death and acclimatory responses to UV
706 stress in Arabidopsis thaliana. Journal of Experimental Botany. 2015;66:6679–95.

707 51. Rusaczonek A, Czarnocka W, Willems P, Sujkowska-Rybkowska M, Van Breusegem F,
708 Karpiński S. Phototropin 1 and 2 influence photosynthesis, UV-C induced photooxidative stress
709 responses, and cell death. Cells. 2021;10:200.

710 52. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity
711 applying an improved ABTS radical cation decolorization assay. Free radical biology and
712 medicine. 1999;26:1231–7.

713 53. Benzie IF, Strain J. [2] Ferric reducing/antioxidant power assay: direct measure of total
714 antioxidant activity of biological fluids and modified version for simultaneous measurement of
715 total antioxidant power and ascorbic acid concentration. In: Methods in enzymology. Elsevier;
716 1999. p. 15–27.

717 54. Swain T, Hillis W. The phenolic constituents of Prunus domestica. I.—The quantitative
718 analysis of phenolic constituents. Journal of the Science of Food and Agriculture. 1959;10:63–8.

719 55. Fukumoto L, Mazza G. Assessing antioxidant and prooxidant activities of phenolic compounds.
720 Journal of agricultural and food chemistry. 2000;48:3597–604.

721 56. Velikova V, Yordanov I, Edreva A. Oxidative stress and some antioxidant systems in acid rain-
722 treated bean plants: protective role of exogenous polyamines. Plant science. 2000;151:59–66.

723 57. Orozco-Cardenas M, Ryan CA. Hydrogen peroxide is generated systemically in plant leaves
724 by wounding and systemin via the octadecanoid pathway. Proceedings of the National Academy
725 of Sciences. 1999;96:6553–7.

726 58. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of
727 protein utilizing the principle of protein-dye binding. Analytical biochemistry. 1976;72:248–54.

728 59. Nakano Y, Asada K. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in
729 spinach chloroplasts. Plant and cell physiology. 1981;22:867–80.

730 60. Aebi H. [13] Catalase in vitro. In: Methods in enzymology. Elsevier; 1984. p. 121–6.

731 61. Beauchamp C, Fridovich I. Superoxide dismutase: improved assays and an assay applicable to
732 acrylamide gels. Analytical biochemistry. 1971;44:276–87.

733 62. Veeranagouda Y, Remaury A, Guillemot J, Didier M. RNA Fragmentation and Sequencing
734 (RF-Seq): Cost-Effective, Time-Efficient, and High-Throughput 3′ mRNA Sequencing Library
735 Construction in a Single Tube. Current Protocols in Molecular Biology. 2019;129:e109.

736 63. Smith T, Heger A, Sudbery I. UMI-tools: modeling sequencing errors in Unique Molecular
737 Identifiers to improve quantification accuracy. Genome research. 2017;27:491–9.

738 64. Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, et al. STAR: ultrafast
739 universal RNA-seq aligner. Bioinformatics. 2013;29:15–21.

740 65. Putri GH, Anders S, Pyl PT, Pimanda JE, Zanini F. Analysing high-throughput sequencing
741 data in Python with HTSeq 2.0. Bioinformatics. 2022;38:2943–5.

742 66. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-
743 seq data with DESeq2. Genome biology. 2014;15:1–21.

744 67. Wu T, Hu E, Xu S, Chen M, Guo P, Dai Z, et al. clusterProfiler 4.0: A universal enrichment
745 tool for interpreting omics data. The innovation. 2021;2.

746 68. Bernacki MJ, Rusaczonek A, Czarnocka W, Karpiński S. Salicylic acid accumulation
747 controlled by LSD1 is essential in triggering cell death in response to abiotic stress. Cells.
748 2021;10:962.

749 69. Czarnocka W, Fichman Y, Bernacki M, Różańska E, Sańko-Sawczenko I, Mittler R, et al.
750 FMO1 is involved in excess light stress-induced signal transduction and cell death signaling. Cells.
751 2020;9:2163.

752 70. Brodersen P, Malinovsky FG, Hématy K, Newman M-A, Mundy J. The role of salicylic acid
753 in the induction of cell death in Arabidopsis acd11. Plant physiology. 2005;138:1037–45.

754 71. Sheikh AH, Tabassum N, Rawat A, Almeida Trapp M, Nawaz K, Hirt H. m6A RNA
755 methylation counteracts dark-induced leaf senescence in Arabidopsis. Plant physiology.
756 2024;194:2663–78.

757 72. ROSANNA C, KERSTIN A, BERND K. Effects of altered a-and b-branch carotenoid
758 biosynthesis on photoprotection and whole-plant acclimation of Arabidopsis to photo-oxidative
759 stress. Plant, Cell and Environment. 2013;36:438–53.

760 73. Xiang N, Zhang B, Hu J, Li K, Guo X. Modulation of carotenoid biosynthesis in maize (Zea
761 mays L.) seedlings by exogenous abscisic acid and salicylic acid under low temperature. Plant Cell
762 Reports. 2024;43:1.

763 74. Wang F, Xu H, He W, Lu Y, Luo H, Guo Q, et al. Exogenous salicylic acid promotes carotenoid
764 accumulation and antioxidant capacity in germinated maize kernels by regulating carotenoid
765 biosynthetic pathway. Food Bioscience. 2024;59:103990.

766 75. Kırgeç Y, Batı-Ay E, Açıkgöz MA. The effects of foliar salicylic acid and zinc treatments on
767 proline, carotenoid, and chlorophyll content and anti-oxidant enzyme activity in Galanthus elwesii
768 Hook. Horticulturae. 2023;9:1041.

769 76. Daruwalla A, Kiser PD. Structural and mechanistic aspects of carotenoid cleavage
770 dioxygenases (CCDs). Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of
771 Lipids. 2020;1865:158590.

772 77. Horváth E, Szalai G, Janda T. Induction of abiotic stress tolerance by salicylic acid signaling.
773 Journal of Plant Growth Regulation. 2007;26:290–300.

774 78. Yoshimoto K, Jikumaru Y, Kamiya Y, Kusano M, Consonni C, Panstruga R, et al. Autophagy
775 negatively regulates cell death by controlling NPR1-dependent salicylic acid signaling during
776 senescence and the innate immune response in Arabidopsis. The Plant Cell. 2009;21:2914–27.

777 79. Wang W, Wang X, Huang M, Cai J, Zhou Q, Dai T, et al. Hydrogen peroxide and abscisic acid
778 mediate salicylic acid-induced freezing tolerance in wheat. Frontiers in plant science. 2018;9:1137.

80. Wang Z, Dong S, Teng K, Chang Z, Zhang X. Exogenous salicylic acid optimizes photosynthesis, antioxidant metabolism, and gene expression in perennial ryegrass subjected to salt stress. Agronomy. 2022;12:1920.

81. Zhang X, Liu Y, Zhang W, Yang W, An S, Guo M, et al. Salicylic acid treatment ameliorates postharvest quality deterioration in 'France'prune (Prunus domestica L.'Ximei') fruit by modulating the antioxidant system. Foods. 2024;13:2871.

82. Jibran R, A Hunter D, P Dijkwel P. Hormonal regulation of leaf senescence through integration of developmental and stress signals. Plant Molecular Biology. 2013;82:547–61.

83. Kanojia A, Gupta S, Benina M, Fernie AR, Mueller-Roeber B, Gechev T, et al. Developmentally controlled changes during Arabidopsis leaf development indicate causes for loss of stress tolerance with age. Journal of experimental botany. 2020;71:6340–54.

84. Guiboileau A, Sormani R, Meyer C, Masclaux-Daubresse C. Senescence and death of plant organs: nutrient recycling and developmental regulation. Comptes rendus biologies. 2010;333:382–91.

85. Sobieszczuk-Nowicka E, Wrzesiński T, Bagniewska-Zadworna A, Kubala S, Rucińska-Sobkowiak R, Polcyn W, et al. Physio-genetic dissection of dark-induced leaf senescence and timing its reversal in barley. Plant Physiology. 2018;178:654–71.

86. Zhang B, Huang S, Guo Z, Meng Y, Li X, Tian Y, et al. Salicylic acid accelerates carbon starvation-induced leaf senescence in Arabidopsis thaliana by inhibiting autophagy through Nonexpressor of pathogenesis-related genes 1. Plant Science. 2023;336:111859.

87. Breeze E, Harrison E, McHattie S, Hughes L, Hickman R, Hill C, et al. High-resolution temporal profiling of transcripts during Arabidopsis leaf senescence reveals a distinct chronology of processes and regulation. The Plant Cell. 2011;23:873–94.

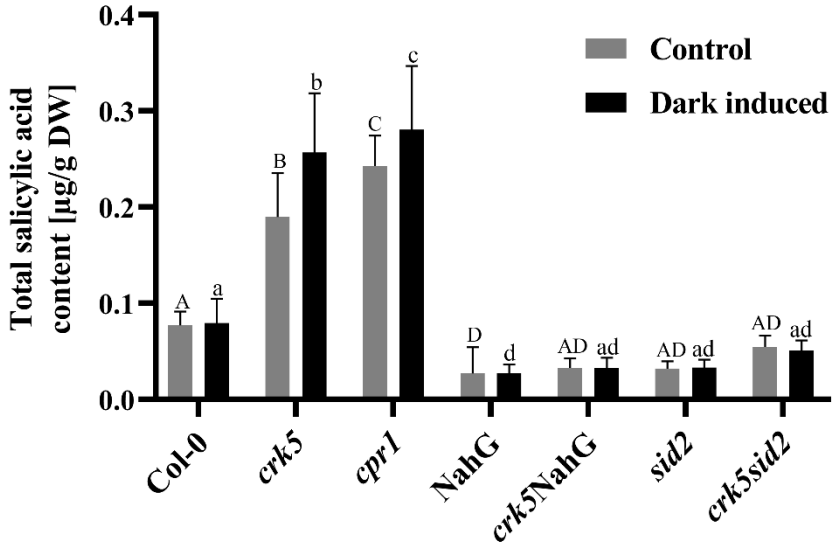


Figure S1. Total salicylic acid (SA) content of the wild-type, *crk5*, c*pr1*, and salicylic acid-synthesis dysfunction *sid2* mutant or salicylic acid-catabolism transgenic NahG line in control and after exposure for 4 days to permanent darkness. Mean values (±SD) were derived from 9 plants (n = 9). Statistical analysis was performed according to a t-test at a level of $p < 0.05$. Letters A, B, C, D, and a, b, c, d above the bars indicate homogenous groups, and values sharing common labels (letters) are not significantly different from each other (Modified from Kamran et al, 2025, accepted in Plant Physiology).

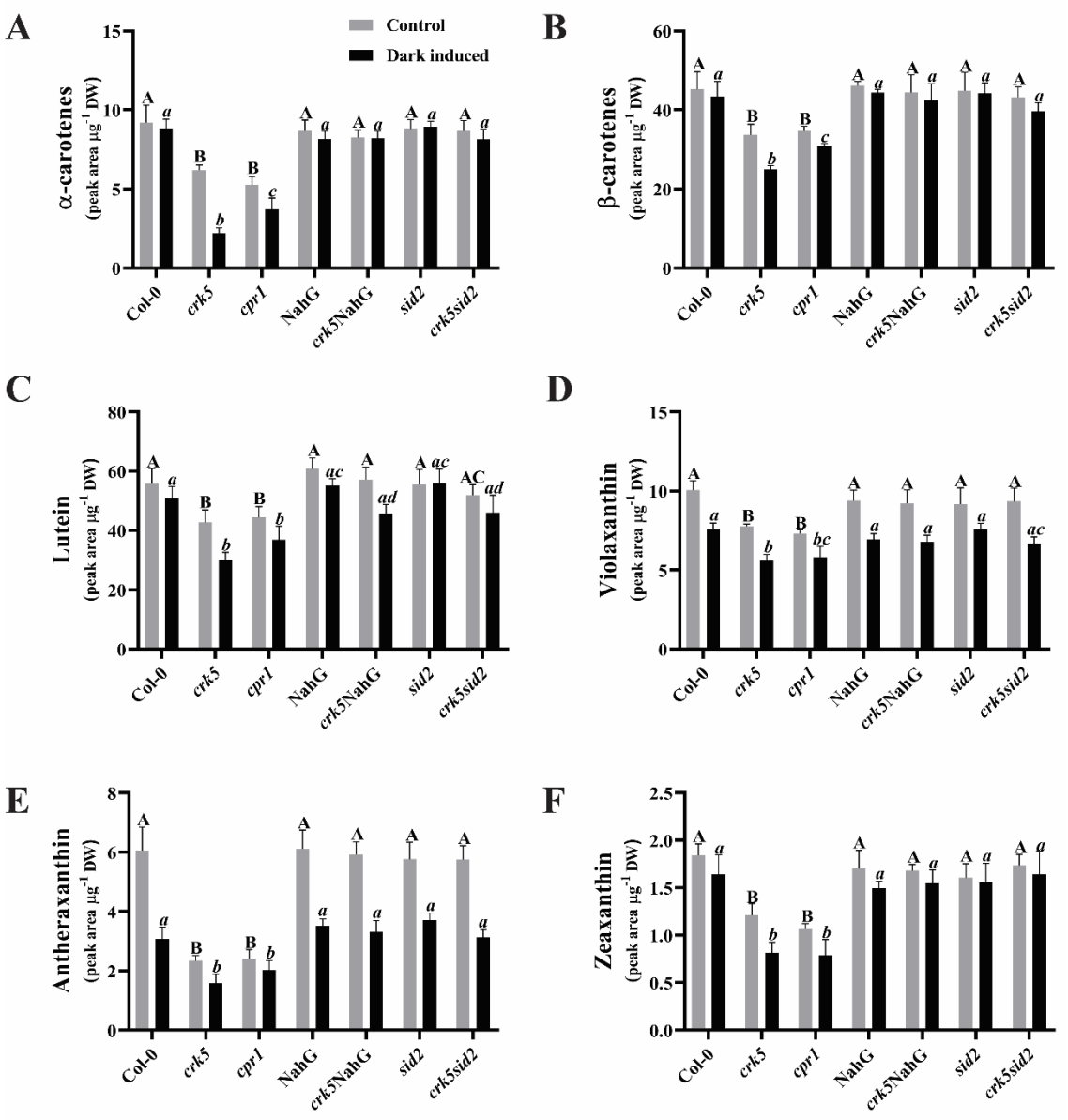


Figure 2. Foliar carotenes and xanthophylls content of wild-type, *crk5*, c*pr1*, and salicylic acid-synthesis dysfunction *sid2* mutant or salicylic acid-catabolism transgenic NahG line in control and after exposure for 4 days to permanent darkness. α-carotenes (**A**), ß-carotenes (**B**), lutein (**C**), violaxanthin (**D**), antheraxanthin (**E**), and zeaxanthin (**F**). Mean values (±SD) were derived from 9 plants (n = 9). Statistical analysis was performed using a t-test at a significance level of $p < 0.05$. Letters A, B, C, and a, b, c, d, above the bars indicate homogenous groups, and values sharing common labels (letters) are not significantly different from each other.

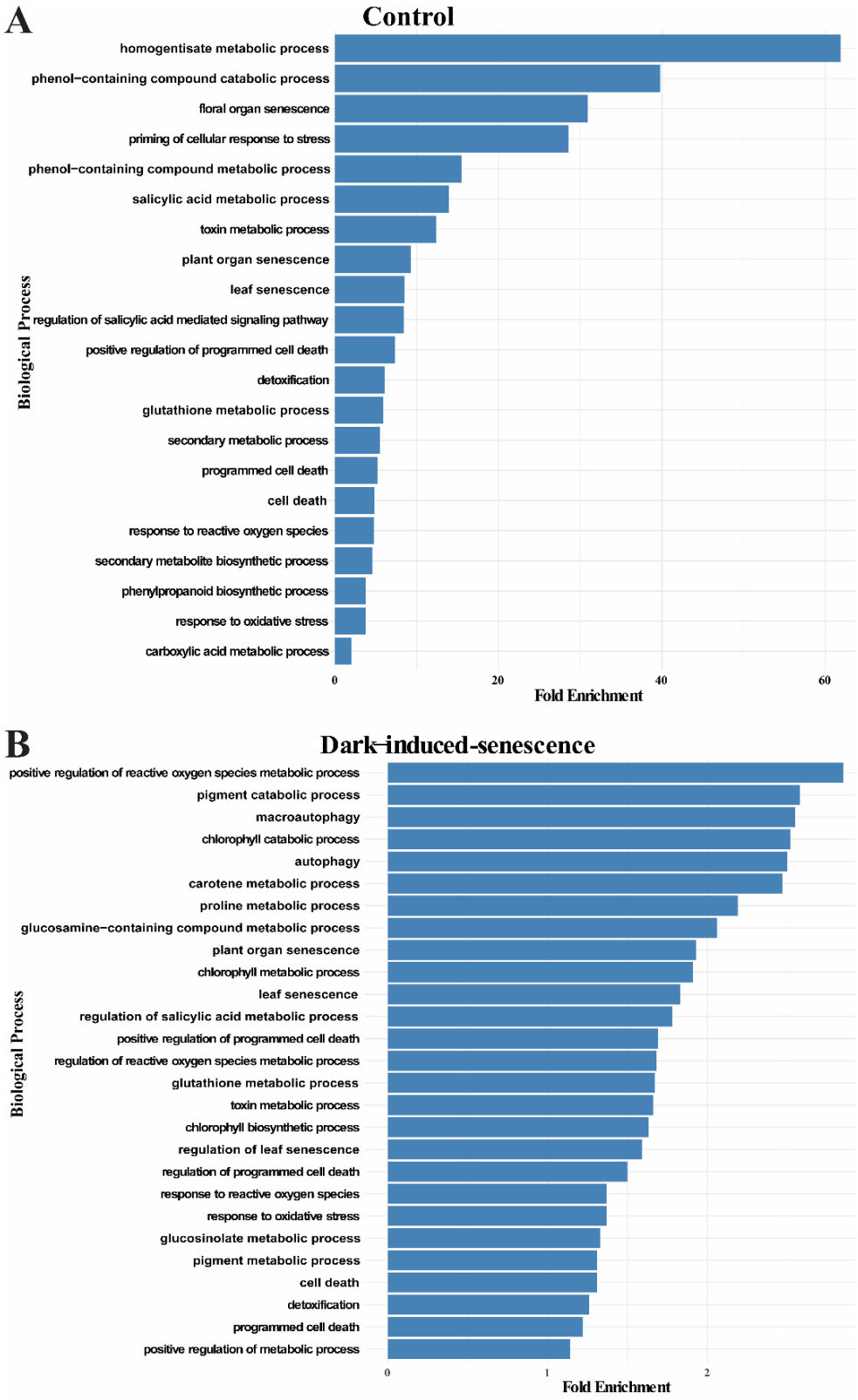


Figure S3. Gene ontology (GO) analysis of genes significantly enriched in *crk5* compared to Col-0 in the RNA-seq experiment in control (**A**) and dark-induced (**B**) conditions.

Table S1. Primers used in this study.

Gene	AGI Code	Sequence LP Primer	Sequence RP Primer
CRK5	AT4G23130	AGGAGATCTCTCGCCAGAATC	CGATAGTCTCTTCACGGCAAC
NahG	Transgene line (M60055)	ACTCTGCCGCTACTCCCATA	CGAGCCCTAGGTACATCTGC
SID2	AT1G74710	AATTGGCAGGGAGACTTACGAA	TCCTCTATCGAATGATTCTATCTCCTT

Table S2. *Accession Numbers* for Fig. 5C and D.

Senescence Marker Genes	AGI Code	SA Signaling Pathway Genes	AGI Code
SAG12	AT5G45890	*AAO3*	AT2G27150
SAG13	AT2G29350	*AOX1a*	AT3G22370
SAG29	AT5G13170	*AOX1d*	AT1G32350
ORE1 (ANAC092)	AT5G39610	*APX6*	AT4G32320
NAP (ANAC029)	AT1G69490	*ARR2*	AT4G16110
WRKY75	AT5G13080	*C4H (CYP73A5)*	AT2G30490
NYE1/SGR1	AT4G22920	*CAT3*	AT1G20630
NYC1	AT4G13250	*CKX2*	AT2G19500
ANAC016	AT1G34180	*DAO1*	AT1G14100
ANAC046	AT3G04060	*GDH2*	AT5G07440
MC1	AT1G02170	*GLN1.4*	AT5G16570
MC2	AT4G25110	*GPX2*	AT2G31570
MC5	AT5G64240	*GSTU26*	AT1G17180
MC8	AT1G16420	*GSTU4*	AT2G29470
VPEγ	AT4G32940	*GSTU41*	AT2G29460
VPEα	AT2G25940	*GUN1*	AT2G31400
VPEδ	AT3G20210	*MAPKKK18*	AT1G05100

ATG4a	AT2G44140	*MDAR1*	AT3G52880
ATG4b	AT3G59950	*MPK3*	AT3G45640
ATG5	AT5G17290	*MPK6*	AT2G43790
ATG7	AT5G45900	*NPR1*	AT1G64280
ATG8a	AT4G21980	*OXI1*	AT3G25250
ATG8e	AT4G16520	*PAL1*	AT2G37040
ATG18a	AT3G62770	*PAL2*	AT3G53260
BI-1	AT5G47120	*PAL4*	AT3G10340
LSD1	AT4G20380	*PR1*	AT2G14610
HRD1A	AT1G18270	*PRX33*	AT3G49120
SPL14	AT1G20980	*PRX52*	AT5G05340
		PRX53	AT5G05340
		PRX71	AT5G64100
		RBOHC	AT5G51060
		RBOHD	AT5G47910
		RBOHE	AT1G19230
		SWEET15	AT5G13170
		TRXh5	AT1G45145

Warsaw, 12.03.2026

Muhammad Kamran

muhammad_kamran@sggw.edu.pl

Department of Plant Genetics, Breeding and Biotechnology, Institute of Biology

Warsaw University of Life Sciences

Biological Sciences Discipline Counsel

of the Warsaw University

of Life Sciences

Statement

I hereby declare that in the publication:

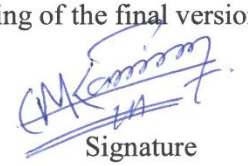
Kamran, M., Burdiak, P., Rusaczonek A., Zarrin Ghalami R., & Karpiński, S. (2026b). CRK5 preserves antioxidant homeostasis and prevents cell death during dark-induced senescence through inhibiting the salicylic acid signaling pathway. Under peer revision in BMC Plant Biology, Springer Nature. **(Publication 3)**

My participation constitutes:

- participation in the conceptualization and experimental design of the study
- generation of double mutant plants through genetic crossing and PCR validations
- plant cultivation
- preparations for the stress treatment conditions (dark-induced senescence)
- collection of material for analysis
- preparation and implementation of experiments involving:
    - phenotypic analysis of the dark-induced plants
    - measurements of the relative ion leakage and trypan blue staining for the cell death analysis in control and dark-induced plants
    - measurements of ROS level through DAB staining in control and dark-induced plants, and participated in preparing samples for ROS scavenger enzymes (APX, CAT, and SOD) and H_2O_2 content
    - RNA-seq library preparation and analysis
- participation in preparing samples and analysis concerning carotenes, xanthophyll, and phenolic content, determination of radical scavenging activities through DPPH and ABTS measurements

- performed the statistical analysis for ion leakage, micro-lesions, pigment content, scavenging activities (DPPH and ABTS), Phenolic content, H_2O_2 content, and antioxidant activities (APX, CAT, and SOD)
- resource collection for the article
- visualization of the data (preparing all the figures and tables)
- drafting of the original manuscript, and the review and editing of the final version.

I estimate my contribution at: 67.5%



Signature

Warsaw, 12.03.2026

Paweł Burdiak

pawel_burdiak@sggw.edu.pl

Department of Plant Genetics, Breeding and Biotechnology, Institute of Biology

Warsaw University of Life Sciences

Biological Sciences Discipline Counsel

of the Warsaw University

of Life Sciences

Statement

regarding co-authorship in the publication with Muhammad Kamran, which is the basis of his doctoral thesis

I hereby declare that I am aware that the publication:

Kamran, M., Burdiak, P., Rusaczonek A., Zarrin Ghalami R., & Karpiński, S. (2026b). CRK5 preserves antioxidant homeostasis and prevents cell death during dark-induced senescence through inhibiting the salicylic acid signaling pathway. Under peer revision in BMC Plant Biology, Springer Nature **(Publication 3),** of which I am a co-author, has been included in Muhammad Kamran's doctoral thesis.

I contributed to:

- participated in the experimental design of the study
- RNA-seq library preparation
- supervised the experimental data
- review and editing of the manuscript.

I estimate my contribution at: 7.5%

Signature

Paweł Burdiak

Warsaw, 12.03.2026

Anna Rusaczonek

anna_rusaczonek@sggw.edu.pl

Department of Botany and Plant Physiology, Institute of Biology

Warsaw University of Life Sciences

Biological Sciences Discipline Counsel

of the Warsaw University

of Life Sciences

Statement

regarding co-authorship in the publication with Muhammad Kamran, which is the basis of his doctoral thesis

I hereby declare that I am aware that the publication:

Kamran, M., Burdiak, P., Rusaczonek A., Zarrin Ghalami R., & Karpiński, S. (2026b). CRK5 preserves antioxidant homeostasis and prevents cell death during dark-induced senescence through inhibiting the salicylic acid signaling pathway. Under peer revision in BMC Plant Biology, Springer Nature **(Publication 3),** of which I am a co-author, has been included in Muhammad Kamran's doctoral thesis.

I contributed to:

- participation in the measurements and analysis of enzymatic (APX, CAR, and SOD) and non-enzymatic (carotenoids and phenolic) antioxidant compounds
- participation in the measurements and analysis of H_2O_2 and radical scavenging activities (DPPH and ABTS)

I estimate my contribution at: 7.5%

Signature

Warsaw, 12.03.2026

Roshanak Zarrin Ghalami

roshanak_zarrin_ghalami@sggw.edu.pl

Department of Plant Genetics, Breeding and Biotechnology, Institute of Biology

Warsaw University of Life Sciences

Biological Sciences Discipline Counsel

of the Warsaw University

of Life Sciences

Statement

regarding co-authorship in the publication with Muhammad Kamran, which is the basis of his doctoral thesis

I hereby declare that I am aware that the publication:

Kamran, M., Burdiak, P., Rusaczonek A., Zarrin Ghalami R., & Karpiński, S. (2026b). CRK5 preserves antioxidant homeostasis and prevents cell death during dark-induced senescence through inhibiting the salicylic acid signaling pathway. Under peer revision in BMC Plant Biology, Springer Nature **(Publication 3),** of which I am a co-author, has been included in Muhammad Kamran's doctoral thesis.

I contributed to:

- participation in the generation of double mutant lines
- participation in the analysis of RNA-seq data

I estimate my contribution at: 5%

Signature

Warsaw, 12.03.2026

Stanisław Karpinski

stanislaw_karpinski@sggw.edu.pl

Department of Plant Genetics, Breeding and Biotechnology, Institute of Biology

Warsaw University of Life Sciences

Biological Sciences Discipline Counsel

of the Warsaw University

of Life Sciences

Statement

regarding co-authorship in the publication with Muhammad Kamran, which is the basis of his doctoral thesis

I hereby declare that I am aware that the publication:

Kamran, M., Burdiak, P., Rusaczonek A., Zarrin Ghalami R., & Karpiński, S. (2026b). CRK5 preserves antioxidant homeostasis and prevents cell death during dark-induced senescence through inhibiting the salicylic acid signaling pathway. Under peer revision in BMC Plant Biology, Springer Nature **(Publication 3),** of which I am a co-author, has been included in Muhammad Kamran's doctoral thesis.

I contributed to:

- securing research funding
- conceptualization and experimental design of the study
- supervised the experimental data
- review and editing of the final version

I estimate my contribution at: 12.5%

S. Karpiński

Signature

List of other achievements

List of other publications

1. Duszyn, M., Burdiak, P., Dąbrowska-Bronk, J., Rusaczonek, A., **Kamran, M.,** Zarrin Ghalami, R., Majnert, A., Bojarczuk, J., Gawroński, P., & Karpiński, S. (2025). From Light Harvesting to Grain Filling: Chlorophyll Fluorescence, Pigment Composition, and Oxidative Status as Discrete Yield Determinants in Rye. *Plants*, *14*(24), 3746.

2. Ghalami, R. Z., Burdiak, P., **Kamran, M.,** Duszyn, M., Rusaczonek, A., Muszyńska, E., & Karpiński, S. (2026). Role of CIA2 and CIL in the Regulation of Chloroplast Development During Photomorphogenesis in Arabidopsis. *Cells*, *15*(4), 333.

3. Szechyńska-Hebda, M., Ghalami, R. Z., **Kamran, M.,** Van Breusegem, F., & Karpiński, S. (2022). To be or not to be? Are reactive oxygen species, antioxidants, and stress signalling universal determinants of life or death? *Cells*, *11*(24), 4105.

List of conferences and summer school

1. **Muhammad Kamran,** Pawel Burdiak, Roshanak Zarrin Ghalami, Maria Duszyn, and Stanislaw Karpinski. The CRK5 kinase oppositely regulates dark-induced senescence and dissipation of absorbed energy due to inhibition of the salicylic acid signaling pathway. The Society for Experimental Biology 2025, Antwerb (Belgium) 8-11/07/2025. (**Poster**)
2. Pawel Burdiak, Maria Duszyn, **Muhammad Kamran,** Roshanak Zarrine Ghalami, and Stanislaw Karpinski. The LSD1 and CRK5 differentially affect foliar temperature in Arabidopsis. Plant Biology Europe Congress in July 2023, Marseille (France) 3-6/07/2023. (**Poster**)
3. Maciej Jerzy Bernacki, Damian Witon, Weronika Czarzocka, Piotr Gawronski, Anna Rusaczonek, Roshanak Zarrine Ghalami, **Muhammad Kamran,** and Stanislaw Karpinski. The Zinc-Finger Thylakoid-Membrane Protein FIP is involved in photosynthesis apparatus adaptation to changing light conditions. The 33rd International Conference on Arabidopsis Research 2023, Chiba (Japan) 5-9/06/2023. (**Poster**)

4. September 11 – 16, 2023, **Summer School at Warsaw University of Life Sciences**. I participated in an international summer school entitled as “from discovery to implementation – utilising genomics for plant breeding”. The program was focused on the journey from basic genetic research to practical application in modern agriculture. The key themes included were: (i) Identifying new genes to drive innovations in plant breeding and biotechnology, and (ii) Utilizing genomics to address phenotypic differences in crops.