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Leucoanthocyanidin Reductase 3 (*PaLAR3*) Locus in Norway Spruce (*Picea abies*) and Its Link to Resistance Against *Heterobasidion parviporum*

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ABSTRACT

Heterobasidion parviporum is a fungal pathogen that is drastically damaging Norway spruce (*Picea abies*) in Europe. The infections will result in root and stem rot, causing significant economic losses for forest owners. Previous studies have shown that the *PaLAR3* gene, which encodes the leucoanthocyanidin reductase enzyme, can increase resistance to *H. parviporum* in Norway spruce. The presence of the B allele at the *PaLAR3* locus has been associated with higher (+)-catechin concentrations and increased enzyme production in inoculation experiments, resulting in inhibited pathogen growth. The control of *H. parviporum* involves a multifaceted approach, including silvicultural and sustainable forestry practices, genetic resistance and chemical/biological control. In this study, we determined the *PaLAR3* genotypes in a representative sample of Norway spruce breeding materials from southern Finland and examined their effect on necrosis caused by *H. parviporum* in spruce stems. The results showed that the homozygous *PaLAR3BB* genotype was present in only 9% of the trees. However, the necrotic area interacted with homozygous *PaLAR3BB* under low-water treatment. These findings support the idea that the *PaLAR3* locus may be a valuable marker for identifying *P. abies* resistance to different strains of *Heterobasidion parviporum*.

1 | Introduction

Norway spruce (*Picea abies* (L.) Karst.) is one of the most economically significant tree species in Europe, playing a crucial role in the forest industry as a source of raw material (Skrøppa 2003). This species contributes to mitigating the effects of climate change through carbon storage and conserving biological diversity, and it is a crucial part of Europe's ecosystem (Niinimäki et al. 2013). *Heterobasidion* root rot, caused by fungi of the genus *Heterobasidion*, is among the most severe threats to the economic use of spruce trees in boreal forests, as the decay can reach up to 12 m in the stem of the infected individuals (Stenlid and Wästerlund 1986). In Finland, two species of *Heterobasidion* are known to exist:

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H. annosum s.s. (Fr.) Bref. and *H. parviporum* Niemelä and Korhonen (Korhonen 1978). These species have distinct host preferences, with Norway spruce being the primary host for *H. parviporum* and Scots pine (*Pinus sylvestris*) being the primary host for *H. annosum* s.s. (Korhonen 1978; Capretti et al. 1990; Gonthier and Garbelotto 2013). The distribution area of *H. parviporum* is assumed to follow Norway spruce to its northernmost habitats in the future (Korhonen et al. 1998; Korhonen and Lipponen 2001).

The primary mode of infection is through spores entering wounds in the tree (Gonthier and Garbelotto 2013), often facilitated by logging activities or natural factors. As the disease progresses, the fungus causes decay in the heartwood, weakening the tree and making it susceptible to wind throw (Netherer et al. 2021). Treating freshly cut stumps with chemical or biological control reduces the risk of spore production and further infections. However, eradication becomes impractical when the pathogen has been established in the host. This, in turn, increases the risk of secondary infections through root contacts. For this reason, we require additional control measures, for example, increased host tree resistance.

As the climate changes, damages caused by fungal pathogens are expected to increase (Seidl et al. 2017), thus threatening the anticipated growth of wood productivity in forests. In addition, more abiotic disturbances, such as wind, snow and drought in forest ecosystems, are expected to rise (Seidl et al. 2017; Senf and Seidl 2021; Patacca et al. 2022). This could have some significant effects on forest health, for example, the wind damage is enhanced by pathogens, such as *H. parviporum*, creating a positive feedback loop and reducing the stability of the trees and quality of the wood material (Krisans et al. 2020). Residuals of Norway spruce colonised by *H. parviporum* can serve as a persistent source of inoculum for multiple years, leading to potential infections in nearby spruce seedlings of the subsequent tree generation (Piri and Hamberg 2015). Also, the climate change-driven impacts on the water supply are likely to worsen (Senf and Seidl 2021) by increasing drought-induced stress reactions in vegetation and making the trees more vulnerable to different pests and pathogens, including *H. parviporum* and *H. annosum* s.s. (Terhonen et al. 2019; Yeoh et al. 2021). However, under a pathogen attack, plants can fight the infection by, for example, producing different toxic metabolites (Franceschi et al. 2005; Keeling and Bohlmann 2006). Therefore, enhancing tree tolerance to pathogens in the changing climate through tree breeding is essential.

An earlier study (Nemesio-Gorriz et al. 2016) discovered an association between *H. parviporum* resistance and Norway spruce's leucoanthocyanidin reductase 3 (*PaLAR3*) gene. A group of variants in this gene, designated as group B alleles, have been reported to improve the resistance of Norway spruce trees as they can limit the fungal *H. parviporum* growth in sapwood (Nemesio-Gorriz et al. 2016). The enzyme production, encoded by the *PaLAR3* gene, increases under a pathogen attack as a defence mechanism against the infection (Jyske et al. 2020). Nemesio-Gorriz et al. (2016) inoculated one fungal strain in 1-year-old Norway spruce twigs and followed the fungal growth in the sapwood. One of the measures of susceptibility of Norway spruce trees against *H. parviporum* is the lesion length around the inoculation point (Lind et al. 2014). Understanding the potential of the *PaLAR3B* allele in the locus to the *Heterobasidion* resistance of Norway spruce would require further inoculation experiments. This study aimed to estimate the prevalence of the *PaLAR3* genotypes in a breeding population of Norway spruce and to determine whether the trees with a homozygotic *PaLAR3BB* or heterozygous *PaLAR3AB* genotype differ in their ability to resist an infection (as measured by the area of necrosis in phloem/sapwood) of two different strains of *H. parviporum*. We hypothesise that, in *PaLAR3B* allele-containing genotypes, the necrosis caused by *H. parviporum* is smaller.

TABLE 1 | Plants used in the experiment and their respective treatments. Hpa1 refers to *Heterobasidion parviporum* strain 1, Hpa2 represents *Heterobasidion parviporum* strain 2, control indicates wounded plants (1.5% Malt Extract Agar) and NT signifies non-treated plants.

Treatment	Number of plants
Hpa1	212
Hpa2	209
Control	209
NT	124
Total	754

2 | Materials and Methods

2.1 | Greenhouse Experiment for Necrosis Assessment

The study material included 754 3-year-old rooted cuttings from 80 Norway spruce clones provided by the Natural Resources Institute Finland (Luke) on 5 March 2020 (Table 1). Each clone had 8–10 ramets. The clones are second-generation breeding material from southern Finland, representing eight full-sib families produced through controlled crossing. The same material was also growing in Luke's Haapastensyrjä field unit (60°37.581′ N, 24°27.581′ E) as 10-year-old saplings from which the young ramets were previously derived.

The experiment was carried out ex situ at the Forest Botany and Tree Physiology greenhouse in Göttingen, Germany, to monitor the environmental parameters during the experiment (as described in Durodola, Blumenstein, and Terhonen 2023). The rooted cuttings were transplanted into 3-L plastic pots filled with 2.5 L of fertilised peat soil (Flora Gard, TKS2 Instant Plus, Hermann Meyer KG, Rellingen, Germany). Before and after the watering experiment (see below), the height and diameter of each sapling were measured to the nearest 0.1 cm, with the height measured again once the growth period had ended (Durodola, Blumenstein, and Terhonen 2023).

Two different fungal strains were used: *H. parviporum* strain 1 (Hpa1: strain number: SB2005 9.16, isolated from a Norway spruce stump) and *H. parviporum* strain 2 (Hpa2: strain number:

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SB 2014 2.69, isolated from a Norway spruce seedling) (collected by Dr. Tuula Piri). The non-treated plants were removed from the necrosis analysis as the aim was focused on the effect of *PaLAR3* genotypes on necrosis caused by *H. parviporum* infection. Therefore, 630 plants were used for the necrosis analysis, categorised into 73 clones and 8 families (ID: 38, 40, 41, 42, 43, 47, 48, 50) (Table 2).

TABLE 2 | *PaLAR3* genotype prevalence (*PaLAR3AA*, *PaLAR3AB* and *PaLAR3BB*) among plant genotypes in necrosis analysis.

Family	Genotype	Number of clones	Percentage (%)
38	AA	5	6.85
38	AB	3	4.11
38	BB	0	0.00
40	AA	10	13.70
40	AB	4	5.48
40	BB	0	0.00
41	AA	5	6.85
41	AB	2	2.74
41	BB	0	0.00
42	AA	14	19.18
42	AB	0	0.00
42	BB	0	0.00
43	AA	4	5.48
43	AB	0	0.00
43	BB	0	0.00
47	AA	2	2.74
47	AB	7	9.59
47	BB	5	6.85
48	AA	9	12.33
48	AB	0	0.00
48	BB	0	0.00
50	AA	0	0.00
50	AB	1	1.37
50	BB	2	2.74
Total 8		73	100.00

Summary of *PaLAR3* prevalence among samples

	Genotype	Number of samples	Percentage (%)
	AA	424	67.30
	AB	143	22.70
	BB	63	10.00
Total		630	100.00

Prior to the inoculations, the fungal isolates were cultured on 1.5% Malt Extract Agar (MEA) for 2 weeks in the dark at +21°C. The inoculation holes were made approximately 10 cm above the stem base by pushing a sterile 5 mm cork borer through the bark to the sapwood surface. Equal-sized plugs from pure cultures of *H. parviporum* (Hpa1 or Hpa2) or control (1.5% MEA) were placed onto the exposed surface and sealed with Parafilm. Mock-inoculated trees with agar inoculation without fungi and non-inoculated trees were added to the experiment as control specimens. The watering treatments started in July 2020 (2020/7/22) and lasted 16 weeks, after which the plants were watered optimally until February 2021. The plants were divided into groups subjected to lower (50% of the optimal) and optimal watering. The amount of watering was adjusted according to the temperature and soil moisture (HH2 device equipped with the ML2x sensor [Delta-T Devices Ltd., Cambridge, UK]) that were monitored and recorded throughout the experiment. The optimally watered group initially received 576 mL twice weekly, while the low water treatment group received 288 mL twice weekly. Subsequently, the water amounts were adjusted to 384 and 192 mL twice weekly. On 18 August 2020, the watering quantity was further reduced to 192 and 96 mL twice weekly for both optimal and low watered groups, respectively. After the experiment, the bark of each specimen was peeled off to reveal the necrotic tissue. The length and width of the necrotic area on each tree were measured from the phloem and the sapwood (Durodola, Blumenstein, and Terhonen 2023). The area of the necrotic lesion was determined by using the equation to count the area of an ellipse ($A = \pi r_1 r_2$), in which the values of the two radii were determined by dividing the total lengths (r_1) and widths (r_2) of the lesion into two. The necrotic areas were calculated both for phloem and sapwood.

2.2 | DNA Extraction, PCR and Gel Electrophoresis for Genotyping PaLAR3

Needle samples were collected from each cutting's original specimen (10-year-old tree) in the Haapastensyrjä field unit as described in Terhonen et al. (2022). The needles were stored in 1.5 mL Eppendorf tubes and kept in a −20°C freezer before the DNA extractions. DNA was extracted using the Qiagen DNeasy Plant Pro Kit (Qiagen, USA). Briefly, 70–100 μg of needle material was transferred into a ready-to-use tissue disruption tube, following the addition of lysis buffers. The samples were then incubated at +65°C for 15 min. The lysis of the samples was done in Tissuelyser (Tissuelyser II, Qiagen, USA) at 24 Hz twice for 2 min. After the lysis, the samples were centrifuged in 14,000 RFC (relative centrifugal force, g-force) for 5 min, and 400 μL of the supernatant was pipetted for DNA extraction. DNA was extracted using a Qiagen DNeasy Plant Pro Kit and Qiacube DNA extraction automate, intended for fully automated DNA processing. After the DNA extraction, the concentration and purity of the DNA were measured using NanoDrop One/One Microvolume UV–Vis Spectrophotometer (Thermo Fisher Scientific, USA).

After the DNA extraction, PCR was carried out using a Thermo Fisher Scientific (USA) DreamTaq Green DNA Polymerase (5 U/μL) kit. *PaLAR3* locus-specific primers

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were designed (Edesi et al. 2021) against the genomic sequence of *PaLAR3* (KX574230.1 and KX574229.1) for one locus and two alleles. To detect *PaLAR3* alleles, 10–40 ng genomic DNA per sample was used in each 12.5 μL PCR reaction. In each reaction, 0.4 μM of locus-specific LAR_ComA primer (5′ GAAATCTGCAGCCAATGGA 3′) (Edesi et al. 2021) and 0.2 μM of each allele-specific primer (LAR_B2: 5′ CTGTATAACCGTAACATCTACTG 3′, and LAR_A: 5S′ GAACGGGTATAAACTCCGT 3′) (Edesi et al. 2021) were included. For each reaction, 200 μM dNTP and 0.2 U/μL DNA polymerase (DreamTaq DNA Polymerase, Thermo Scientific, USA) were used. The total reaction volume was adjusted for 12.5 μL with autoclaved MQ water. The PCR conditions were 95°C for 5 min, followed by 35 cycles of 30 s at 95°C, 30 s at 54°C, 30 s at 72°C and final elongation for 10 min at 72°C. Isolates with a known *PaLAR3AB* genotype were used as positive controls (Terhonen et al. 2022). The presence of *PaLAR3A* and *PaLAR3B* alleles or both was determined by the visual detection made by ultraviolet transillumination of DNA amplicons on a 1.5% agarose gel, stained with ethidium bromide (1–1.5 h, 120 V) (Terhonen et al. 2022). The *PaLAR3A* allele formed a band with a size of 110 bp and *PaLAR3B*, a size of 200 bp (Edesi et al. 2021; Terhonen et al. 2022).

2.3 | Additional DNA Isolation and qPCR-Based Validation of PaLAR3 Genotypes

The DNA extraction process was conducted following the method described by Pirttilä et al. (2001) using fresh needles (50–100 mg) ground in liquid nitrogen. The DNA quality was tested using a nanodrop spectrophotometer. The extraction process involved the addition of prewarmed LiCl and CTAB extraction buffer to the sample, followed by adding chloroform and isoamyl alcohol and centrifugation. The DNA was then precipitated with isopropanol and absolute ethanol, washed in 70% ethanol and dissolved in water. The DNA templates were diluted to half of their volume with water before being used in quantitative PCR (qPCR) using EvaGreen dye (Solis BioDyne, Estonia) on a Rotor-GeneQ device (Qiagen, USA). The validation of *PaLAR3* alleles was performed using allele-specific primers, as described by Nemesio-Gorriz et al. (2016). The qPCR program consisted of an initial denaturation step of 15 min at 95°C, followed by 40 cycles of 15 s at 95°C, 20 s at 60°C and 30 s at 72°C. The melting curves were also analysed, ranging from 62°C to 99°C.

2.4 | Statistical Methods

First, we assessed the distribution of data sets in the R statistical software (R Core Team, 2023) by employing the Shapiro–Wilk test. Since the data did not follow a normal distribution, we utilised the Levene test to examine the homogeneity of variances. The Kruskal–Wallis test was used for homoscedastic data (PaLAR3 genotypes and watering treatments). In instances where the data exhibited heteroscedasticity (unequal variance), we applied Welch's ANOVA (inoculation treatment). Subsequently, post hoc analyses were conducted when there were variations in a dependent variable among treatments. We utilised Dunn–Bonferroni or Games–Howell multiple comparison tests depending on the specific assessment method (Kruskal–Wallis or Welch–ANOVA, respectively). To analyse necrosis, we studied how certain factors (*PaLAR3* genotypes—*PaLAR3AA*, *PaLAR3AB*, *PaLAR3BB*; inoculation types—two strains of *H. parviporum* and control; water treatment—low and optimal) affect the lesion area in the sapwood. For this analysis, we used generalised linear mixed models with the lme4 package (Bates et al. 2015) in R. Additionally, for plant growth, we looked at how the *PaLAR3* genotypes and water treatment influenced plant diameter and starting height. In our models, we considered and included the origin of Norway spruce saplings (family) as a random factor, as only one family had all *PaLAR3* genotypes. Therefore, we could not use 'family' as an explanatory variable.

3 | Results

3.1 | Genotype

The most prevalent genotype among the samples was the homozygous *PaLAR3AA* genotype, which was found in 424 samples (67.3%). The heterozygous genotype *PaLAR3AB* was found in 143 samples (22.7%), while 63 samples had the homozygous *PaLAR3BB* genotype (10%) (Table 2).

3.2 | Necrosis

The necrosis model shows significant differences between the inoculation treatments ($p = <2e\text{-}16$). Also, significant differences in the lesion area in sapwood based on the individual effects of *PaLAR3* genotypes and water treatment were shown (Table 3). The interaction effects suggest that the influence of certain factors such as *Heterobasidion* strains, *PaLAR3* genotypes and water treatments on the lesion area in sapwood can vary depending on the presence of other factors. Some of these interactions are significant, showing that the effect of one variable depends on the levels of others.

The interaction effect between factors Hpa2 (*H. parviporum* strain 2) and genotypes BB (*PaLAR3BB*) is significant ($p = 0.0167$) (Figure 1A), indicating that the smaller necrotic areas caused in the sapwood by *H. parviporum* strain two may be influenced by the presence of genotype *PaLAR3BB*. Similarly, the interaction effect between *H. parviporum* strain 1 (Hpa1) in the optimal watered plants was significant ($p = 0.0160$), suggesting that the impact of Hpa1 on the lesion area is significantly affected by optimum watering compared to the low watering treatment (Figure 1B). Overall, regardless of the water treatments, a consistent pattern of decline in lesion area is evident across *PaLAR3* genotypes (from *PaLAR3AA* to *PaLAR3BB*) due to *H. parviporum* inoculation (Figure 1C).

3.3 | Growth

Similar to the necrosis results, the most prevalent genotype among the samples was the homozygous *PaLAR3AA* found in 500 samples (66%). The heterozygous *PaLAR3AB* genotype was found in 185 samples (25%), while 69 samples had the homozygous *PaLAR3BB* genotype (9%) (Table 4).

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TABLE 3 | Variations in the lesion area within the sapwood of Norway spruce plants, which were subjected to inoculation with two *H. parviporum* strains (including a mock control), *PaLAR3* genotypes (AA, AB and BB) under different water treatments (low and optimum). The data analysis employed generalised mixed linear models, with statistical significance at $p < 0.05$.

	Estimate	SE	*t*	*p*
Intercept	2.79100	0.05877	47.487	**< 2e-16**
Inoculation Hpa1[a]	0.73045	0.06179	11.822	**< 2e-16**
Inoculation Hpa2[b]	0.81263	0.06210	13.085	**< 2e-16**
Genotypes AB[c]	−0.09148	0.10110	−0.905	0.3655
Genotypes BB[d]	0.14381	0.14539	0.989	0.3226
Watering Optimum[e]	0.13031	0.06643	1.962	**0.0498**
Inoculation Hpa1: Genotypes AB[f]	0.03265	0.13673	0.239	0.8113
Inoculation Hpa2: Genotypes AB[g]	0.03129	0.13817	0.226	0.8209
Inoculation Hpa1: Genotypes BB[h]	−0.27439	0.19406	−1.414	0.1574
Inoculation Hpa2: Genotypes BB[i]	−0.46471	0.19418	−2.393	**0.0167**
Inoculation Hpa1: Watering Optimum[j]	−0.22518	0.09343	−2.410	**0.0160**
Inoculation Hpa2: Watering Optimum[k]	−0.10206	0.09380	−1.088	0.2766
Genotypes AB: Watering Optimum[l]	0.23060	0.13427	1.717	0.0859
Genotypes BB: Watering Optimum[m]	−0.14050	0.18482	−0.760	0.4472
Inoculation Hpa1: Genotypes AB: Watering Optimum[n]	−0.19418	0.18709	−1.038	0.2993
Inoculation Hpa2: Genotypes AB: Watering Optimum[o]	−0.26206	0.18814	−1.393	0.1637
Inoculation Hpa1: Genotypes BB: Watering Optimum[p]	0.11115	0.26054	0.427	0.6697
Inoculation Hpa2: Genotypes BB: Watering Optimum[q]	0.49251	0.26076	1.889	0.0589

Note: The intercept represents the baseline, that is, mock control, AA genotypes and low-watering genotypes. The superscript letters denote the differences/interaction with reference to the intercept.
[a]Interaction between intercept/mock control and Hpa1.
[b]Interaction between control and Hpa2.
[c]Difference between genotypes AA and AB.
[d]Difference between genotypes AA and BB.
[e]Difference between low and optimal watering.
[f]Interaction effect between Hpa1and genotypes AB.
[g]Interaction effect between Hpa2 and genotypes AB.
[h]Interaction effect between Hpa1and genotypes BB.
[i]The interaction effect between Hpa2 and genotypes BB.
[j]Interaction effect between Hpa1and optimum watering.
[k]Interaction effect between Hpa2 and optimum watering.
[l]Interaction effect between genotype AA and optimum watering.
[m]Interaction effect between genotype BB and optimum watering.
[n]Interaction effect between Hpa1, genotype AB and optimum watering.
[o]Interaction effect between Hpa2, genotype AB and optimum watering.
[p]Interaction effect between Hpa1, genotype BB and optimum watering.
[q]Interaction effect between Hpa2, genotype BB and optimum watering.

3.4 | Starting Height

The initial heights of plants significantly differed among the *PaLAR3* genotypes ($p = 0.002$). These findings indicate that plants carrying the *PaLAR3BB* genotypes demonstrated a notably greater height than those with the *PaLAR3AA* ($p = 3.91e\text{-}3$) and AB ($p = 1.86e\text{-}3$) genotypes (Figure 2). No statistically significant differences were observed between the plants with *PaLAR3AA* and AB genotypes (Figure 2), and no significant differences occurred in the diameter among *PaLAR3* genotypes ($p = 0.61$).

4 | Discussion

We investigated the prevalence of *PaLAR3* genotypes in clonally propagated Norway spruce saplings and discovered a predominant presence of the homozygous *PaLAR3AA* genotype, constituting 66% of the samples. *PaLAR3AB* and *PaLAR3BB* genotypes accounted for 25% and 9%, respectively. We observed a significant decrease in sapwood lesion area only for the *PaLAR3BB* genotype under reduced watering. This contradicts our hypothesis that homozygous and heterozygous *PaLAR3B* genotypes differ in their resistance to *H. parviporum*.

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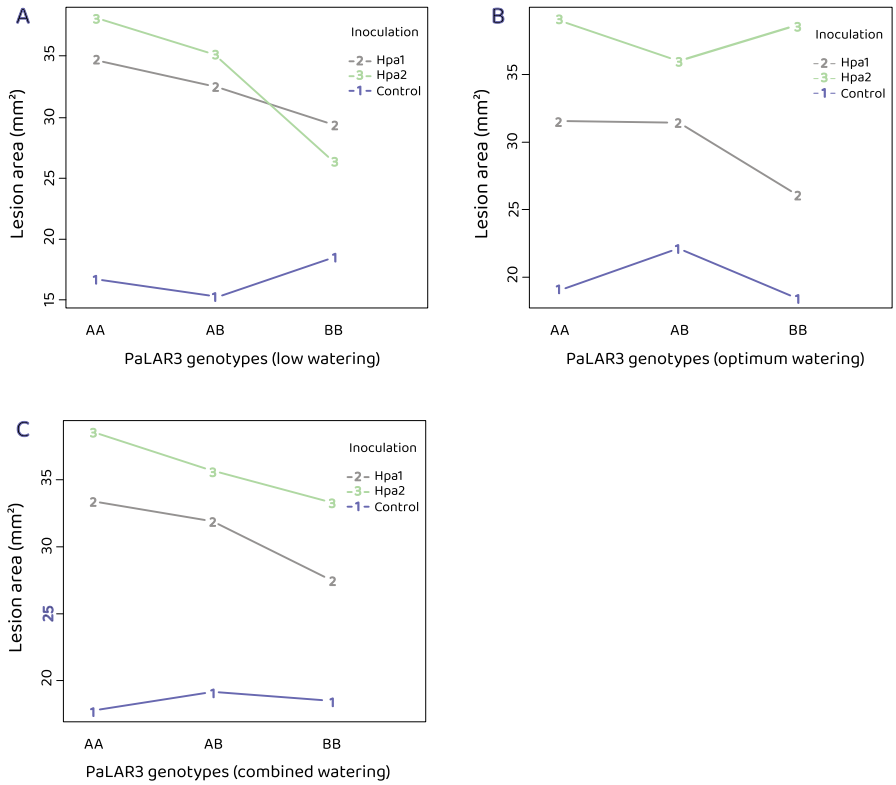

FIGURE 1 | Lesion area in sapwood between Norway spruce *PaLAR3* genotypes due to *Heterobasidion parviporum* strain 1 (Hpa1), *H. parviporum* strain 2 (Hpa2) and the mock-control in (A) low-watered plants, (B) optimally watered plants and (C) combined, that is, all plants.

TABLE 4 | *PaLAR3* genotype prevalence among plant genotypes in growth analysis.

PaLAR3 genotype	Number of samples	Percentage (%)
PaLAR3AA	500	66
PaLAR3AB	185	25
PaLAR3BB	69	9
Total	754	100

The results of the necrosis model reveal substantial differences among the inoculation treatments, indicating an evident influence of the strain's impact on the observed necrosis area (Simonen 2023). However, we found marginally significant differences when assessing the individual effects of *PaLAR3* genotypes and water treatment on the lesion area in the sapwood. This implies that the specific *PaLAR3* genotypes and water treatment levels may contribute meaningfully to the observed variations in the lesion area in the sapwood. In addition, *PaLAR3* might be a factor contributing to drought tolerance in the trees as well. Moreover, examining interaction effects underscores the complexity of factors influencing the lesion area. Some of these interaction effects were statistically significant, indicating that the impact of one variable is contingent upon the levels of others. For instance, the interaction effect between *H. parviporum* strain 2 (Hpa2) and genotype *PaLAR3BB* is noteworthy, suggesting that the reduced necrosis caused by *H. parviporum* strain 2 in the sapwood may be influenced by the presence of the *PaLAR3BB* genotype under limited water treatment. Simonen (2023) also noted that *H. parviporum* exhibited similar growth across all *PaLAR3* genotypes. These findings emphasise the intricate interplay of multiple factors in determining the extent of necrosis in the sapwood. Understanding these interactions is crucial for a more comprehensive grasp of the dynamics involved in response to *H. parviporum* strains, the influence of specific plant genotypes and environmental conditions such as water availability.

The observed height differences among genotypes highlight the potential role of the *PaLAR3* gene in modulating plant growth. The specific attributes associated with the *PaLAR3BB* genotype seem to contribute to enhancement in plant height. As demonstrated by Simonen (2023), plants carrying the *PaLAR3BB* genotype exhibited greater growth in height compared to plants with alternative genotypes. This finding aligns with the notion that specific genetic variations can significantly influence observable characteristics or phenotypic traits, such as the height of plants (Zeltiņš et al. 2022; Chen et al. 2018). These results may have implications for plant breeding and genetic selection strategies. If the *PaLAR3BB* genotype consistently results in taller plants, this genetic trait could be targeted for cultivation in breeding programmes aimed at enhancing plant height. However, further research is necessary to explore the underlying mechanisms by which the *PaLAR3* gene influences plant growth and to ascertain whether other factors may interact with these genotypic effects.

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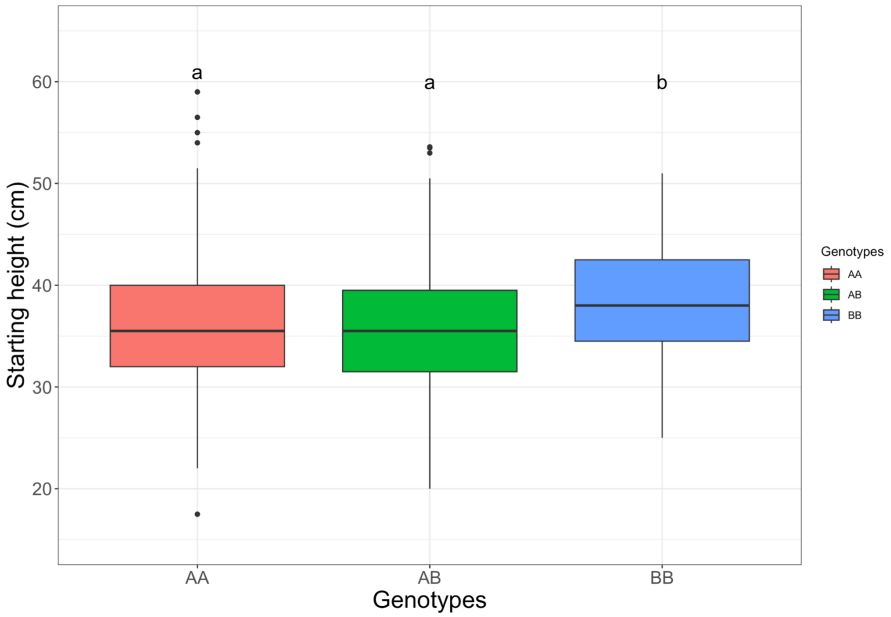

FIGURE 2 | A boxplot comparing the starting height (cm) of the Norway spruce saplings at the beginning of the experiment. Each box represents a different *PaLAR3* genotype (AA in red, AB in green and BB in blue). The letters above the boxes determine the significantly different groups, where group 'a' is significantly different from group 'b' ($p = 0.002$). The line in each box represents the median and the whiskers show the distribution of the individual samplings.

Gene interactions in terrestrial plants are complex, and efforts to investigate them have been made during the past decade (Zhou and Zhang 2020). Since the *P. abies* genome has a comparatively high fraction of repetitive sequences in its overall huge and complex genome, as typical for conifers (Bernhardsson et al. 2019), it is a challenging species in which to explore expressed genes and to map their functional traits. Two-thirds of the total *P. abies* genome size (12 Gbp out of 20 Gbp) have been assembled, containing the majority of expressed genes (Nystedt et al. 2013; De La Torre et al. 2014). Nevertheless, discovering other candidate genes related to the defence against fungal pathogens is a fairly unexplored field, and the potential for discovering other genes is high in the future. So far, in addition to *PaLAR3*, other candidate genes have been associated with resistance against *H. parviporum* infections in Norway spruce (Elfstrand et al. 2020; Chaudhary et al. 2020), and it has become evident that the root rot resistance in Norway spruce is a quantitative trait (Lind et al. 2014; Capador-Barreto et al. 2021).

As climate change not only supports the further spread of fungal pathogens but also increases abiotic stress, such as drought, the challenges for spruce trees will become manifold. In addition to genes resistant to pathogens such as *Heterobasidion* spp., the genetic adaptation to drought as a physiological response will also strengthen the trees (Yeoh et al. 2021). Studies by Depardieu et al. (2021, 2020) have shown that 285 genes in spruce are linked to adaptation to climate, and 110 genes can be connected to drought response. Furthermore, *P. abies* has a strong production of terpenes and phenolics for defence against bark beetles and pathogens (Danielsson et al. 2011; Keeling and Bohlmann 2006). Those diterpene acids inhibit the growth of fungal pathogens and may even kill them (Kusumoto et al. 2014). Studies by Schiebe et al. (2012) and Zhao et al. (2011) have shown that differences in Norway spruce genotypes with a higher defence against fungal infections are correlated with a higher terpene response. Mainly (+)-3-carene was observed to be highly induced by *H. parviporum* inoculation (Danielsson et al. 2011; Zhao et al. 2010).

5 | Conclusion

In homozygous *PaLAR3BB* genotypes, the necrotic area was smaller (under limited water) than those with the homozygous *PaLAR3AA* and heterozygous *PaLAR3AB* genotypes, even though they were present in fewer quantities than their counterparts. The observed patterns in growth parameters emphasise the intricate interplay between genetic factors and tree physiology. These findings enhance our understanding of the genetic basis of resistance to *H. parviporum*. It suggests that just having the *PaLAR3BB* genotype alone does not automatically make Norway spruce more resistant to this infection, and there could be other factors involved that make it more complicated. Our findings, however, provide a foundation for further exploration of the molecular mechanisms underlying these interactions in the resilience of the trees towards biotic and abiotic stress factors, giving valuable information for breeding.

Author Contributions

E.T., B.D. and J.H. designed the study. B.D., M.K. and N.H. performed the experiments. B.D. analysed the data, and B.D. and N.H. wrote the first draft of the manuscript. M.H. provided the sapling material used in this study. T.P. provided the fungal strains used in this study. E.T., J.H. and K.B. conceived the study. B.D., E.T., K.B., M.H., M.K. and N.H. edited the manuscript. B.D. and N.H. revised the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data supporting the findings of this study are available from the authors upon reasonable request.

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