Plant Physiology and Biochemistry 229 (2025) 110497

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Methyl jasmonate seed treatment enhances Norway spruce seedling resistance to *Botrytis cinerea* via a multitude of defense responses

Ngan Bao Huynh [a], Axel Schmidt [b], Taina Pennanen [c], Jonathan Gershenzon [b], Melissa H. Mageroy [a,*]

[a] Division of Plant Health and Biotechnology, Norwegian Institute for Bioeconomy Research, Ås, Norway
[b] Department of Biochemistry, Max Planck Institute of Chemical Ecology, Jena, Germany
[c] Natural Resources Institute Finland (Luke), Latokartanonkaari 9, Helsinki, 00790, Finland

ARTICLE INFO

Keywords:
Norway spruce
Plant resistance
Seed priming
Botrytis cinerea
Plant defense

ABSTRACT

Methyl jasmonate (MeJA) is known to effectively protect Norway spruce (*Picea abies*) against pests and pathogens. However, MeJA application to spruce saplings can significantly reduce growth and is not feasible to use in protecting older trees due to cost. Seed treatment with MeJA or other priming stimulants with fewer negatives could be a practical solution to enhance Norway spruce resistance. Therefore, we assessed the potential of Norway spruce seed treatment with MeJA, pipecolic acid (PipA), lignan (Li), and chitosan (Chi) in enhancing the resistance of the emerged seedlings against *Botrytis cinerea*. For the first time, MeJA seed treatment was shown to reduce the mortality of the seedlings effectively after *B. cinerea* infection, with a growth reduction as a side effect. To understand the mechanisms underlying this phenomenon, we quantified phenolics, defense hormones, and differential transcript expressions. MeJA seed treatment increased the concentration of the flavan-3-ols catechin and proanthocyanidin B1. Transcriptomic data suggested an increase in oxidative stress protection, cell wall reinforcement, and pathogenesis-related protein production. Our data also suggested an antagonistic relationship in hormonal signaling between abscisic acid (ABA) and jasmonic acid (JA)/ethylene (ET). Overall, our findings indicated MeJA seed treatment enhanced resistance of young seedlings against *B. cinerea* via a multitude of defense responses, modulated by complex regulatory systems.

1. Introduction

Norway spruce (*Picea abies* (L.) H. Karst.) is an ecologically and economically important species for European forestry (Hannerz and Ekström, 2023). Spruce is an important source of wood for timber construction and pulp for paper production (Hannerz and Ekström, 2023). Additionally, it is a key species in the boreal forest that provides many ecological services, such as essential habitats for many other organisms, carbon sequestration, and erosion protection (Caudullo et al., 2016). To meet the high demand for Norway spruce, seedlings are produced in large quantities in nurseries in Nordic countries (Solvin et al., 2023). Conditions such as high density, cold storage, or high humidity in nurseries can increase the risk of fungal disease outbreaks (Petäistö, 2006; Lilja et al., 2010). One of the major damaging fungal diseases in seedling production is grey mold caused by *Botrytis* spp. (Petäistö et al., 2004; Lilja et al., 2010). The development of resistance to extant fungicides is contributing to a growing problem with *Botrytis* outbreaks in forest nurseries (Nielsen et al., 2024). These challenges spark the need to explore alternatives to using fungicides to protect Norway spruce seedlings in nurseries.

An alternative to traditional plant protection with fungicides is to enhance a plant's defense system. Norway spruce has a complex defense system against pathogens and herbivores, comprised of physical and chemical defenses. Structural components, such as lignin or suberin polymers, act as mechanical barriers by making the tissues tougher and preventing penetration (Krokene, 2015). Additionally, resinous terpenes can trap insects, preventing the spread of pathogens, and sealing wounds (Krokene, 2015). Terpenes are also toxic to pests and pathogens (Krokene, 2015). Moreover, phenolics, especially flavonoids, display great antifungal and feeding-deterrent properties (Hammerbacher et al., 2020).

Norway spruce defense responses can also be temporally categorized (Krokene, 2015). Constitutive or innate defenses provide constant protection as the first barrier, while inducible or acquired defenses are only

* Corresponding author.
E-mail address: melissa.mageroy@nibio.no (M.H. Mageroy).

https://doi.org/10.1016/j.plaphy.2025.110497
Received 3 June 2025; Received in revised form 29 July 2025; Accepted 7 September 2025
Available online 8 September 2025

activated in response to a stimulus and enhance the level of protection (Krokene, 2015). Inducible defenses include the accumulation of terpenes and phenolics or anatomical changes such as traumatic resin duct formation or swelling of polyphenolic cells (Krokene, 2015).

Inducible defenses are further classified by the timing of their response to the stimulus. Directly inducible defenses are initiated immediately after exposure to a stimulus, like an insect attack or pathogen infection (Wilkinson et al., 2019). These defenses may return to their original level after a short period or be maintained to face prolonged danger or to guard against future threats (Wilkinson et al., 2019). The latter, also known as prolonged induction, was thought to be the main mechanism of conifer long-term resistance in trees with sub-lethal fungal infection or after application of the jasmonic acid derivative methyl jasmonate (MeJA) (Christiansen et al., 1999; Krokene et al., 2000; Mageroy et al., 2020a; Erbilgin et al., 2006; Zas et al., 2014; Zeneli et al., 2006).

MeJA-induced resistance (MeJA-IR) in conifers has been studied for over 20 years, and this treatment has shown significant decreases in pest/pathogen damage across all studies (Huynh et al., 2024b). In a recent study, MeJA treatment did not induce defense chemicals but provided the same resistance to bark beetle attacks as sub-lethal fungal inoculation (Mageroy et al., 2020a). This low induction but high resistance indicated that MeJA could prime Norway spruce defenses for greater induction on subsequent attack (Martinez-Medina et al., 2016; Mauch-Mani et al., 2017). If we can effectively harness defense priming in Norway spruce, it could be a promising method for future sustainable plant protection.

MeJA-IR in conifers has mostly been studied in 1-year-old or older plants (Huynh et al., 2024b). Older plants require a higher effective dose of MeJA, which can be costly due to the high price of MeJA (Huynh et al., 2024b). However, seed treatment with a low dose of MeJA has not been well explored. Enhanced resistance and tolerance to biotic and abiotic stresses from seed treatment by MeJA or JA has been studied in other species like tomatoes (Giovannini et al., 2024; Król et al., 2015; Worrall et al., 2012), melon (Buzi et al., 2004) and rice (Bhavanam and Stout, 2021). Vivas et al. (2012) tested MeJA treatment on maritime pine (*Pinus pinaster*) seeds but at a high concentration, inducing intoxication. For Norway spruce, only one study has tested seed treatment with nicotinamide and JA (Berglund et al., 2015). Their results indicated that seed treatment can somewhat reduce pine weevil attacks. These studies demonstrated the potential of seed priming, which would be a cheaper and more practical way for future use of MeJA in Norway spruce protection.

Previous studies on MeJA seed priming in other species observed increased phenolic compounds and chitinase, peroxidase, and lipoxygenase activities (Bhavanam and Stout, 2021; Buzi et al., 2004; Król et al., 2015). The molecular mechanisms of MeJA-IR and priming in Norway spruce have only been studied in 48-year-old trees and 2-year-old seedlings (Mageroy et al., 2020b; Wilkinson et al., 2022). Both studies gave insights into the mechanism of MeJA-IR, including complex defense hormonal regulation, priming of gene encoding pathogenesis-related (PR) proteins, and epigenetic regulatory mechanisms (Wilkinson et al., 2022; Mageroy et al., 2020b). Questions such as whether these mechanisms are consistent in seed treatment, or what epigenetic changes occur in the genome, remain unanswered. Epigenetic changes can be studied using formaldehyde-assisted isolation of regulatory elements (FAIRE) to investigate chromatin accessibility (Baum et al., 2020).

Even though MeJA treatment can enhance conifer resistance, it also significantly reduces growth (Huynh et al., 2024b). Therefore, finding better alternative priming stimulants is of interest. We previously tested β-aminobutyric acid (BABA), a known priming stimulant in angiosperms, on 2-year-old Norway spruce. However, our results suggested that BABA was not an effective and suitable alternative to MeJA (Huynh et al., 2024a). Another potential chemical is pipecolic acid (PipA), a Lys-derived non-protein amino acid, which plays a critical role in acquiring plant immunity and regulates SA biosynthesis (Bernsdorff et al., 2015). Many studies have shown that foliar application of PipA can enhance resistance in tobacco, tomatoes, and cucumber against bacterial and fungal infections (Vogel-Adghough et al., 2013; Pazarlar et al., 2021; Zhang et al., 2020). Chitosan (Chi), a polysaccharide from the deacetylation of chitin from crustaceans, fungi, or insect sources, is another potential candidate due to its antimicrobial characteristics (Aranaz et al., 2021). Chi is recognized as a defense elicitor in plants, triggering several defense responses when applied and enhancing resistance (Riseh et al., 2022; Malerba and Cerana, 2016). Many studies have shown that foliar application and seed treatment of Chi can reduce the damage from pathogens in various crop species. i.e. tomatoes, wheat, or cucumbers (El Hadrami et al., 2010; Riseh et al., 2022). An abundant class of phenolic derivatives in Norway spruce wood called lignan (Li) could also enhance resistance. Lignans have been previously recognized for their role in enhancing resistance in various plant species against a broad spectrum of pests and pathogens (Ražná et al., 2021). However, there are no studies on foliar application of lignans for enhanced resistance, although a recent study showed that Norway spruce seeds grown in substrates with added lignans had improved germination rates (Adamczyk et al., 2022).

In this study, we assessed the potential of Norway spruce seed treatment with MeJA, PipA, Li, and Chi to enhance the resistance of the emerged seedlings against *Botrytis cinerea*. Furthermore, we used transcriptomics, FAIRE-qPCR, and quantification of defensive metabolites, enzymatic activity, and hormones to investigate the underlying mechanisms of enhanced resistance provided by seed treatment.

2. Materials and Methods

2.1. Experimental design

The experiment was divided into two batches for easier handling (Fig. 1). The first batch of seedlings was subjected to fungal assay, morphology, and FAIRE-qPCR for chromatin accessibility analysis. The

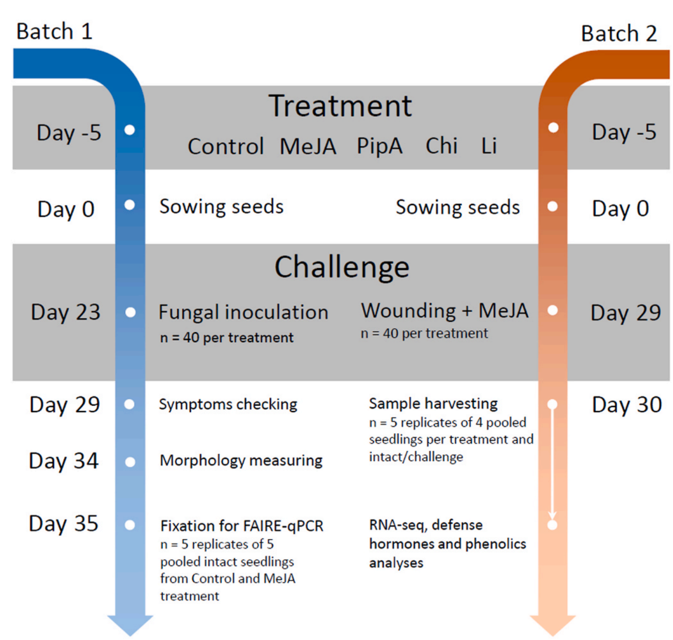

Fig. 1. Overview of the experiment timeline. Norway spruce seeds were treated with methyl jasmonate (MeJA), pipecolic acid (PipA), chitosan (Chi) and lignan (Li) then propagated into seedlings. In batch 1, seedlings were subjected to morphology assessment, fungal assay and chromatin accessibility analysis. In batch 2, seedlings were subjected to a challenge of wounding with MeJA application for RNA-seq and metabolite analyses. The day indicates the number of days post-sowing.

second batch of seedlings was subjected to a challenge for RNA-seq, and defense hormones and phenolics analyses. A detailed timeline for each batch is presented in Fig. 1.

2.2. Plant materials

Norway spruce seeds (seed lot F15-16 from Kilen (59.31° N, 8°47′E, 89 m a.s.l.)) were purchased from Skogfrøverket. Seeds were surface sterilized with 3.5 % sodium hypochlorite for 5 min with constant stirring. Then they were rinsed thoroughly under running tap water for 5–10 min until the smell of sodium hypochlorite completely dissipated. Seeds were then transferred to a 1-L bottle filled with sterilized ddH_2O (sdH_2O). They were shaken gently for approximately 22 h, with a water change after the first 6 h.

After sterilization, seeds were soaked overnight in 200 ml solution of different chemicals: 0.5 mM MeJA, 0.5 mM PipA, 0.0005 % (w/v) Chi (diluted from a stock of 10 mg/ml Chi in 1 M citric acid), and 0.0005 % (w/v) Lignan (200 ml of solution for each treatment). Lignan type LP (Adamczyk et al., 2022) solution was made the day before to dissolve better. The concentration of MeJA was selected based on pilot experiments and a meta-analysis of previous studies involving MeJA treatment in conifers (Huynh et al., 2024b). Since the other compounds (PipA, Li, and Chi) have not been tested on spruce before, we chose to apply them at concentrations similar to those used for MeJA to allow for a meaningful comparison. After soaking, seeds were strained and rinsed with sdH_2O in a laminar flow hood and placed on sterile filter papers (Ø10 cm) in a Petri dish for each treatment. The seeds were then cold stratified at 4 °C for 5 days before sowing.

Seeds were planted in Phytatrays (Sigma-Aldrich, Massachusetts, USA, product number; P5929, lot number; MKCT4636, 9.9 x 8.3 x 4.3 (L x W x H) cm) filled with sterile vermiculite in a laminar flow hood. Using sterile forceps, seeds were placed into the vermiculite to a depth of 5 mm. For batch 1, each Phytatray contained 40 seeds, while in batch 2, each Phytatray contained 48 seeds, with the seeds placed in a 5 x 8 or 6 x 8 grid, respectively. The trays were then closed and placed in growth chambers at 24.5 °C and 16 h of light per day. Light irradiance is 60 μmol $m^{-2}.s^{-1}$ on top of the Phytatray lid, and 50 μmol $m^{-2}.s^{-1}$ under the Phytatray lid at the base of the seedlings. Germination percentage was calculated for seeds in batch 1 by dividing the number of germinated seeds 19 days after sowing by the total number of seeds sown and multiplying by 100.

2.3. *Botrytis cinerea* assay

B. cinerea, strain FG 48 (Nielsen et al., 2024), was revived from cryo-storage (−80 °C) onto 3.9 % potato dextrose agar petri plates. The culture plates were allowed to grow on the bench at room temperature for 6 days, after which they were put under UV light for an additional 10 days. On the day of inoculation, 5 mL of sdH_2O was added to the culture plate and spores were gently scraped into the water with a sterile metal spatula. The spore solution was then taken from the plate using a pipette and placed into a 50 mL falcon tube. Spores were quantified using a hemocytometer (Sigma-Aldrich, Massachusetts, USA, product number; Z359629). The spore solution was then diluted to a final concentration of 10^6 spores/ml with 0.1 % Tween- 20 in sdH_2O. The same solution without fungal spores was prepared for the mock inoculum.

Twenty-three days after sowing, 40 seedlings in each treatment were inoculated with *B. cinerea*. Seedlings were placed on top of a sterile filter paper in a Petri dish (Ø10cm). Each dish contained 5 seedlings. The roots were covered with half a piece of sterile filter paper and watered with 2 ml sdH_2O (Fig. S1A). Then 5 ml of the prepared *B. cinerea* spore solution was added onto the needles. The same procedure was done for the mock-inoculated seedlings, except that the mock inoculum was used. The needles were pushed down slightly with pipette tips to ensure full submergence. The plates were sealed tightly with parafilm and placed in a dark place at room temperature for 6 days.

After 6 days, the inoculated seedlings were assessed. The health states of the seedlings were split into three categories: (1) healthy, in which the seedlings were all green and firm; (2) symptomatic, in which the seedlings had some discoloration in the needles and the stems, with some mild loss of structure integrity and needles; (3) dead, in which the seedlings were extremely mushy, heavily discolored, and completely lost their structure integrity (Fig. S1B).

2.4. Morphology analysis

Stem and root lengths of 20 seedlings from each treatment from batch 1 were assessed 34 days after sowing. The individual seedling picture was taken on a grid surface with a known scale. The seedlings were then measured through the images in the program ImageJ (version 1.53k, National Institutes of Health, USA; http://imagh.nih.gov/ij). The stem was measured from the base of the needles down to where the green color turned brown. The root was measured from the end of the stem to the root tip (Fig. S2).

2.5. Challenge and harvesting for transcriptomic and metabolites analyses

To determine the different immediate defense responses, seedlings were subjected to wounding with MeJA application as a challenge. Five seedlings were placed in each Petri dish lined with sterile filter paper. For the challenged seedlings, all the needles were smashed using a sterile metal spatula. Another piece of filter paper was then placed on top of them. Finally, 5 ml of 1 mM MeJA +0.1 % Tween in sdH20 was added to mimic an attack signal. The intact seedlings were sandwiched between the 2 filter papers with the addition of 5 ml of 0.1 % Tween in sdH_2O. The Petri dishes were closed tightly with parafilm and placed back in the growth room. After 24 h, the seedlings were harvested by flash freezing in liquid nitrogen and then stored at −80 °C. Whole seedlings were then ground into a fine powder using liquid nitrogen in mortar and pestle. Each bioreplicate was pooled from 4 seedlings.

2.6. Defense hormone and phenolic analysis

2.6.1. Extraction

Phenolics and phytohormones were analyzed from the same extraction, containing internal standards (IS) for both analyses. About 100 mg of fresh spruce tissue was extracted in 1 ml solvent of methanol in a 2-ml Eppendorf tube, containing 40 ng D4-SA (Santa Cruz Biotechnology, USA), 40 ng D6-JA (HPC Standards GmbH, Germany), 40 ng D6-ABA (Toronto Research Chemicals, Toronto, Canada), and 8 ng D6-JA-Ile (HPC Standards GmbH) as IS for phytohormones and 20 μg Apigenin-7-glucoside (Carl Roth GmbH, Karlsruhe, Germany) as IS for phenolics. The homogenates were shaken for 1 h at room temperature and then pellet centrifuged at 14000 rpm (Eppendorf centrifuge 5417 R) at room temperature for 15 min. The supernatant was transferred to an LC-MS vial. The sample solid residue was then re-extracted in the same manner with 500 μl of the same solvent, and then the supernatant was added to the same LC-MS vial.

2.6.2. Phytohormones analysis

The extract was first used for defense hormone analysis, which was performed by LC-MS/MS as in (Heyer et al., 2018) on an Agilent 1260 series HPLC system (Agilent Technologies) with the modification that a tandem mass spectrometer QTRAP 6500 (SCIEX, Darmstadt, Germany) was used. Chromatographic separation was achieved on a Zorbax Eclipse XDB-C18 column (50 × 4.6 mm, 1.8 μm, Agilent Technologies). Water containing 0.05 % formic acid and acetonitrile were employed as mobile phases A and B, respectively. The elution profile was: 0–0.5 min, 10 % B; 0.5–4.0 min, 10–90 % B; 4.0–4.02 min, 90–100 % B; 4.02–4.5 min, 100 % B and 4.51–7.0, min 10 % B. Flow rate was kept at 1.1 ml/min and the column temperature was maintained at 25 °C. The mass spectrometer was equipped with a Turbo spray ion source operated in negative ionization mode. The ion spray voltage was maintained at −4500 eV. The turbo gas temperature was set at 650 °C. Nebulizing gas was set at 60 psi, curtain gas at 40 psi, heating gas at 60 psi, and collision gas was set to "medium". The mass spectrometer was operated in multiple reaction monitoring (MRM) mode, details of the instrument parameters and response factors for quantification can be found in Table S1. Since we observed that the peaks for both D6-labeled JA and D6-labeled JA-Ile standards (HPC Standards GmbH, Cunnersdorf, Germany) contained 40 % of the corresponding D5-labeled compounds, the sum of the peak areas of D5-and D6-labeled compounds were used for quantification. For quantification of salicylic acid glucoside, the internal D4-SA standard was used applying an experimentally determined response factor of 8.3.

2.6.3. Phenolics analysis

After finishing with the hormones analysis, the same extract was used for phenolics analysis. Phenolic analysis was performed by LC-MS/MS as in (Huang et al., 2017) on an Agilent 1200 series HPLC system (Agilent Technologies) coupled to an API3200 tandem mass spectrometer (AB Sciex, Darmstadt, Germany) with modifications. Chromatographic separation was achieved on a Zorbax Eclipse XDB-C18 column (50 × 4.6 mm, 1.8 μm, Agilent Technologies). Water containing 0.05 % formic acid and acetonitrile were employed as mobile phases A and B, respectively. The elution profile was: 0–1.0 min, 0 % B; 1.0–7.0 min, 0–65 % B; 7.0–7.02 min, 65–100 % B; 7.02–8.0 min, 100 % B and 8.01–10.0, min 0 % B. Flow rate was kept at 1.1 ml/min and the column temperature was maintained at 25 °C. The mass spectrometer was equipped with a Turbo spray ion source operated in negative ionization mode. The ion spray voltage was maintained at −4200 eV. The turbo gas temperature was set at 500 °C. Nebulizing gas was set at 60 psi, curtain gas at 30 psi, heating gas at 60 psi, and collision gas was set to 6 psi. The mass spectrometer was operated in multiple reaction monitoring (MRM) mode, details of the instrument parameters and response factors for quantification can be found in Table S2.

2.7. Total nucleic acid extraction

For total nucleic acid extraction, 15–20 mg of tissue per sample were aliquoted into a 2 ml tube. To each tube, 680 μl of lysis buffer was added. The lysis buffer contained 650 μl CTAB buffer (3 % w/v CTAB (Merck KGaA Darmstadt, Germany, 219374-100 GM); 100 mM Tris HCl pH 7; 50 mM EDTA; 1.4 M NaCl), 13 mg polyvinylpyrrolidone (PVP) (Sigma-Aldrich, Massachusetts, USA, product number: PVP40, lot number: WXBD6511V) and 1.3 μl β-mercaptoethanol (BME) (Sigma-Aldrich, Massachusetts, USA, product number; M3148, lot number; BCBL6953V). The tubes were then incubated at 65 °C for 15 min in a ThermoShaker at 1000 rpm. After incubation, 40 μl of 10 % sodium dodecyl sulphate (Sigma-Aldrich, Massachusetts, USA, product number L4390; lot number SLBB0911V) was added and vortex vigorously until the homogenate turned white and foamy. The tubes were then centrifuged at 15000 rpm at 4 °C for 15 min. After centrifugation, the supernatant was transferred to a new tube and 0.5 volume of 7.5 M lithium chloride was added to the supernatant to precipitate the total nucleic acid. The tubes were then incubated for 2–3 h at −20 °C. After incubation, the supernatant was pellet centrifuged for 1 h at 15000 rpm at 4 °C. The supernatant was discarded, and the pellet was subjected to two washes with 70 % ethanol and dried briefly at room temperature before resuspended with 30 μl of nuclease-free water.

2.8. Transcriptome analysis

Total nucleic acid samples were checked for quantity and quality on a Nanodrop 2000 (Thermo Scientific, Massachusetts, USA, catalogue number; ND2000) equipped with NanoDrop 2000/2000c software (version 1.6.198) specifying for RNA. Samples were then sent to BGI Tech Solutions (Tai Po, Hong Kong). The samples were then subjected to DNase treatment and quality/quantity check before building the libraries following BGI protocol. Across 50 samples, 1.79 billion 150 bp paired-end (PE) clean reads were generated in total, with the minimum, maximum, and mean number of read pairs per sample being 30.6, 36.2, and 35.8 million, respectively. For all samples, ≥97.9 % of nucleotides had a Phred quality score of >20.

Clean reads were aligned to the Norway spruce reference transcriptome from Mageroy et al. (2020) using the bowtie 2 package v2.3.1 (Langmead and Salzberg, 2012). The transcriptome was indexed by bowtie2-build function, then they were aligned using the bowtie function with the parameter settings: '–very sensitive' '-q' '-k 10'. The featureCounts function from the Rsubread R package v2.0.1 (Liao et al., 2014, 2019) was used to count uniquely aligned fragments for each gene. The options specified were: 'isGTFAnnotationFile = TRUE' and 'isPairedEnd = TRUE'. The Gene Transfer Format (GTF) reference transcriptome annotation file was retrieved from Wilkinson et al. (2022).

Further analyses were adapted from the pipeline in Wilkinson et al. (2022), with some modifications. Count tables from featureCounts were pre-filtered to only keep genes with ≥100 reads counts across all samples. After filtering, the read counts were transformed with a variance stabilizing transformation (vst) using the function vst from the package DESeq2 (Love et al., 2014) with these specifications: 'blind = TRUE', 'nsub = 1000', 'fitType = "parametric"'. The transformed data was then subjected to a principle component analysis (PCA) and heatmap analysis of sample-to-sample distances. PCA was performed by the plotPCA function from DESeq2 package, with these specifications: 'intgroup = c ("Treatment","challenge")', 'ntop = 45543', 'returnData = TRUE'. PCA plot was generated using "ggplot" function from ggplot2 (v 3.5.1) package (Wickham, 2016). A heatmap showing sample-to-sample distances was made using the package "pheatmap" (v 1.0.12) (Kolde, 2019), with default options.

After exploring the global pattern, differential expression (DE) analysis was performed in RStudio using the function "DESeq" from the package DESeq2 (v 1.44.0) (Love et al., 2014). Genes showing significantly changed expression level between treatment and challenge with adjusted $p < 0.05$ were selected from the result table using the "results" function with specifications: 'alpha = 0.05', 'cooksCutoff = T', 'lfcThreshold = 0', 'contrast = c("treatment",x,y)'. The option 'contrast' is pre-defined with only interesting contrasting pairs.

Based on these results, we decided to continue the analysis for only MeJA and control treatment. Agglomerative hierarchical clustering of genes by expression pattern was performed on transformed count data by "vst" function with options: 'blind = FALSE', 'nsub = 1000', 'fitType = "parametric"'. The clusters are then shown by a heatmap showing each genes's Z-score transformed count coupling with a dendrogram made from Ward's minimum variance method. This was done by the function "aheatmap" from the "NMF" package v0.28 (Gaujoux and Seoighe, 2010). The same transformed counts data was used to make gene expression profile plots from each cluster, using the "ggplot" function from "ggplot2" package.

Annotated protein domains from Wilkinson et al. (2022) were used for the Pfam enrichment analysis using the function "enrichment" from the "bc3net" package v1.0.4 (DE MATOS SIMOES and EMMERT-STREIB, 2016). A Pfam protein signature is considered enriched when its adjusted p-value ≤0.05 (Benjamini and Hochberg, 1995). The fold-enrichment plots displaying top 5 significantly enriched protein signatures were made using ggplot2.

2.9. Chromatin openness assessment

Formaldehyde-assisted isolation of regulatory DNA elements (FAIRE) was used to assess the chromatin openness of target genes selected based on metabolite and transcriptome analysis. Methods were adapted from a FAIRE protocol developed for Arabidopsis leaves (Baum et al., 2020). Seedlings were harvested 35 days after sowing and fixed with formaldehyde. Five bioreplicates of 5 pooled seedlings were prepared for each seed treatment (Fig. 1) by cutting the seedlings into small pieces and placing them in a 15 ml Falcon tube. Twelve ml of the crosslinking buffer [3 % formaldehyde (252549, Sigma-Aldrich) in 400 mM sucrose, 10 mM HEPES (pH 7.8) (H3375-250G, Sigma-Aldrich), 0.1 mM phenylmethylsulphonyl fluoride (10837091001, Roche) and 5 mM BME] were added to each tube, ensuring the tissues were completely submerged in the buffer. Tubes were left open and put on ice in the vacuum chamber. The vacuum was applied for three rounds of 20 min, releasing the vacuum after each round. After the third round, the crosslinking process was quenched by adding 650 μl of 2.5 M glycine into each tube, for a final glycine concentration of 128 mM. Then, another three rounds of 20-min vacuum were applied, similar to the previous crosslinking process. After the last vacuum round, tissues were washed thoroughly with tap water to remove any remaining buffer. They were then dried thoroughly, flash-frozen in liquid nitrogen, ground into fine powder, and stored at −80 °C for further steps.

For chromatin extraction, 25–30 mg of tissues per sample was aliquoted into a 2 ml tube, with two technical replicates required for each bioreplicate. To each tube, 900 μl of total nucleic acid extraction lysis buffer (see above) were added and tubes were vortex to mix thoroughly. The tubes were then incubated at 37 °C for 1 h in a ThermoShaker at 1400 rpm. The tubes were then centrifuged at 15000 rpm at 4 °C for 15 min. After centrifugation, the supernatant from the two technical replicates was pooled into one new 2 ml Eppendorf tube. The pooled supernatant was then aliquoted equally into four 1.5 ml Bioruptor® Microtubes (Cat. No. C30010016, Diagenode), approximately 300 μl per tube. Tubes were then subjected to sonication using the Bioruptor® Pico sonication device (Cat. No. B01080010, Diagenode) coupled with a Bioruptor Water Cooler (Diagenode) for temperature control. The sonication was done with 20 cycles of 45 s on and 45 s off in a 4 °C water bath. This setting was optimal for producing fragments ranging from 200 to 600 bp. The machine was rested for 20 min between each round, allowing complete cool down for higher sonication efficiency. After sonication, all aliquots from each bioreplicate were combined into one 2 ml Eppendorf tube. The combined supernatant of each bioreplicate was then split equally into two 2 ml tubes, with approximately 500 μl per tube. One tube was de-crosslinked by incubating at 65 °C for 24 h. The other tube was stored at −20 °C until the de-crosslinking was finished.

After de-crosslinking, DNA was extracted from crosslinked and de-crosslinked samples. To each tube 200 μl was added of an extraction buffer [100 mM EDTA, 100 mM Tris-HCl and 100 mM NaCl]. Next, 700 μl of phenol:chloroform:isoamyl with the ratio of 25:24:1 (77617, Sigma-Aldrich), were added to each tube and vortexed thoroughly for 20 s until the homogenate was white. The tubes were then centrifuged at 15000 rpm at 4 °C for 15 min. After centrifugation, the upper aqueous phase was carefully transferred to a new 2 ml Eppendorf tube without disrupting the interphase and the organic phase. To each tube, an equal volume of chloroform was added and vortexed vigorously. The tubes were then centrifuged again at 15000 rpm at 4 °C for 15 min. After centrifugation, the upper aqueous phase was transferred to a new 2 ml Eppendorf tube. To precipitate the DNA, 0.1 volume of sodium acetate 3 M and 0.8 to 1 volume of isopropanol was added to each tube. Tubes were then incubated on ice for 20 min. After incubation, the supernatant was pellet centrifuged for 1 h at 15000 rpm at 4 °C. The supernatant was then discarded, and the pellet was subjected to two washes with 70 % ethanol and dried briefly at room temperature before resuspended with 30 μl of nuclease-free water. The extracted DNA was diluted to 5 ng μl^{-1} for qPCR quantification.

To assess the transcriptional accessibility of selected targeted genes, qPCR was performed at several positions within 2000 bp upstream from the transcription start site (TSS) and the gene body. Primers were designed in Primer3 (primer3.ut.ee) using the available genome at Plant GenIE.org. The full list of primers can be found in Table S3. Quantification of chromatin openness was performed in ViiA7 Real-Time PCR System (Applied Biosystems, ThermoFisher Scientific, USA) with the associated QuantStudio Real-Time PCR software (v1.3, Applied Biosystems). A 10 μl reaction volume was used, with 5 μl Fast SYBR Green Master Mix (Applied Biosystems), 2 μl nuclease-nuclease free water, 1 μl of forward and reverse primers (4 μM) and 2 μl of the diluted DNA. The thermocycle conditions started with the denaturation stage with 1 cycle of 95 °C for 20 s, followed by 40 cycles of 95 °C for 1 s, followed by 20 s at 60 °C for annealing and extension; and finally the melt curve stage of 1 cycle of 95 °C for 15 s, 60 s at 60 °C, and 15 s at 95 °C.

The recovery ratio of free DNA to total DNA for each primer was calculated using this formula:

$$Recovery\ Ratio = 2^{-\left(Ct_{crosslinked\ replicate} - Ct_{\ decrosslinked\ replicate}\right)}$$

The relative recovery ratio was normalized to the recovery ratio for the housekeeping gene Actin:

$$Relative\ recovery\ ratio = \frac{Test\ region\ Recovery\ Ratio}{Actin\ Recovery\ Ratio}$$

2.10. Statistical analysis

All statistical analyses were performed in R (version 4.1.2) via RStudio (version 2024.1.9, build 394). All plots were made using the packages "ggplot2" (Wickham, 2016) and "patchwork" (Pedersen, 2024).

Germination data from different seed treatments were analyzed by logistic regression using the function "glm" from the package "stats" with the specification of the family as "binomial". Morphology data from different seed treatments were assessed using one-way ANOVA by the function "lm" (from the "stats" package). ANOVA tables with type II errors for the models were obtained using the function "Anova" from the "car" package (Fox and Weisberg, 2019). All models were checked visually for assumptions of normality, constant variance, and independence using the function "plot". Both analyses were then followed by a Tukey's pairwise post-hoc test using the function "emmeans" from the package "emmeans" (Lenth, 2023).

Effects of seed treatments on the resistance against *B. cinerea* were assessed by cumulative link model using the function "clm" from the package "ordinal" (Christensen, 2022) and function "Anova.clm" from the package "RVAideMemoire" (Herve, 2023). Model assumptions were checked using the "nominal_test" and "scale_test" functions. The model was then subjected to Tukey's post-hoc pairwise comparison using the function "emmeans" from the package "emmeans" (Lenth, 2023).

Two-way ANOVA models with interaction were initially used to assess the effects of seed treatment and challenge on defense responses (phenolics and defense hormones concentrations). The model was built using the function "lm" (from the "stats" package). ANOVA tables with type II errors for the models were obtained using the function "Anova" from the "car" package (Fox and Weisberg, 2019). The interaction term was removed from the model if it was insignificant. All models were checked visually for assumptions of normality, constant variance, and independence using the function "plot". Models that failed the normality assumption were subjected to log or squared root transformation of the dependent variables. All models were then subjected to Tukey's pairwise post-hoc test using the function "emmeans" from the package "emmeans" (Lenth, 2023), to compare across all combinations of different seed treatments and challenges. Models that could not fulfill the normality assumption even after transformation were then subjected to a non-parametric approach, Aligned Rank Transformation (ART) ANOVA, using the function "art" followed by ART contrast test using the function "art.con" from the package "ARTool" (Kay et al., 2021).

3. Results

In this study, we treated Norway spruce seedlings with MeJA, PipA, Chi and Li to evaluate their effects on seedling germination rate and morphology. We also investigated whether these treatments enhanced seedling resistance to *Botrytis cinerea*. To further understand the underlying mechanisms of this enhanced resistance, we analyzed phenolics and hormone levels, transcriptomics data, and chromatin openness.

3.1. Effects of seed treatment on germination percentage and seedling length

To determine the effect of seed treatment on seed germination, the final germination percentage was assessed 19 days after sowing. Only Chi treatment significantly improved the germination percentage compared to control seeds (Fig. S3). MeJA and PipA treatment slightly decreased the final germination percentage but was not statistically significant compared to control seeds (Fig. S3). Li treatment significantly increased the final germination percentage compared to PipA treatment, but not compared to control seeds (Fig. S3).

Effects of seed treatment on seedling growth were evaluated by measuring seedling length, including roots and stem length 34 days after sowing. Seedlings from seeds treated with MeJA had significantly shorter root and stem lengths than all other treatments (Fig. 2). The reduced length of stems and roots resulted in the reduction of the total length of seedlings from MeJA treatment (Fig. 2). Other seed treatments did not affect seedling root or stem length compared to control.

3.2. Effects of seed treatment on seedlings resistance to Botrytis cinerea infection

To determine if seed treatment enhanced seedlings resistance to fungal disease, seedlings were inoculated with *B. cinerea* 23 days after sowing. Disease severity was recorded 6 days after inoculation.

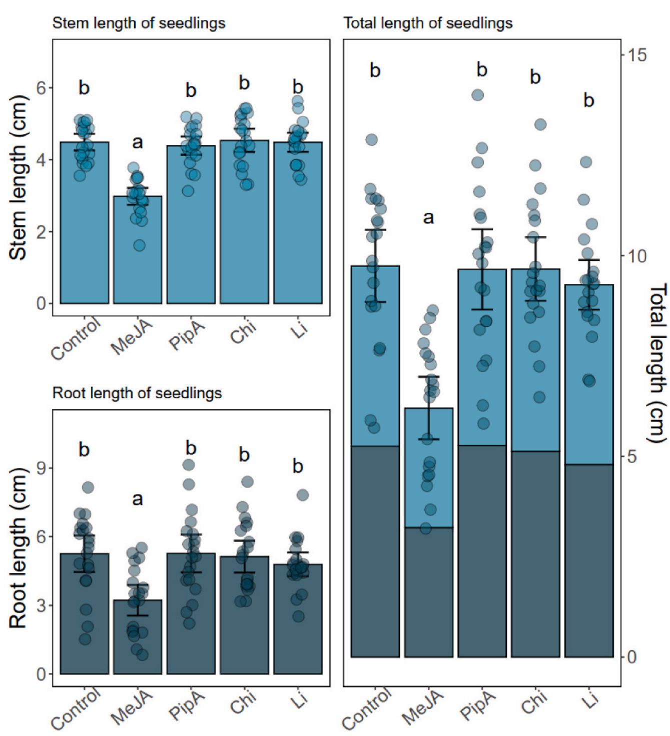

Fig. 2. Mean stem length (light blue bars), root length (dark blue bars), and total length of treated Norway spruce seeds at 34 days after sowing. Seeds were treated with water (Control), methyl jasmonate 0.5 mM (MeJA), pipecolic acid 0.5 mM (PipA), chitosan 0.0005 % (Chi) and lignan LP 0.0005 % (Li). Error bars show a 95 % confidence interval for the and circles represent individual data points (n = 20 per treatment). Treatments with different letters are significantly different (1-way ANOVA, followed by Tukey's post hoc test, $p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Seedlings grown from MeJA-treated seeds had significantly lower mortality than seedlings from control seeds, also the lowest mortality rate (20 %) among all other treatments (Fig. 3). Additionally, the treatment had the highest number of healthy seedlings after inoculation (22.5 %) (Fig. 3), which indicated higher resistance towards *B. cinerea*.

PipA treatment had a similar amount of healthy seedlings as control seeds while having a slightly lower mortality rate. Both Li and Chi treatments had more healthy seedlings than control (12.5 % and 17.5 %, respectively), but the mortality rate was not significantly reduced. However, the overall effect of these treatments on seedling resistance to *B. cinerea* was not significant compared to control (Fig. 3).

3.3. Effects of seed treatment on phytohormones

To further understand the defense mechanisms of spruce seedlings to B. cinerea challenge, we also quantified their defense hormone levels (Fig. 4 and S4). SA concentration did not differ much between treatment and challenge. Intact seedlings from PipA and Li seed treatment had significantly lower SA concentrations than those from the control treatment. Challenged seedlings from Chi seeds had lower SA concentrations than challenged seedlings from the control treatment. SA glucoside concentration was significantly higher in intact seedlings than in challenged ones across all seed treatments. Only challenged seedlings from PipA-treated seeds had significantly lower SA glucoside concentrations than those from all other seed treatments. ABA was generally present in higher concentration in intact seedlings than challenged seedlings across all seed treatments. Both intact and challenged seedlings from control seeds had higher ABA concentrations than those from MeJA- and Chi-treated seeds (Fig. S4). PipA- and Li-treated seeds did not have different ABA concentrations compared to control seeds, in both intact and challenged seedlings.

In the intact seedlings, the concentration of JA and JA-Ile did not

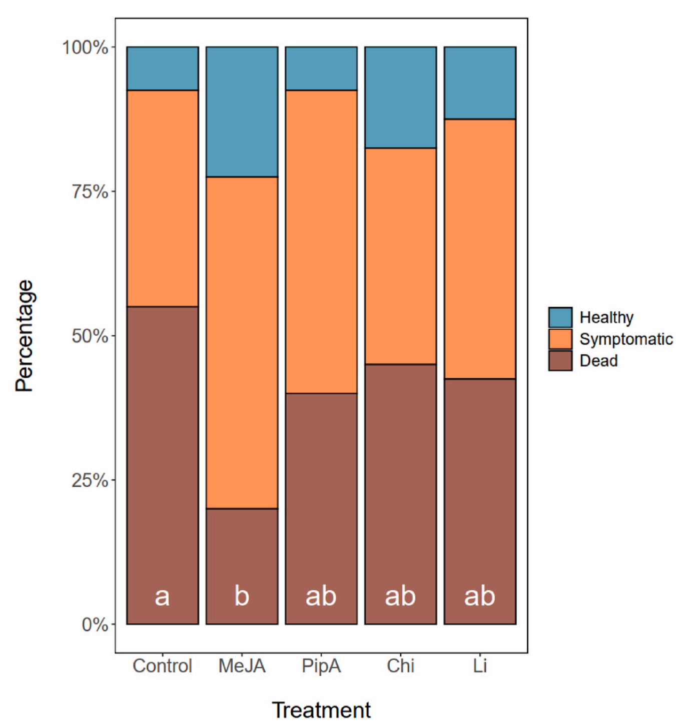

Fig. 3. Disease severity 6 days after *Botrytis cinerea* inoculation. Norway spruce seedlings from treated seeds were inoculated with *B. cinerea* 23 days after germination (n = 40 per treatment). Seed treatments were water (Control), methyl jasmonate 0.5 mM (MeJA), pipecolic acid 0.5 mM (PipA), chitosan 0.0005 % (Chi) and lignan LP 0.0005 % (Li). Treatments with different letters are significantly different (cumulative link model, followed by Tukey's post hoc test, $p < 0.05$).

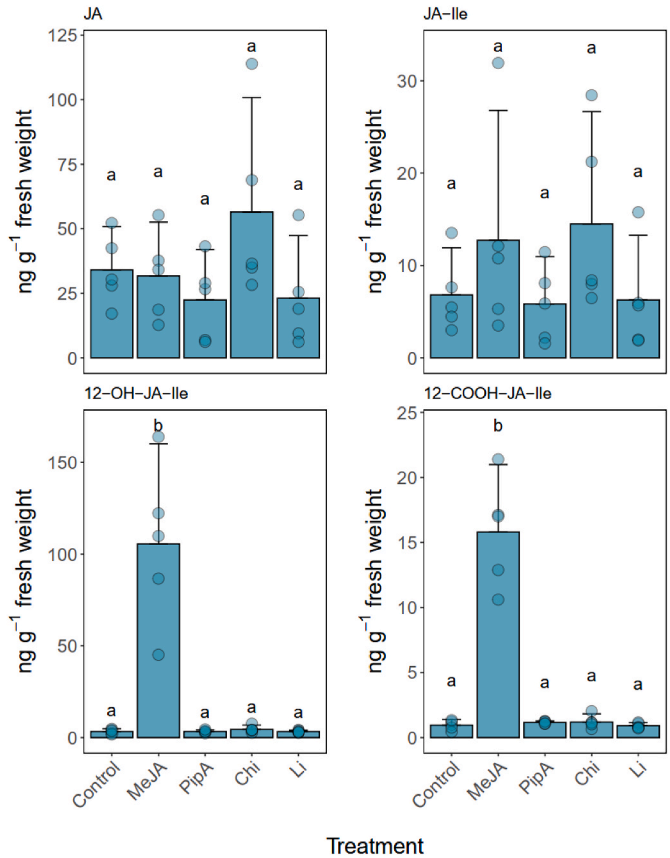

Fig. 4. Mean concentrations of jasmonic acid (JA), JA isoleucine (JA-Ile), 12-OH-JA-Ile, and 12-COOH-JA-Ile in Norway spruce seedlings, propagated from seeds treated with water (Control), methyl jasmonate 0.5 mM (MeJA), pipecolic acid 0.5 mM (PipA), chitosan 0.0005 % (Chi), and lignan 0.0005 % (Li). Twenty-nine days after sowing, seedlings were challenged by wounding and treatment with MeJA. For each treatment, 20 intact (shown here) and 20 challenged (see Supplementary Information Fig. S1) seedlings were harvested 24 h after challenge. Each of the 5 bioreplicates was pooled from 4 seedlings. Error bars show 95 % confidence intervals and circles represent individual data points (n = 5 per treatment). For each compound, seed treatments with different letters are significantly different (2-way ANOVA with log transformation for 12-OH-JA-Ile, and squared root transformation for JA-Ile and 12-COOH-JA-Ile, followed by Tukey's HSD post hoc test, $p < 0.05$).

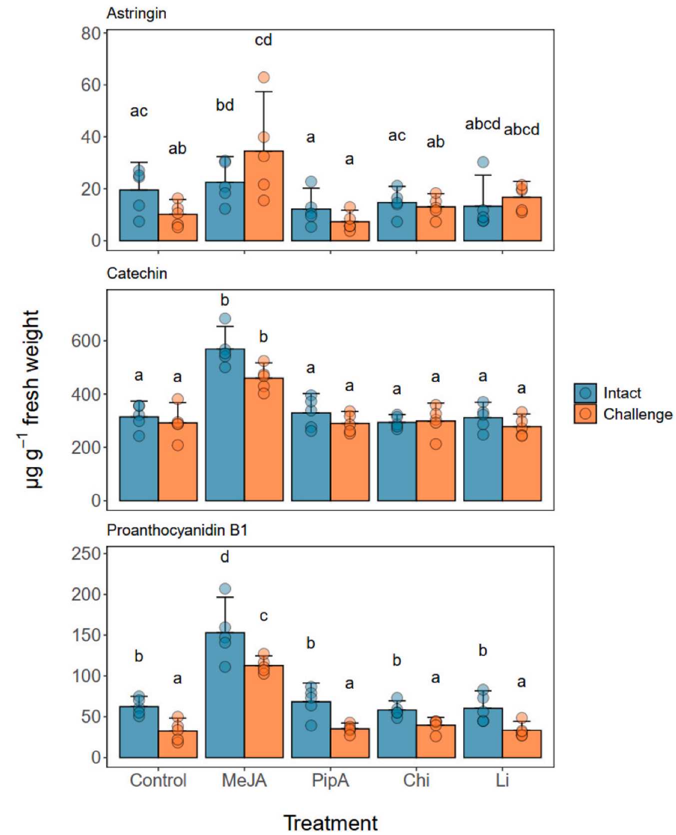

Fig. 5. Mean concentrations of astringin, catechin, and proanthocyanidin B1 in Norway spruce seedlings, propagated from seeds treated with water (Control), methyl jasmonate 0.5 mM (MeJA), pipecolic acid 0.5 mM (PipA), chitosan 0.0005 % (Chi), and lignan 0.0005 % (Li). Twenty-nine days after sowing, seedlings were challenged by wounding and treatment with MeJA. For each treatment, 20 intact (blue bars) and 20 challenged (orange bars) seedlings were harvested 24 h after challenge. Each of the 5 bioreplicates was pooled from 4 seedlings. Error bars show 95 % confidence intervals and circles represent individual data points (n = 5 per treatment and challenge). For each compound, seed treatments and challenges with different letters are significantly different (2-way ANOVA, with squared root transformation for astringin and proanthocyanidin B1, followed by Tukey's HSD post hoc test, $p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

differ between treatments. However, intact seedlings from MeJA-treated seeds had much higher concentrations of catabolized JA-ILE products,12-OH-JA-Ile and 12-COOH-JA-Ile, than those from other treatments (Fig. 4). As seedlings were challenged by wounding and application of MeJA, all JA-related hormone concentrations in challenged seedlings were significantly higher than in intact seedlings and did not differ between treatments (Fig. S5).

3.4. Effects of seed treatment on phenolics

In addition to defense hormone levels, we also investigated the composition of some major spruce phenolic compounds. Astringin concentrations in intact and challenged seedlings from MeJA-treated seeds were higher than those from control seeds (Fig. 5). Seedlings from other seed treatments did not have different astringin concentrations than those from the control treatment. Intact and challenged seedlings from MeJA-treated seeds also had the highest concentration of the flavan-3-ol catechin among all treatments (Fig. 5). However, there was no significant difference in catechin concentration between intact and challenged seedlings within treatments. Additionally, the concentration of proanthocyanidin B1 was higher in intact seedlings than in challenged seedlings across all treatments. Furthermore, seedlings from MeJA-treated seeds had the highest proanthocyanidin B1 concentration of all seed treatments (Fig. 5).

3.5. Transcriptional response to seed treatment and challenge

To understand how seed treatment can enhance resistance to *B. cinerea*, transcriptomics analysis was performed on intact whole seedlings and 24 h after challenge seedlings by wounding and applying 1 mM MeJA. Principal component analysis (PCA) was conducted to explore the global patterns of the data. Samples were mostly separated by the challenge on PC1 with a 78 % variance (Fig. 6). The intact samples were very similar between treatments, except for two replicates from the MeJA treatment. After challenge, seedlings from MeJA-treated seeds distinctly grouped from other treatments accounting for 3 % of the total variance. The analysis suggested that MeJA seed treatment induced a shift in challenged seedlings' transcriptome, but not other treatments.

Since only MeJA seed treatment induced a shift in the global transcriptome of challenged seedlings, we decided to perform subsequent analyses only on intact (I) and challenged (W) seedlings from control (C) and MeJA-treated (MJ) seeds. The pairwise comparisons were used to

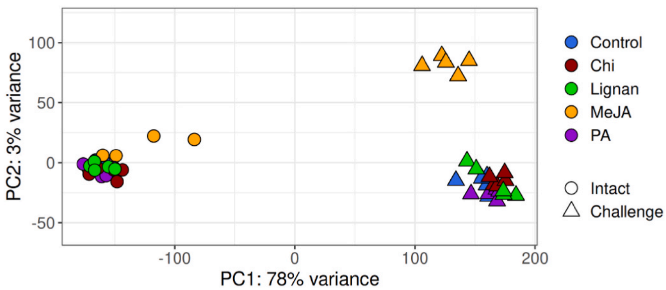

Fig. 6. Principle component analysis showed how seed treatments of Norway spruce (control (blue), MeJA (yellow), Lignan (green), Chi (red), and PipA (purple)) and challenge (intact vs wounded) impact whole seedlings transcriptome 24 h after challenge (n = 5 per treatment and challenge). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

identify transcripts with significantly different expressions ($p.adj < 0.05$) between at least two of the four groups (C_I, C_W, MJ_I, and MJ_W). Only relevant pairwise comparisons between the four groups were performed. Most differentially expressed (DE) transcripts were from comparisons between intact and challenged (i.e. _W vs _I) groups, consistent with the PCA results (Fig. S6). The comparison between MJ_I and C_I had the lowest number of DE transcripts. The second lowest number of DE transcripts was found in the comparison between MJ_W and C_W (Fig. S6). However, there were nearly eight times more DE transcripts in the comparison between MJ_W and C_W than between MJ_I and C_I. This suggested a substantial impact of MeJA seed treatment on Norway spruce seedling response to challenge.

The transcriptional response to seed treatment and challenge was further explored by grouping all DE transcripts into 12 clusters based on their expression patterns (Fig. 7A). A mean expression profile for transcripts within each cluster was generated for each treatment group (Fig. 7B). Finally, protein family (Pfam) enrichment analysis was performed for each cluster (Fig. 7C, Fig. S7, and Supplementary Data Set 2). Although the PCA showed that the challenge induced the most changes in the seedling transcriptome, our primary interest was in the effect of seed treatments on the transcriptome of both intact and challenged seedlings. Therefore, we concentrated on expression patterns that differed between seed treatments in either intact or challenged seedlings (MJ_I vs C_I and MJ_W vs C_W).

Transcripts that were differently expressed between MJ_I and C_I seedlings were found in clusters 4 and 6. Transcripts in cluster 4 were more highly expressed in MJ_I seedlings than in C_I seedlings (Fig. 7B). Among enriched Pfams in this cluster, we found five Pfams relating to pathogenesis-related (PR) proteins, with three Pfams relating to chitinase. We also found three Pfams relating to JA biosynthesis, two Pfams relating to ethylene biosynthesis, two Pfams relating to terpenes biosynthesis, and one Pfam relating to regulating proanthocyanin pathways. Additionally, we found seven other Pfams relating to defense and tolerance against abiotic, biotic, and oxidative stress (Supplementary Data Set 2). Three of these mentioned Pfams appeared among the top five most enriched Pfams (Fig. 7C). Those were “divergent CCT motif” relating to JA biosynthesis, “Barwin family” relating to PR protein, and “animal haem peroxidase” relating to oxidative stress protection (Fig. 7C).

In contrast to cluster 4, transcripts in cluster 6 had a lower expression in MJ_I seedlings than in C_I seedlings (Fig. 7B). Many of the enriched Pfams in this cluster were related to growth and development, including six Pfams relating to photosynthesis, three Pfams relating in growth hormone auxin, and three Pfams relating to chloroplast development. Additionally, we observed several Pfams related to the cell wall and cell integrity, such as “cellulose synthase”, “pectate lyase”, “cellulase (glycosyl hydrolase family 5)”, and “glycosyl hydrolase family 9”. Pfams “UDP-glucuronosyl and UDP-glucosyl transferase”, which can contribute to anthocyanin and flavone/flavanol biosynthesis, were also enriched in this cluster.

Cluster 1, 2, 3, 5, 7, 8, 9, 10, 11, and 12 contained transcripts that showed differences between challenged seedlings from different seed treatments (Fig. 7B). Among these clusters, clusters 3 and 10 included transcripts that had a higher expression in MJ_W seedlings in comparison to C_W seedlings and comparison to intact seedlings. These clusters indicate that MeJA seed treatment primed an upregulation of transcripts in response to challenge. Many Pfams relating to defense and tolerance to biotic, abiotic, and oxidative stress were enriched in these clusters. Among these Pfams, three were related to terpene biosynthesis, two were related to drought tolerance, two were related to the phenylpropanoid pathway and one was related to JA biosynthesis. We also observed some enriched Pfams relating to intracellular transportation and maintaining cell homeostasis. For example, two of the top five most enriched Pfams in cluster 10 are “VHS domain” and “exocyst complex component Sec10”, and one of the top five in cluster 3 is “GDP dissociation inhibitor”.

We also observed several transcripts relating to callose synthase 9, 10, 11, and 12 in these clusters, although we did not see their Pfams enriched. However, we observed two Pfams that may relate to the pathway in cluster 3, which were “sucrose synthase” and “UTP–glucose-1-phosphate uridylyltransferase”.

In contrast to clusters 3 and 10, clusters 5 and 12 contained transcripts that were downregulated in MJ_W seedlings compared to C_W seedlings and intact seedlings (Fig. 7B). These clusters indicate that MeJA seed treatment also primed a downregulation of transcripts in response to challenge. Most of the enriched Pfams in these two clusters were related to RNA metabolism and regulating, including Pfams such as “DYW family of nucleic acid deaminases”, “mTERF” and “PPR repeat”. Interestingly, two Pfams relating to chromatin modification were enriched in cluster 12, namely Pfam “linker histone H1 and H5 family” and Pfam “ZF-HD protein dimerization region”.

Another interesting pattern, “MeJA-induced opposite response to challenge”, was observed in clusters 9 and 11 (Fig. 7B). In this pattern, DE transcripts in MJ_W seedlings had the opposite expression direction compared to C_W. In cluster 9, we observed a down-regulation in seedlings from MeJA-treated seeds and up-regulation in seedlings from control seeds in response to challenge. Most of the enriched Pfams in this cluster were related to metabolism, regulation and translation of RNA. As in clusters 5 and 12, these Pfams included “RNA processing motif (RRM)”,”KH domain” and “mTERF”. In addition, two of the top five enriched Pfams were related to ribosomal protein, including “ribosome biogenesis protein Nop16” and “ribosomal protein S19e”. We also noted some Pfams involving in chromatin modification, including “C-terminus of histone H2A”, “acetyltransferase (GNAT) family” or “nucleosome assembly protein (NAP)” among the enriched Pfams in cluster 9. In contrast to cluster 9, DE transcripts in cluster 11 showed an up-regulation in seedlings from MeJA-treated seeds and down-regulation in seedlings from control seed in response to challenge. Most enriched Pfams in cluster 11 are directly or indirectly involved in defense against biotic and abiotic stress. These Pfams included “lipoxygenase”, which can be involve in JA-biosynthesis, and “catalase”, “caleosin-related protein” and “ferritin-like domain” which can be involved in response to oxidative stress.

3.6. Effect of seed treatment on chromatin openness

After investigating the transcriptome and the metabolites, we used FAIRE-qPCR to examine if changes in chromatin accessibility around the transcription start site (TSS) contributed to changes in the MeJA-primed response to challenge. We selected two *callose synthase 9* (*MA_10135474g0010* and *MA_10433373g0020*), *callose synthase 11* (*MA_10437131g0040*), *callose synthase 12* (*MA_10437131g0040*), and *phenylalanine ammonia-lyase* (*PAL*) (*MA_10437131g0040*) because their transcripts exhibited a MeJA-induced primed-up response to challenge

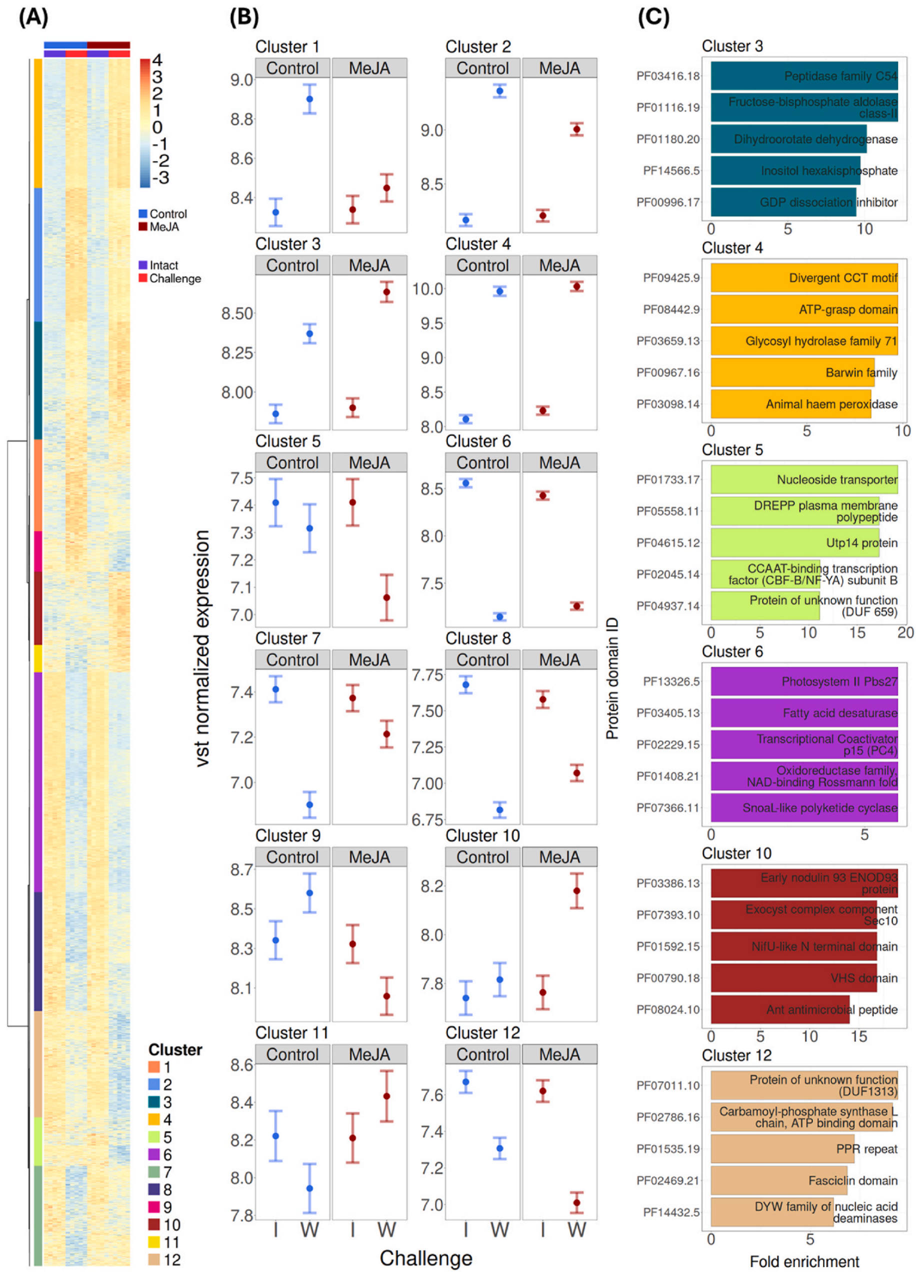

Fig. 7. Expression clustering and Pfams enrichment analysis of intact and challenged seedlings from treated seeds. **(A)** A heatmap of all genes which were significantly differentially expressed (adjusted *p*-value <0.05) in at least one of the five pairwise comparisons between treatment groups (Fig. S6). Treatment groups are indicated at the top of the heatmap (n = 5). Genes were grouped by expression pattern using Pearson sample-to-sample distances, and Ward's method clustering. The heatmap displays the outcome of the clustering with each row being a different gene and the expression values being represented by z-scores. The dendrogram was divided up into 12 clusters which are indicated by the colored boxes to the left of the heatmap. **(B)** The mean expression profiles for treatment groups within each cluster. The vst normalized mean expression is given for intact (I) and challenged (W) seedlings from control (blue) and MeJA (red) treated seeds. The error bars depict 95 % confidence intervals. **(C)** Five Pfams with the highest fold-enrichment in each cluster are displayed. See Supplemental Information Fig. S7 for the plots of all other clusters. See Supplemental Data Set 2 for all significantly overrepresented protein signatures. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

pattern, identified in cluster 3 or 10. Additionally, we chose genes involved in flavan-3-ol biosynthesis such as *leucoanthocyanidin reductase* (*LAR*) (*MA_10181288g0010*), *anthocyanidin synthase* (*ANS*) (*MA_10435917g0010*), and *dihydroflavonol 4-reductase* (*DFR*) (*MA_170159g0010*) due to the high concentration of catechin and proanthocyanin B1 in seedlings from MeJA-treated seeds. There was great variability between individual samples from the same seed treatment (Fig. S8). Thus, we could not see any significant differences in the chromatin accessibility between treatments.

4. Discussion

A recent meta-analysis showed that exogenous application of MeJA can significantly decrease the damage of pests and pathogens in *Picea* spp. and *Pinus* spp. (Huynh et al., 2024b). Although MeJA effectively enhances conifer resistance, its high cost and the intensive labor required for treating saplings and mature trees make it impractical for forestry protection (Huynh et al., 2024b). Additionally, this MeJA-induced resistance comes at the cost of reduced growth. However, this side effect should not preclude the use of MeJA, as it is typically transient and will diminish over time (Zas et al., 2014).

The meta-analysis also highlighted that enhancing conifer resistance through MeJA treatment of seeds or treatment with other chemicals has not been well-explored. Thus, we treated Norway spruce seeds with MeJA, PipA, Li, and Chi and evaluated their effects on seedling morphology, resistance, and defense responses. We observed reduced growth and increased resistance to *B. cinerea* in seedlings from MeJA-treated Norway spruce seeds, but not in seedlings from other seed treatments. To further understand the mechanisms underlying this phenomenon, we quantified phenolics, phytohormones, enzymatic activity, and differential transcript expression. MeJA seed treatment changed several defense responses in seedlings both before and after challenge. Our results indicated MeJA seed treatment induced seedling resistance via a multitude of defense responses, including cell wall reinforcement, increased oxidative stress protection, and increased production of antimicrobial compounds.

4.1. MeJA penetrates Norway spruce seed coats and affects emerging seedlings

The first evidence that MeJA seed treatment affects the emerged seedlings was the reduction in seedlings' roots and stem length. This result aligns with the commonly observed growth reduction in conifers after exogenous application of MeJA (Huynh et al., 2024b). Transcriptome analysis of intact seedlings also indicated that Pfams relating to growth hormones and photosynthesis factors were enriched among down-regulated transcripts in seedlings from MeJA-treated seeds compared to control ones (Supplementary Data Set 2). The down-regulation of these transcripts could contribute to the reduction in seedlings' length. Among the other seed treatments, only Chi had an observable positive effect on seedling emergence (Fig. S3). In addition to negative effects on growth, MeJA treatment of young plants has been shown to impair wound healing (Chen et al., 2023). Although we did not observe such effects in our study, these and other potential impacts on plant vigor and development warrant further investigation in long-term studies involving plants derived from MeJA-treated seeds.

4.2. Seed treatment with MeJA can enhance seedlings resistance to *Botrytis cinerea*

Previous works on seed treatment with MeJA or JA to conifer seeds did not give solid evidence of enhanced resistance in the emerged seedlings (Berglund et al., 2015; Vivas et al., 2012). However, enhanced resistance and tolerance to biotic and abiotic stresses after MeJA seed treatment have been reported in other species. For example, MeJA treatment of tomato seeds can hinder the performance of pests, like fruit worms (Paudel et al., 2014) or leaf miners (Strapasson et al., 2014), and increase resistance to the fungus *Fusarium oxysporum* (Król et al., 2015). Enhanced tolerance to abiotic stresses, such as osmotic stress, drought stress and heavy metal stress, was also observed in seedlings from MeJA-treated rice grains (*Oryza sativa*, L.), maize kernels (*Zea mays*) and pigeon peas (*Cajanus cajan*) (Tayyab et al., 2020; Kaushik et al., 2022; Sheteiwy et al., 2018). In this study, we observed enhanced resistance of seedlings from MeJA-treated seeds against *B. cinerea*. MeJA seed treatment significantly reduced the mortality of 23-day-old seedlings six days post-inoculation (Fig. 3). This is the first study showing that MeJA seed treatment effectively enhanced conifer seedling resistance (Huynh et al., 2024b).

All other chemicals could not significantly reduce the mortality of seedlings when infected with *B. cinerea* (Fig. 3). However, Chi seed treatment might have some more potential as it has the second most number of healthy seedlings after *B. cinerea* infection. Chi seed treatment was reported to effectively enhance resistance of the emerged seedlings in several plant species (Orzali et al., 2014; Agbodjato et al., 2016; Bhaskara Reddy et al., 1999; Rodríguez et al., 2007; Benhamou et al., 1994). The insignificant protection effect of Chi treatment might have been due to the low concentration of Chi used in this study. Studies on tomato, wheat, rice, and maize showed enhanced resistance after seed treatment used Chi concentrations ranging from 0.05 % to 1 % (Orzali et al., 2014; Agbodjato et al., 2016; Bhaskara Reddy et al., 1999; Rodríguez et al., 2007; Benhamou et al., 1994). These are much higher concentrations than what was used in our study, 0.0005 % and these seeds have a thinner seed coat than Norway spruce. Furthermore, chitosan's molecular weight, origin, and degree of deacetylation can affect its bioactivity and permeability (Dubin et al., 2021; Li et al., 2020). Thus, seed treatment with a higher concentration of lower molecular weight chitosan is needed to determine if chitosan can protect Norway spruce seedlings.

4.3. MeJA-induced resistance to *B. cinerea* is underpinned by a multitude of defense responses

B. cinerea is a necrotrophic pathogen that has multilayered strategies to infect and kill the plant host. It can secrete various cell wall degrading enzymes to soften the plant cell wall and make it more permeable (van Kan, 2005; Bi et al., 2023). Additionally, it induces accumulation of reactive oxygen species (ROS) in the host plant, which can lead to oxidative burst and triggering hypersensitive response and eventual cell death (AbuQamar et al., 2017; Bi et al., 2023). To assess how MeJA-treatment of Norway spruce seeds hinders the ability of *B. cinerea* to infect and kill seedlings, we quantified metabolites, enzymatic activity, and differential transcript expression in intact and challenged seedlings from MeJA-treated and control seeds.

As ROS accumulation and oxidative burst are the main triggers leading to HR and cell death, a plant must defend itself against *B. cinerea* by detoxifying and decreasing ROS accumulation. In our transcriptomic data, we saw several enriched Pfams related to oxidative stress response in clusters 3, 4, 10, and 11 (Supplementary Data Set 2). This phenomenon indicated an increase in oxidative stress protection in seedlings from MeJA-treated seeds, both before (cluster 4) and after (clusters 3, 10, and 11) challenge. For example, enriched Pfams in cluster 4 included: "animal haem peroxidase", "NADH: flavin oxidoreductase/NADH oxidase family", "glutathione peroxidase", "glycosyl oxidase N-terminus", and "GMC oxidoreductase". Similarly, the Pfams "thioredoxin-like", "glutathione S-transferase, C-terminal domain", "lipoxygenase", "catalase" and "caleosin related protein" were enriched in clusters 3, 10, and 11. These Pfams are important for the detoxification and reduction of ROS, which play an important role in plant resistance against *B. cinerea* (Hanano et al., 2023; Mata-Pérez and Spoel, 2019; De Gara et al., 2003; Gullner et al., 2017; Lehmann et al., 2015; Tyagi et al., 2022). MeJA seed treatment was also previously reported to increase protection from oxidative stress in pigeon peas (Kaushik et al., 2022). Additionally, MeJA treatment reduced *B. cinerea* infection in postharvest grapes by enhancing the activity of peroxidase and transcripts of catalase (Jiang et al., 2015). Taken together, this suggests that MeJA seed treatment could have increased seedling resistance by enhancing oxidative stress protection.

Another plant mechanism to cope with oxidative stress is the accumulation of phytoalexins and antioxidants, such as flavonoids and tannins (Elad, 1997). In our study, seedlings from MeJA-treated seeds had higher concentrations of the flavan-3-ols catechin and proanthocyanidin B1 in both intact and challenged tissues compared to controls (Fig. 5). Flavan-3-ols are effective antifungal compounds in Norway spruce (Hammerbacher et al., 2014, 2019, 2020). However, we did not see a consensus in the expression profiles of transcripts relating to flavan-3-ols biosynthesis. Flavan-3-ols are synthesized and stored in parenchyma polyphenolic cells (Krokene, 2015; Krekling et al., 2000; Krokene et al., 2008). Thus, these compounds may have been synthesized at an earlier time point and stored, which could contribute to the lack of correlation between transcriptomic and metabolite data.

We also observed a slight reduction of catechin and a significant reduction of proanthocyanidin B1 after the challenge, in seedlings from both control and MeJA-treated seeds. In a previous study, MeJA treatment of 2-year-old Norway spruce seedlings primed the induction of catechin and MeOH-soluble tannins in response to subsequent wounding (Huynh et al., 2024a). The reduction we observed could be explained by the polymerization of these molecules into condensed tannin under an enhanced oxidative process caused by the challenge (Hernández et al., 2006).

Changes in cell wall composition may have also contributed to increased resistance against *B. cinerea* in seedlings from MeJA-treated seeds. During infection, *B. cinerea* can secrete several enzymes to degrade and soften the cell wall, including pectin methyl esterase (PME), pectate lyase, and cellulase (Blanco-Ulate et al., 2016a; Elad, 1997). Interestingly, we observed Pfams related to these enzymes enriched in cluster 6 (Supplementary Data Set 2), indicating transcripts of these enzymes were downregulated in seedlings from MeJA-treated seeds even before the challenge. Increased expression and activity of PME has been associated with host susceptibility to *B. cinerea* (Bacete et al., 2018; Blanco-Ulate et al., 2016a), while silencing plant pectate lyase can increase tomato resistance to *B. cinerea* (Yang et al., 2017). Furthermore, inhibition of cellulose biosynthesis has been reported to signal cell wall damage and lead to the production of JA, lignin, and several resistance responses (Bacete et al., 2018). Thus, the decreased expression of these enzymes in intact seedlings from MeJA-treated seeds may contribute to enhanced cell wall integrity and later resistance to *B. cinerea*.

In contrast to transcripts in cluster 6, transcripts in cluster 3 showed a primed increase in expression in challenged seedlings from MeJA-treated seeds. We saw an enrichment of Pfam "glycosyl hydrolase family 1", which includes the enzyme β-glucosidase, in cluster 3. Some β-glucosidases are reported to have an important role in plant defense against pathogens by contributing to cell wall lignification and activating several chemical defense compounds (Morant et al., 2008; Dharmawardhana et al., 1995). Additionally, we observed the enrichment of the Pfams "plant O-methyltransferase dimerization domain" and "O-methyltransferase" in MeJA-primed cluster 10. These Pfams might be related to the phenylpropanoid pathway and lignin biosynthesis in spruce (Porth et al., 2011), which provided further evidence that MeJA seed treatment may prime cell wall lignification upon challenge.

Furthermore, we observed several transcripts of *callose synthase 9, 10, 11*, and 12 that followed a MeJA-primed increased expression pattern (Supplementary Data Set 1). Callose deposition is an important defense against phytopathogens (Wang et al., 2022). Callose deposition can thicken the cell wall and control the pore size of plasmodesmata by creating papillae (Li et al., 2023). This phenomenon can control the cell wall permeability, thus preventing the penetration and spread of the pathogen (Li et al., 2023). Even though an increase in *callose synthase* transcripts was primed, their associated Pfams were not enriched in the same clusters. However, the Pfams "sucrose synthase" and "UTP–glucose-1-phosphate uridylyltransferase" were enriched in cluster 3, primed-up response to challenge. Sucrose synthase and UTP–glucose-1-phosphate uridylyltransferase can both increase the amount of UDP-glucose, a precursor for callose biosynthesis (Verma and Hong, 2001; Wenqi, 2024; Kleczkowski, 1994). Thus, the response of these two Pfams after MeJA priming could have contributed to the primed increased response of different callose synthase transcripts.

Another factor known to regulate callose is ROS level. Changes in the ratio of ROS types and a high presence of singlet oxygen and superoxide radicals are associated with callose deposition (González-Bosch, 2018). Changes in ROS-related activity in seedlings from MeJA-treated seeds were implicated by the transcriptomic data. These changes could contribute to the regulation of callose biosynthesis. The actual regulation and induction of callose deposition and its role in Norway spruce defense require further research, including ROS quantification and callose localization.

MeJA-induced resistance to *B. cinerea* in Norway spruce seedlings could also be related to PR proteins. Several PR proteins Pfams, such as "Barwin family", "chitinase class I", "chitin recognition protein", "glycosyl hydrolase family 18", and "thaumatin family" (Supplementary Dataset 2), were enriched in cluster 4 (upregulated in MeJA-intact seedlings). This indicates that intact seedlings from MeJA-treated seeds had increased defensive potential prior to challenge. Previous studies in 2-year-old Norway spruce also reported that numerous PR genes were primed or prolongedly upregulated after MeJA treatment (Mageroy et al., 2020b; Wilkinson et al., 2022). PR proteins play important roles in plant defense against pathogens, including *B. cinerea*, by hydrolyzing fungal cell walls and signaling infection (Elad, 1997; Blanco-Ulate et al., 2016b; van Loon, 1997; van Loon et al., 2006). Thus, an increase in the expression of these PR proteins in intact seedlings from the MeJA treatment could have contributed to enhanced resistance to subsequent fungal infection.

4.4. *MeJA-induced resistance is regulated via hormonal signaling*

Hormonal signaling is important in regulating defense responses. In our study, we saw a significant reduction in ABA in seedlings from MeJA-treated seeds compared to control, in both intact and wounded seedlings (Fig. S4). The role of ABA in plant-pathogen interaction is dependent on the host, the pathogens, and the environmental conditions, and therefore is still under discussion (Mauch-Mani and Mauch, 2005; Blanco-Ulate et al., 2016b). On one hand, many studies have reported that ABA decreases plant resistance (AbuQamar et al., 2017; Elad, 1997; Flors et al., 2005; Mauch-Mani and Mauch, 2005). ABA-deficient mutants of tomato and *Arabidopsis thaliana* are more resistant to *B. cinerea* than wildtype (AbuQamar et al., 2017; Mauch-Mani and Mauch, 2005). On the other hand, ABA is required for callose deposition, which is important in tomato basal resistance and BABA-induced resistance against *B. cinerea* (Asselbergh and Höfte, 2007; Flors et al., 2005). Lower ABA concentrations in seedlings from MeJA-treated seeds observed in our study could indicate a negative role of ABA in Norway spruce defense against *B. cinerea*. However, a previous study on mature Norway spruce trees indicated that increased ABA signaling might contribute to increased resistance to *Heterobasidion annonsum* (Kovalchuk et al., 2019). Thus, it would be hasty to draw any conclusion about the role of ABA in Norway spruce resistance.

JA/ET signaling is also important in defense against pathogens. We observed the enrichment of Pfams relating to JA biosynthesis and regulation, such as "divergent CCT motif", "tify domain", and "NADH: flavin oxidoreductase/NADH oxidase family", and Pfams relating to ethylene biosynthesis and regulation, such as "ethylene insensitive 3" and "aminotransferase class I and II", in cluster 4 (Supplementary Data Set 2). This indicated an increase in the transcriptional activity of these defense hormones in intact seedlings from MeJA-treated seeds. These data strengthened the evidence of the synergistic relationship between JA and ET in plant defense (Li et al., 2019; Xu et al., 1994). Although we observed an increase in transcriptional activity of JA biosynthesis in intact seedlings from MeJA-treated seeds, we did not observe an increase in JA or JA-Ile. This phenomenon might be explained by the rapid negative feedback loop that regulates JA signaling. In this pathway, the JASMONATE ZIM-DOMAIN 3 (JAZ3) represses the transcription factor MYC2, which regulates JA-response genes. In the presence of JA-Ile, JAZ3 is recruited to CORONATINE INSENSITIVE 1 (COI1), leading to its degradation by the 26S proteasome (Chini et al., 2007). The freed MYC2 can then activate JA-response genes, including JAZ3, which again binds to MYC2 suppressing further signaling (Chini et al., 2007). The rapid turnover of JA-Ile may have prevented the detection of elevated JA-Ile at the timepoint evaluated in our study. However, we did observe a significant increase in JA-Ile degradation products, 12-OH-JA-Ile and 12-COOH-JA-Ile (Fig. 4). An increase in JA-Ile breakdown products could indicate a previous rise in JA-Ile, as they are produced from JA-Ile via the ω-oxidation pathway (Koo and Howe, 2012). Additionally, overexpression of the JA-Ile producing gene, *JASMONATE RESISTANT 1* (*JAR1*), resulted in a moderate increase in JA-Ile, but a substantial increase in 12-OH-JA-Ile and 12-COOH-JA-Ile (Mahmud et al., 2022). These products can modulate the JA signaling and biosynthesis (Jimenez-Aleman et al., 2019). It is also hypothesized that these breakdown products are recycled via esterification to CoA and β-oxidization in the peroxisome (Koo and Howe, 2012).

Plant defenses can also be regulated at the epigenetic level. Multiple studies have demonstrated that epigenetic mechanisms play a critical role in regulating defense priming (Conrath, 2011; Mauch-Mani et al., 2017; Pastor et al., 2013; Wilkinson et al., 2019). In our study, several Pfams related to chromatin modification were enriched in MeJA-altered response to challenge clusters 9 and 12 (Fig. 7 and Supplementary Data Set 2). This indicates down-regulation of transcriptional activity relating to these Pfams in challenged seedlings from MeJA-treated seeds than control one. Previous studies on MeJA-induced resistance in Norway spruce also observed several epigenetic regulators, including chromatin modification, to be differentially expressed after MeJA or after wounding in MeJA-treated plants (Mageroy et al., 2020b; Wilkinson et al., 2022). To further explore this we used FAIRE-qPCR to examine the openness of some MeJA-primed transcripts. Our data showed great variability between individual samples, leading to a non-conclusive pattern for each target gene. The variability between individual samples might stem from the imperfect method, as the protocol was adapted from one used for Arabidopsis (Baum et al., 2020). Individual samples can also behave very differently, even with the same genotypes (Wilkinson et al., 2023). The method should be revised, or a higher resolution method for epigenetic studies should be used in future research.

Overall, our findings showed MeJA seed treatment can effectively protect Norway spruce seedlings against *B. cinerea* through a multitude of defense mechanisms. Effective seed treatment can drastically reduce the chemical and labor costs of applying MeJA, and strengthen its potential usage in forest protection. However, research on MeJA-induced resistance in conifers has predominantly focused on the immediate benefits to the tree itself, often neglecting the potential interactions with other biotic and abiotic factors. To enhance the practicality of MeJA seed treatment in forest protection, future studies should prioritize investigating the durability of MeJA-IR over extended periods and its ecological impact.

CRediT authorship contribution statement

Ngan Bao Huynh: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Axel Schmidt:** Writing – review & editing, Supervision, Conceptualization. **Taina Pennanen:** Writing – review & editing, Resources. **Jonathan Gershenzon:** Writing – review & editing, Supervision, Resources, Conceptualization. **Melissa H. Mageroy:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Funding

This work was funded by the Norwegian Research Council (project no. 324129).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank Michael Reichelt for his assistance with the metabolite analysis. We thank Veronica Quynh Thi Phan for her assistance with the laboratory work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2025.110497.

Data availability

Data will be made available on request.

References

Abuqamar, S., Moustafa, K., Tran, L.S., 2017. Mechanisms and strategies of plant defense against *botrytis cinerea*. Crit. Rev. Biotechnol. 37, 262–274. https://doi.org/10.1080/07388551.2016.1271767.

Adamczyk, B., Adamczyk, S., Kitunen, V., Hytönen, T., Mäkipää, R., Pennanen, T., 2022. Variation in the chemical quality of woody supplements for nursery growing media affects growth of tree seedlings. N. For. 53, 797–810. https://doi.org/10.1007/s11056-021-09887-6.

Agbodjato, N.A., Noumavo, P.A., Adjanohoun, A., Agbessi, L., Baba-Moussa, L., 2016. Synergistic effects of plant growth promoting rhizobacteria and chitosan on in vitro seeds germination, greenhouse growth, and nutrient uptake of maize (*Zea mays* L.). Biotechnology Research International, 7830182. https://doi.org/10.1155/2016/7830182, 2016.

Aranaz, I., Alcántara, A.R., Civera, M.C., Arias, C., Elorza, B., Heras Caballero, A., Acosta, N., 2021. Chitosan: an overview of its properties and applications. Polymers 13, 3256. https://doi.org/10.3390/polym13193256.

Asselbergh, B., Höfte, M., 2007. Basal tomato defences to *Botrytis cinerea* include abscisic acid-dependent callose formation. Physiol. Mol. Plant Pathol. 71, 33–40. https://doi.org/10.1016/j.pmpp.2007.10.001.

Bacete, L., Mélida, H., Miedes, E., Molina, A., 2018. Plant cell wall-mediated immunity: cell wall changes trigger disease resistance responses. Plant J. 93, 614–636. https://doi.org/10.1111/tpj.13807.

Baum, S., Reimer-Michalski, E.M., Jaskiewicz, M.R., Conrath, U., 2020. Formaldehyde-assisted isolation of regulatory DNA elements from *Arabidopsis* leaves. Nat. Protoc. 15, 713–733. https://doi.org/10.1038/s41596-019-0277-9.

Benhamou, N., Lafontaine, P.J., Nicole, M., 1994. Induction of systemic resistance to fusarium crown and root-rot in tomato plants by seed treatment with Chitosan. Phytopathology 84, 1432–1444. https://doi.org/10.1094/Phyto-84-1432.

Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. Roy. Stat. Soc. B 57, 289–300.

Berglund, T., Lindström, A., Aghelpasand, H., Stattin, E., Ohlsson, A.B., 2015. Protection of spruce seedlings against pine weevil attacks by treatment of seeds or seedlings with nicotinamide, nicotinic acid and Jasmonic acid. Forestry: Int. J. Financ. Res. 89, 127–135. https://doi.org/10.1093/forestry/cpv040.

Bernsdorff, F., Döring, A.-C., Gruner, K., Schuck, S., Bräutigam, A., Zeier, J., 2015. Pipecolic acid orchestrates plant systemic acquired resistance and defense priming via salicylic acid-dependent and -Independent pathways. Plant Cell 28, 102–129. https://doi.org/10.1105/tpc.15.00496.

Bhaskara Reddy, M.V., Arul, J., Angers, P., Couture, L., 1999. Chitosan treatment of wheat seeds induces resistance to Fusarium graminearum and improves seed quality. J. Agric. Food Chem. 47, 1208–1216. https://doi.org/10.1021/jf981225k.

Bhavanam, S., Stout, M., 2021. Seed treatment with jasmonic acid and methyl jasmonate induces resistance to insects but reduces plant growth and yield in rice, *Oryza sativa*. Front. Plant Sci. 12, 691768. https://doi.org/10.3389/fpls.2021.691768.

Bi, K., Liang, Y., Mengiste, T., Sharon, A., 2023. Killing softly: a roadmap of *Botrytis cinerea* pathogenicity. Trends Plant Sci. 28, 211–222. https://doi.org/10.1016/j.tplants.2022.08.024.

Blanco-Ulate, B., Labavitch, J.M., Vincenti, E., Powell, A.L.T., Cantu, D., 2016a. Hitting the Wall: plant cell walls during *Botrytis cinerea* infections. In: FILLINGER, S., ELAD, Y. (Eds.), Botrytis – the Fungus, the Pathogen and its Management in Agricultural Systems. Springer International Publishing, Cham. https://doi.org/10.1007/978-3-319-23371-0_18.

Blanco-Ulate, B., Vincenti, E., Cantu, D., Powell, A.L.T., 2016b. Ripening of tomato fruit and susceptibility to *Botrytis cinerea*. In: FILLINGER, S., ELAD, Y. (Eds.), Botrytis – the Fungus, the Pathogen and its Management in Agricultural Systems. Springer International Publishing, Cham. https://doi.org/10.1007/978-3-319-23371-0_19.

Buzi, A., Chilosi, G., DE Sillo, D., Magro, P., 2004. Induction of resistance in melon to *Didymella bryoniae* and *Sclerotinia sclerotiorum* by seed treatments with Acibenzolar-S-methyl and Methyl Jasmonate but not with salicylic acid. J. Phytopathol. 152, 34–42. https://doi.org/10.1046/j.1439-0434.2003.00798.x.

Caudullo, G., Tinner, W., DE Rigo, D., 2016. *Picea Abies* in Europe: Distribution, Habitat, Usage and Threats.

Chen, Y., Björkman, C., Bylund, H., Björklund, N., Högberg, K.-A., Puentes, A., 2023. Healing of bark wounds in Norway spruce seedlings can be negatively affected by treatment with methyl jasmonate. Trees (Berl.) 37, 1369–1384. https://doi.org/10.1007/s00468-023-02428-y.

Chini, A., Fonseca, S., Fernández, G., Adie, B., Chico, J.M., Lorenzo, O., García-Casado, G., López-Vidriero, I., Lozano, F.M., Ponce, M.R., Micol, J.L., Solano, R., 2007. The JAZ family of repressors is the missing link in jasmonate signalling. Nature 448, 666. https://doi.org/10.1038/nature06006.

Christensen, R.H.B., 2022. ordinal—Regression models for ordinal data. R package version 11–16, 2022.Available: https://CRAN.R-project.org/package=ordinal.

Christiansen, E., Krokene, P., Berryman, A.A., Franceschi, V.R., Krekling, T., Lieutier, F., Lönneborg, A., Solheim, H., 1999. Mechanical injury and fungal infection induce acquired resistance in Norway spruce. Tree Physiol. 19, 399–403. https://doi.org/10.1093/treephys/19.6.399.

Conrath, U., 2011. Molecular aspects of defence priming. Trends Plant Sci. 16, 524–531. https://doi.org/10.1016/j.tplants.2011.06.004.

De Gara, L., De Pinto, M.C., Tommasi, F., 2003. The antioxidant systems vis-à-vis reactive oxygen species during plant–pathogen interaction. Plant Physiol. Biochem. 41, 863–870. https://doi.org/10.1016/S0981-9428(03)00135-9.

De Matos Simoes, R., Emmert-Streib, F., 2016. bc3net: gene regulatory network inference with Bc3net. R package version, 1.

Dharmawardhana, D.P., Ellis, B.E., Carlson, J.E., 1995. A [beta]-Glucosidase from Lodgepole Pine Xylem Specific for the Lignin Precursor coniferin. Plant Physiology 107, 331–339. https://doi.org/10.1104/pp.107.2.331.

Dubin, A., Likhanov, A., Klyachenko, O., Subin, A., Kluvadenko, A., 2021. Effect of chitosan formulations of different biological origin on tobacco (*Nicotiana tabacum* l.) PR-genes expression. J. Microbiol. Biotechnol. Food Sci. 9, 1141–1144. https://office2.jmbfs.org/index.php/JMBFS/article/view/4488.

El Hadrami, A., Adam, L.R., EL Hadrami, I., Daayf, F., 2010. Chitosan in plant protection. Mar. Drugs 8, 968–987. https://doi.org/10.3390/md8040968.

Elad, Y., 1997. Responses of plants to infection by *Botrytis cinerea* and novel means involved in reducing their susceptibility to infection. Biol. Rev. 72, 381–422. https://doi.org/10.1017/S0006323197005057.

Erbilgin, N., Krokene, P., Christiansen, E., Zeneli, G., Gershenzon, J., 2006. Exogenous application of methyl jasmonate elicits defenses in Norway spruce (*Picea abies*) and reduces host colonization by the bark beetle *Ips typographus*. Oecologia 148, 426–436. https://doi.org/10.1007/s00442-006-0394-3.

Flors, V., Ton, J., Jakab, G., Mauch-Mani, B., 2005. Abscisic acid and callose: team players in defence against pathogens? J. Phytopathol. 153, 377–383. https://doi.org/10.1111/j.1439-0434.2005.00987.x.

Fox, J., Weisberg, S., 2019. An {R} Companion to Applied Regression. Available: https://socialsciences.mcmaster.ca/jfox/Books/Companion/.

Gaujoux, R., Seoighe, C., 2010. A flexible R package for nonnegative matrix factorization. BMC Bioinf. 11, 1–9.

Giovannini, L., Pagliarani, C., Cañizares, E., Sillo, F., Chitarra, W., DE Rose, S., Zampieri, E., Ioannou, A., Spanos, A., Vita, F., González-Guzmán, M., Fotopoulos, V., Arbona, V., Balestrini, R., 2024. Mycorrhization and chemical seed priming boost tomato stress tolerance by changing primary and defence metabolic pathways. J. Exp. Bot. https://doi.org/10.1093/jxb/erae457.

González-Bosch, C., 2018. Priming plant resistance by activation of redox-sensitive genes. Free Radic. Biol. Med. 122, 171–180. https://doi.org/10.1016/j.freeradbiomed.2017.12.028.

Gullner, G., Zechmann, B., Künstler, A., Király, L., 2017. The signaling roles of glutathione in plant disease resistance. In: HOSSAIN, M.A., MOSTOFA, M.G., DIAZ-VIVANCOS, P., BURRITT, D.J., FUJITA, M., TRAN, L.-S.P. (Eds.), Glutathione in Plant Growth, Development, and Stress Tolerance. Springer International Publishing, Cham. https://doi.org/10.1007/978-3-319-66682-2_15.

Hammerbacher, A., Kandasamy, D., Ullah, C., Schmidt, A., Wright, L.P., Gershenzon, J., 2019. Flavanone-3-hydroxylase plays an important role in the biosynthesis of spruce phenolic defenses against bark beetles and their fungal associates. Front. Plant Sci. 10, 208. https://doi.org/10.3389/fpls.2019.00208.

Hammerbacher, A., Paetz, C., Wright, L.P., Fischer, T.C., Bohlmann, J., Davis, A.J., Fenning, T.M., Gershenzon, J., Schmidt, A., 2014. Flavan-3-ols in Norway spruce: biosynthesis, accumulation, and function in response to attack by the bark beetle-associated fungus *Ceratocystis polonica*. Plant Physiology 164, 2107. https://doi.org/10.1104/pp.113.232389.

Hammerbacher, A., Wright, L.P., Gershenzon, J., 2020. Spruce phenolics: biosynthesis and ecological functions. In: PORTH, I.M., DE LA TORRE, A.R. (Eds.), The Spruce Genome. Springer International Publishing, Cham. https://doi.org/10.1007/978-3-030-21001-4_12.

Hanano, A., Blée, E., Murphy, D.J., 2023. Caleosin/peroxygenases: multifunctional proteins in plants. Ann. Bot. 131, 387–409. https://doi.org/10.1093/aob/mcad001.

Hannerz, M., Ekström, H., 2023. Nordic Forest Statistics 2023 - Resources, Industry, Trade, Prices, Environment and Climate.

Hernández, I., Alegre, L., Munné-Bosch, S., 2006. Enhanced oxidation of flavan-3-ols and proanthocyanidin accumulation in water-stressed tea plants. Phytochemistry 67, 1120–1126. https://doi.org/10.1016/j.phytochem.2006.04.002.

Herve, M., 2023. RVAideMemoire: testing and plotting procedures for biostatistics. R package version 0.9-83. Available: https://CRAN.R-project.org/package=RVAideMemoire.

Heyer, M., Reichelt, M., Mithöfer, A., 2018. A holistic approach to analyze systemic jasmonate accumulation in individual leaves of arabidopsis rosettes upon wounding. Front. Plant Sci. 9. https://doi.org/10.3389/fpls.2018.01569, 2018.

Huang, J., Reichelt, M., Chowdhury, S., Hammerbacher, A., Hartmann, H., 2017. Increasing carbon availability stimulates growth and secondary metabolites via modulation of phytohormones in winter wheat. J. Exp. Bot. 68, 1251–1263. https://doi.org/10.1093/jxb/erx008.

Huynh, N.B., Krokene, P., Nybakken, L., Česna, V., Mageroy, M.H., 2024a. β-aminobutyric acid does not induce defenses or increase Norway spruce resistance to the bluestain fungus *Grosmannia penicillata*. Physiol. Plantarum 176, e70009. https://doi.org/10.1111/ppl.70009.

Huynh, N.B., Krokene, P., Puentes, A., Mageroy, M.H., 2024b. Over 20 years of treating conifers with methyl jasmonate: Meta-analysis of effects on growth and resistance. For. Ecol. Manag. 561, 121893. https://doi.org/10.1016/j.foreco.2024.121893.

Jiang, L., Jin, P., Wang, L., Yu, X., Wang, H., Zheng, Y., 2015. Methyl jasmonate primes defense responses against *Botrytis cinerea* and reduces disease development in harvested table grapes. Sci. Hortic. 192, 218–223. https://doi.org/10.1016/j.scienta.2015.06.015.

Jimenez-Aleman, G.H., Almeida-Trapp, M., Fernández-Barbero, G., Gimenez-Ibanez, S., Reichelt, M., Vadassery, J., Mithöfer, A., Caballero, J., Boland, W., Solano, R., 2019. Omega hydroxylated JA-Ile is an endogenous bioactive jasmonate that signals through the canonical jasmonate signaling pathway. Biochim. Biophys. Acta Mol. Cell Biol. Lipids 1864, 158520. https://doi.org/10.1016/j.bbalip.2019.158520.

Kaushik, S., Sharma, P., Kaur, G., Singh, A.K., AL-Misned, F.A., Shafik, H.M., Sirhindi, G., 2022. Seed priming with methyl jasmonate mitigates copper and cadmium toxicity by modifying biochemical attributes and antioxidants in *Cajanus cajan*. Saudi J. Biol. Sci. 29, 721–729. https://doi.org/10.1016/j.sjbs.2021.12.014.

Kay, M., Elkin, L., Higgins, J., Wobbrock, J., 2021. ARTool: aligned rank transform for nonparametric factorial ANOVAs. R package version 0.11.1. Available: https://github.com/mjskay/ARTool.

Kleczkowski, L., 1994. Glucose activation and metabolism through UDP-glucose pyrophosphorylase in plants. Phytochemistry 37, 1507–1515. https://doi.org/10.1016/s0031-9422(00)89568-0.

Kolde, R., 2019. Pheatmap: prettyheatmaps. Available: https://cran.r-project.org/package=pheatmap.

Koo, A.J., Howe, G.A., 2012. Catabolism and deactivation of the lipid-derived hormone jasmonoyl-isoleucine. Front. Plant Sci. 3, 19. https://doi.org/10.3389/fpls.2012.00019.

Kovalchuk, A., Zeng, Z., Ghimire, R.P., Kivimäenpää, M., Raffaello, T., Liu, M., Mukrimin, M., Kasanen, R., Sun, H., Julkunen-Tiitto, R., Holopainen, J.K., Asiegbu, F.O., 2019. Dual RNA-seq analysis provides new insights into interactions between Norway spruce and necrotrophic pathogen *Heterobasidion annosum* s.l. BMC Plant Biol. 19, 2. https://doi.org/10.1186/s12870-018-1602-0.

Krekling, T., Franceschi, V.R., Berryman, A.A., Christiansen, E., 2000. The structure and development of polyphenolic parenchyma cells in Norway spruce (*Picea abies*) bark. Flora 195, 354–369. https://doi.org/10.1016/S0367-2530(17)30994-5.

Krokene, P., 2015. Chapter 5 - conifer defense and resistance to bark beetles. In: VEGA, F. E., HOFSTETTER, R.W. (Eds.), Bark Beetles. Academic Press, San Diego. https://doi.org/10.1016/B978-0-12-417156-5.00005-8.

Krokene, P., Nagy, N.E., Krekling, T., 2008. Traumatic resin ducts and polyphenolic parenchyma cells in conifers. In: SCHALLER, A. (Ed.), Induced Plant Resistance to Herbivory. Springer Netherlands, Dordrecht. https://doi.org/10.1007/978-1-4020-8182-8_7.

Krokene, P., Solheim, H., Långström, B., 2000. Fungal infection and mechanical wounding induce disease resistance in Scots pine. Eur. J. Plant Pathol. 106, 537–541. https://doi.org/10.1023/A:1008776002248.

Król, P., Igielski, R., Pollmann, S., Kępczyńska, E., 2015. Priming of seeds with methyl jasmonate induced resistance to hemi-biotroph *Fusarium oxysporum* f.sp. lycopersici in tomato via 12-oxo-phytodienoic acid, salicylic acid, and flavonol accumulation. J. Plant Physiol. 179, 122–132. https://doi.org/10.1016/j.jplph.2015.01.018.

Langmead, B., Salzberg, S.L., 2012. Fast gapped-read alignment with Bowtie 2. Nat. Methods 9, 357–359.

Lehmann, S., Serrano, M., L'Haridon, F., Tjamos, S.E., Metraux, J.-P., 2015. Reactive oxygen species and plant resistance to fungal pathogens. Phytochemistry 112, 54–62. https://doi.org/10.1016/j.phytochem.2014.08.027.

Lenth, R.V., 2023. Emmeans: estimated marginal means, aka least-squares means. R package version 1.8.9. Available: https://CRAN.R-project.org/package=emmeans.

Li, K., Xing, R., Liu, S., Li, P., 2020. Chitin and chitosan fragments responsible for plant elicitor and growth stimulator. J. Agric. Food Chem. 68, 12203–12211. https://doi.org/10.1021/acs.jafc.0c05316.

Li, N., Han, X., Feng, D., Yuan, D., Huang, L.-J., 2019. Signaling crosstalk between salicylic acid and Ethylene/Jasmonate in plant defense: do we understand what they are whispering? Int. J. Mol. Sci. 20, 671. https://doi.org/10.3390/ijms20030671.

Li, N., Lin, Z., Yu, P., Zeng, Y., DU, S., Huang, L.J., 2023. The multifarious role of callose and callose synthase in plant development and environment interactions. Front. Plant Sci. 14, 1183402. https://doi.org/10.3389/fpls.2023.1183402.

Liao, Y., Smyth, G.K., Shi, W., 2014. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics 30, 923–930.

Liao, Y., Smyth, G.K., Shi, W., 2019. The R package Rsubread is easier, faster, cheaper and better for alignment and quantification of RNA sequencing reads. Nucleic acids research 47 e47-e47.

Lilja, A., Poteri, M., Petäistö, R.-L., Rikala, R., Kurkela, T., Kasanen, R., 2010. Fungal diseases in Forest nurseries in Finland. Silva Fenn. 44, 525–545. https://doi.org/10.14214/sf.147.

Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15, 550.

Mageroy, M.H., Christiansen, E., Långström, B., Borg-Karlson, A.-K., Solheim, H., Björklund, N., Zhao, T., Schmidt, A., Fossdal, C.G., Krokene, P., 2020a. Priming of inducible defenses protects Norway spruce against tree-killing bark beetles. Plant Cell Environ. 43, 420–430. https://doi.org/10.1111/pce.13661.

Mageroy, M.H., Wilkinson, S.W., Tengs, T., Cross, H., Almvik, M., Pétriacq, P., Vivian-Smith, A., Zhao, T., Fossdal, C.G., Krokene, P., 2020b. Molecular underpinnings of methyl jasmonate-induced resistance in Norway spruce. Plant Cell Environ. 43, 1827–1843. https://doi.org/10.1111/pce.13774.

Mahmud, S., Ullah, C., Kortz, A., Bhattacharyya, S., Yu, P., Gershenzon, J., Vothknecht, U.C., 2022. Constitutive expression of JASMONATE RESISTANT 1 induces molecular changes that prime the plants to better withstand drought. Plant Cell Environ. 45, 2906–2922. https://doi.org/10.1111/pce.14402.

Malerba, M., Cerana, R., 2016. Chitosan effects on plant systems. Int. J. Mol. Sci. 17, 996. https://doi.org/10.3390/ijms17070996.

Martinez-Medina, A., Flors, V., Heil, M., Mauch-Mani, B., Pieterse, C.M., Pozo, M.J., Ton, J., VAN Dam, N.M., Conrath, U., 2016. Recognizing plant defense priming. Trends Plant Sci. 21, 818–822. https://doi.org/10.1016/j.tplants.2016.07.009.

Mata-Pérez, C., Spoel, S.H., 2019. Thioredoxin-mediated redox signalling in plant immunity. Plant Sci. 279, 27–33. https://doi.org/10.1016/j.plantsci.2018.05.001.

Mauch-Mani, B., Baccelli, I., Luna, E., Flors, V., 2017. Defense priming: an adaptive part of induced resistance. Annu. Rev. Plant Biol. 68, 485–512. https://doi.org/10.1146/annurev-arplant-042916-041132.

Mauch-Mani, B., Mauch, F., 2005. The role of abscisic acid in plant–pathogen interactions. Curr. Opin. Plant Biol. 8, 409–414. https://doi.org/10.1016/j.pbi.2005.05.015.

Morant, A.V., Jørgensen, K., Jørgensen, C., Paquette, S.M., Sánchez-Pérez, R., Møller, B. L., Bak, S., 2008. β-Glucosidases as detonators of plant chemical defense. Phytochemistry 69, 1795–1813. https://doi.org/10.1016/j.phytochem.2008.03.006.

Nielsen, K.A.G., Skårn, M.N., Talgø, V., Pettersson, M., Fløistad, I.S., Strømeng, G.M., Brurberg, M.B., Stensvand, A., 2024. Fungicide-Resistant *Botrytis* in Forest nurseries May impact disease control in Norway spruce. Plant Dis. 108, 139–148. https://doi.org/10.1094/pdis-01-23-0037-re.

Orzali, L., Forni, C., Riccioni, L., 2014. Effect of chitosan seed treatment as elicitor of resistance to *Fusarium graminearum* in wheat. Seed Sci. Technol. 42. https://doi.org/10.15258/sst.2014.42.2.03.

Pastor, V., Luna, E., Mauch-Mani, B., Ton, J., Flors, V., 2013. Primed plants do not forget. Environ. Exp. Bot. 94, 46–56. https://doi.org/10.1016/j.envexpbot.2012.02.013.

Paudel, S., Rajotte, E.G., Felton, G.W., 2014. Benefits and costs of tomato seed treatment with plant defense elicitors for insect resistance. Arthropod-Plant Interactions 8, 539–545. https://doi.org/10.1007/s11829-014-9335-y.

Pazarlar, S., Sanver, U., Cetinkaya, N., 2021. Exogenous pipecolic acid modulates plant defence responses against *Podosphaera xanthii* and *Pseudomonas syringae* pv. lachrymans in cucumber (*Cucumis sativus* L.). Plant Biol. 23, 473–484. https://doi.org/10.1111/plb.13243.

Pedersen, T.L., 2024. Patchwork: the composer of plots. R package. Available:, version 1.3.0. https://CRAN.R-project.org/package=patchwork.

Petäistö, R.-L., 2006. *Botrytis cinerea* and Norway spruce seedlings in cold storage. Balt. For. 12.

Petäistö, R.-L., Heiskanen, J., Pulkkinen, A., 2004. Susceptibility of Norway spruce seedlings to grey Mould in the greenhouse during the first growing season. Scand. J. For. Res. 19, 30–37. https://doi.org/10.1080/02827580310019581.

Porth, I., Hamberger, B., White, R., Ritland, K., 2011. Defense mechanisms against herbivory in *Picea*: sequence evolution and expression regulation of gene family members in the phenylpropanoid pathway. BMC Genom. 12, 608. https://doi.org/10.1186/1471-2164-12-608.

Ražná, K., Nôžková, J., Vargaová, A., Harenčár, Ľ., Bjelková, M., 2021. Biological functions of lignans in plants. Agriculture (Pol'nohospodárstvo) 67, 155–165. https://doi.org/10.2478/agri-2021-0014.

Riseh, R.S., Hassanisaadi, M., Vatankhah, M., Babaki, S.A., Barka, E.A., 2022. Chitosan as a potential natural compound to manage plant diseases. Int. J. Biol. Macromol. 220, 998–1009. https://doi.org/10.1016/j.ijbiomac.2022.08.109.

Rodríguez, A.T., Ramírez, M.A., Cárdenas, R.M., Hernández, A.N., Velázquez, M.G., Bautista, S., 2007. Induction of defense response of *Oryza sativa* L. against *Pyricularia grisea* (Cooke) Sacc. by treating seeds with chitosan and hydrolyzed chitosan. Pestic. Biochem. Physiol. 89, 206–215. https://doi.org/10.1016/j.pestbp.2007.06.007.

Sheteiwy, M.S., Gong, D., Gao, Y., Pan, R., Hu, J., Guan, Y., 2018. Priming with methyl jasmonate alleviates polyethylene glycol-induced osmotic stress in rice seeds by regulating the seed metabolic profile. Environ. Exp. Bot. 153, 236–248. https://doi.org/10.1016/j.envexpbot.2018.06.001.

Solvin, T., Fløistad, I.S., Proschowsky, G.F., Leisgaard, T., Ylioja, T., Tynkkynen, M., Skulason, B., Bjorgvinsson, H.S., Stokke, E., Myhre, M.F., Edvardsson, E., Uggla, C., 2023. Statistics: Forest Seeds and Plants in the Nordic Region, vol.2023. NordGen Publication Series. https://doi.org/10.53780/QOUB7866, 02.

Strapasson, P., Pinto-Zevallos, D.M., Paudel, S., Rajotte, E.G., Felton, G.W., Zarbin, P.H. G., 2014. Enhancing plant resistance at the seed stage: low concentrations of methyl Jasmonate reduce the performance of the leaf miner *Tuta absoluta* but do not alter the behavior of its predator *Chrysoperla externa*. J. Chem. Ecol. 40, 1090–1098. https://doi.org/10.1007/s10886-014-0503-4.

Tayyab, N., Naz, R., Yasmin, H., Nosheen, A., Keyani, R., Sajjad, M., Hassan, M.N., Roberts, T.H., 2020. Combined seed and foliar pre-treatments with exogenous methyl jasmonate and salicylic acid mitigate drought-induced stress in maize. PLoS One 15, e0232269. https://doi.org/10.1371/journal.pone.0232269.

Tyagi, S., Shah, A., Karthik, K., Rathinam, M., Rai, V., Chaudhary, N., Sreevathsa, R., 2022. Reactive oxygen species in plants: an invincible fulcrum for biotic stress mitigation. Appl. Microbiol. Biotechnol. 106, 5945–5955. https://doi.org/10.1007/s00253-022-12138-z.

Van Kan, J.A.L., 2005. Infection Strategies of *Botrytis Cinerea*, pp. 77–90. https://doi.org/10.17660/ActaHortic.2005.669.9.

Van Loon, L.C., 1997. Induced resistance in plants and the role of pathogenesis-related proteins. Eur. J. Plant Pathol. 103, 753–765. https://doi.org/10.1023/A:1008638109140.

Van Loon, L.C., Rep, M., Pieterse, C.M.J., 2006. Significance of inducible defense-related proteins in infected plants. Annu. Rev. Phytopathol. 44, 135–162. https://doi.org/10.1146/annurev.phyto.44.070505.143425.

Verma, D.P., Hong, Z., 2001. Plant callose synthase complexes. Plant Mol. Biol. 47, 693–701. https://doi.org/10.1023/a:1013679111111.

Vivas, M., Martín, J., Gil, L., Solla, A., 2012. Evaluating methyl jasmonate for induction of resistance to *Fusarium oxysporum, F. circinatum* and *Ophiostoma novo-ulmi*. Forest Systems 21, 289–299. https://doi.org/10.5424/fs/2012212-02172.

Vogel-Adghough, D., Stahl, E., Návarová, H., Zeier, J., 2013. Pipecolic acid enhances resistance to bacterial infection and primes salicylic acid and nicotine accumulation in tobacco. Plant Signal. Behav. 8, e26366. https://doi.org/10.4161/psb.26366.

Wang, B., Andargie, M., Fang, R., 2022. The function and biosynthesis of callose in high plants. Heliyon 8, e09248. https://doi.org/10.1016/j.heliyon.2022.e09248.

Wenqi, Z., 2024. An overview of UDP-Glucose pyrophosphorylase in plants. Tropical Plant Biology 18, 10. https://doi.org/10.1007/s12042-024-09379-9.

Wickham, H., 2016. ggplot2: elegant graphics for data analysis. Available: https://ggplot2.tidyverse.org.

Wilkinson, S., Dalen, L., Skrautvol, T., Ton, J., Krokene, P., Mageroy, M., 2022. Transcriptomic changes during the establishment of long-term methyl jasmonate-induced resistance in Norway spruce. Plant Cell Environ. 45. https://doi.org/10.1111/pce.14320.

Wilkinson, S.W., Hannan Parker, A., Muench, A., Wilson, R.S., Hooshmand, K., Henderson, M.A., Moffat, E.K., Rocha, P.S.C.F., Hipperson, H., Stassen, J.H.M., López Sánchez, A., Fomsgaard, I.S., Krokene, P., Mageroy, M.H., Ton, J., 2023. Long-lasting memory of jasmonic acid-dependent immunity requires DNA demethylation and ARGONAUTE1. Nat. Plants 9, 81–95. https://doi.org/10.1038/s41477-022-01313-9.

Wilkinson, S.W., Magerøy, M.H., López Sánchez, A., Smith, L.M., Furci, L., Cotton, T.A., Krokene, P., Ton, J., 2019. Surviving in a hostile world: plant strategies to resist pests and diseases. Annu. Rev. Phytopathol. 57, 505–529. https://doi.org/10.1146/annurev-phyto-082718-095959.

Worrall, D., Holroyd, G.H., Moore, J.P., Glowacz, M., Croft, P., Taylor, J.E., Paul, N.D., Roberts, M.R., 2012. Treating seeds with activators of plant defence generates long-lasting priming of resistance to pests and pathogens. New Phytol. 193, 770–778. https://doi.org/10.1111/j.1469-8137.2011.03987.x.

Xu, Y., Chang, P., Liu, D., Narasimhan, M.L., Raghothama, K.G., Hasegawa, P.M., Bressan, R.A., 1994. Plant defense genes are synergistically induced by ethylene and methyl Jasmonate. Plant Cell 6, 1077–1085. https://doi.org/10.1105/tpc.6.8.1077.

Yang, L., Huang, W., Xiong, F., Xian, Z., Su, D., Ren, M., Li, Z., 2017. Silencing of Sl, which encodes a pectate lyase in tomato, confers enhanced fruit firmness, prolonged shelf-life and reduced susceptibility to grey mould. Plant Biotechnol. J. 15, 1544–1555. https://doi.org/10.1111/pbi.12737.

Zas, R., Björklund, N., Nordlander, G., Cendán, C., Hellqvist, C., Sampedro, L., 2014. Exploiting jasmonate-induced responses for field protection of conifer seedlings against a major forest pest, *Hylobius abietis*. For. Ecol. Manag. 313, 212–223. https://doi.org/10.1016/j.foreco.2013.11.014.

Zeneli, G., Krokene, P., Christiansen, E., Krekling, T., Gershenzon, J., 2006. Methyl jasmonate treatment of mature Norway spruce (*Picea abies*) trees increases the accumulation of terpenoid resin components and protects against infection by *Ceratocystis polonica*, a bark beetle-associated fungus. Tree Physiol. 26, 977–988. https://doi.org/10.1093/treephys/26.8.977.

Zhang, H., Qiu, Y., Li, M., Song, F., Jiang, M., 2020. Functions of pipecolic acid on induced resistance against *Botrytis cinerea* and *Pseudomonas syringae* pv. tomato DC3000 in tomato plants. J. Phytopathol. 168, 591–600. https://doi.org/10.1111/jph.12938.