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SHORT COMMUNICATION

Allele *Pa*LAR3B in root rot resistance locus does not influence the infection pressure by *Heterobasidion parviporum* through root contacts

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Abstract

The most destructive root rot pathogen of Norway spruce (*Picea abies*) in Finland is *Heterobasidion parviporum*. After primary infection, this pathogen spreads from tree to tree through root contacts and eventually causes high levels of root and stem rot. Locus *Pa*LAR3 has been associated with root rot resistance of Norway spruce, and higher resistance in Norway spruce against *H. parviporum* has been noted when trees have allele *Pa*LAR3B instead of the more common *Pa*LAR3A. In this study we tested if trees with homo- or heterozygous *Pa*LAR3B allele can better resist the infection through root contacts. The study was established in 2018 in a site where spruce trees had grown at a dense spacing for 10 years. The 72 trees were cut down and a mixture of four *H. parviporum* strains was inoculated on the stumps. After 3 years, 459 trees surrounding the inoculated stumps were harvested and analysed. Based on the presence of conidiospores, forty percent (40%) of the trees were infected by *H. parviporum*. The homozygous *Pa*LAR3B genotype was found only in 1% of the trees. There were no differences in infection rates between the genotypes AA and AB (or BB). Similarly, the distance from inoculated stump or the diameter of the tree did not influence the infection rate.

KEYWORDS
genetic marker, infection, Norway spruce, root contact, root rot

1 | INTRODUCTION

Heterobasidion annosum s.l. is considered as the most harmful and economically most significant root rot pathogen in coniferous forests (Asiegbu et al., 2005). Extensive logging has created habitats favourable for this pathogen, which has dramatically increased the occurrence of infections in the conifer forests of northern Europe. Primary infections by *Heterobasidion* species are established when airborne basidiospores land and germinate on freshly exposed wood substrate (Redfern & Stenlid, 1998). Spore deposition is followed by rapid colonization of the wood material. After the pathogen is introduced, it is impossible to eradicate. Silvicultural control of root rot is challenging because the pathogen can spread to neighbouring trees through root contacts (Piri et al., 2021; Redfern & Stenlid, 1998). Furthermore, the pathogen can remain viable and infective in stumps for decades (Redfern & Stenlid, 1998), resulting

in an inoculum source for new tree generations (Piri et al., 2021). Considering the economic importance of Norway spruce (*Picea abies* [L.] H. Karst.) in boreal forestry, it is imperative to study the effective measures that can be implemented to mitigate the spread of this pathogen. One potential method to control the pathogen would be to develop more resistant forest regeneration materials through selective breeding.

Rapid advancements in molecular research have provided insights into the Norway spruce–*H. parviporum* Niemelä and Korhonen pathosystem through screening of resistance markers based on genetic components (Nemesio-Gorriz et al., 2016). In one defined locus the ability of a tree to control fungal growth in sapwood is based on *Pa*LAR3 (Nemesio-Gorriz et al., 2016). This locus encodes for an enzyme that catalyses the formation of the 3-flavanol (+)-catechin (Nemesio-Gorriz et al., 2016). Nemesio-Gorriz et al. (2016) showed that *Pa*LAR3 has two allelic lineages: *Pa*LAR3A and *Pa*LAR3B, and that allele *Pa*LAR3B in both hetero- and homozygous state increases the resistance (less fungal growth in the sapwood) of Norway spruce against *H. parviporum* (Nemesio-Gorriz et al., 2016).

The aim of this study was to determine whether Finnish Norway spruce breeding materials with different *Pa*LAR3 genotypes could resist *H. parviporum* infection through root contact. The *Pa*LAR3 genotype for each tree chosen for this study was determined, and the results used directly to evaluate the benefits of including *Pa*LAR3-gene based selection in tree resistance breeding and development of effective control methods against this pathogen.

2 | MATERIAL AND METHODS

2.1 | Field settings

The study material consisted of 10-year-old trees originating from controlled crosses between plus trees of Norway spruce. In 2008, hedges of 3837 spruce seedlings were planted in the Haapastensyrjä field unit (60°37.581′N, 24°27.581′E) of the Natural Resources Institute Finland. In 2018, 72 trees were randomly chosen and felled. The stumps of these trees were artificially inoculated with a mixture of four local *H. parviporum* strains: Rusutjärvi 23.2 and Rusutjärvi 104 L1 (isolated from spruce stumps) and **Solböle 2.69 and Solböle 9.1 (isolated from spruce seedlings)**. The treatment suspension was prepared by washing conidiospores off four fully colonized malt extract cultures (one of each strain) into 0.5 L of sterile water. The working suspension was diluted to contain c. 300 spores ml^{-1} and applied onto stump surfaces using a hand sprayer.

In September 2021, a total of 459 trees (from 31 full-sib families) adjacent to each inoculated stump were felled, and the freshly cut stumps (as wood discs) near the ground collected. Based on visual inspection, sample trees were healthy and vigorous. The average distance from the trees to the inoculation point was 61 cm. Similarly, the diameter of the collected stumps was measured (average 4.6 cm). The bark was removed, and the stump discs washed under running tap water, incubated in clean plastic bags for 10 to 14 days, and the presence of *H. parviporum* was determined under a microscope based on visualization of conidiophores.

2.2 | DNA extraction and *Pa*LAR3 allele genotyping

Needles from all the sample trees were collected and stored at −20°C until processed. DNA was extracted from 70 to 100 µg of needle material, with DNeasy Plant Pro Kit Plant (Qiagen) following the manufacturer's instructions. PCR was done as in Edesi et al. (2021) with minor modifications. Briefly, the PCR protocol was as follows: 1X PCR Buffer, 200 µM dNTP, 0.5 µM primer 1 (LAR_ComA: 5'–GAAATCTGCAGCCAATGGA–3') (Edesi et al., 2021), 0.25 µM primer 2 (LAR_B2: 5'–CTGTATAACCGTAACATCTACTG–3') (Edesi et al., 2021), 0.25 µM primer 3 (LAR_A: 5'–GAACGGG TATAAACTCCGT–3') (Edesi et al., 2021), 0.2 U/µl DNA polymerase (DreamTaq DNA Polymerase, Thermo Scientific™), 5 µl of crude DNA as template; the reaction volume was adjusted to 12, 5 µl with autoclaved MQ H_2O.

PCR conditions were 95°C for 5 min, 35 cycles of 95°C for 30s, 55°C for 30s, 72°C for 1 min and 72°C for 10 min. Possible contaminations were determined with a negative control using sterile water as a template; isolates with a known AB allele combination were used as positive controls. The presence of A and/or B allele was determined by the visual detection made by ultraviolet transillumination of DNA amplicons on a 1.5% agarose gel (1–1.5 h, 120 V). The *Pa*LAR3A allele formed a band with a size of 110 bp and *Pa*LAR3B as size 200 bp (Edesi et al., 2021).

2.3 | Statistical analyses

Data were analysed using the statistical package SPSS, version 28.0 (IBM Corporation, New York, NY, USA). A generalized linear model (logistic regression for binary) was constructed to evaluate the fixed effects of *Pa*LAR3A and *Pa*LAR3B allele, family of the genotype, distance from inoculation point and diameter of the stump (without bark) on the presence of *H. parviporum* in the stump (Yes/No). Differences were considered significant if p-value ≤.05.

3 | RESULTS AND DISCUSSION

3.1 | Genotyping of *Pa*LAR3 alleles

The frequencies of *Pa*LAR3A and PaLAR3B alleles were 84% and 16%, respectively. Only 1% (i.e. six out of 459) of the collected trees were homozygous for *Pa*LAR3B (Table 1). 30% were heterozygous (AB) and 69% homozygous for *Pa*LAR3A allele (Figure 1, Table 1).

3.2 | Presence of *Heterobasidion parviporum* in stumps

No significant difference in the presence of *H. parviporum* in the stump (infection occur/do not occur) was observed, based on the *Pa*LAR3 genotype (AA, AB, BB) ($p = .782$), family ($p = .441$), distance ($p = .435$) or stump diameter ($p = .983$). The number of infected stumps between *Pa*LAR3 alleles was: 41% in AA, 39% in AB and 33% in BB (Figure 1). From all stumps 40% were found to be infected.

Heterobasidion parviporum spreads among Norway spruce trees via root contacts (Redfern & Stenlid, 1998) and finding novel, effective control methods to prevent this problem would be highly beneficial. Planting infested sites with more resistant conifer trees could decrease the infection rate through root-to-root contacts. The prospect of increasing the degree of pathogen resistance by selective tree breeding is highly dependent on the availability of genetic markers which could be used to track down and select individuals of desirable resistance qualities in breeding materials. Due to the quantitative mode of the inheritance, successful selection for root rot resistance requires several effective markers associated both at reduced fungal growth, as well as the tree's ability to resist new infections (Lind et al., 2014).

The allele *Pa*LAR3B was earlier reported to limit *H. parviporum* growth in Norway spruce sapwood in trees homo- or heterozygous for the allele and to be associated with production of higher amounts of (+) catechin in bark (Nemesio-Gorriz et al., 2016). In this study we showed that over a 3-year-time period *H. parviporum* was able to infect ca. 40% of trees in a densely planted field. However, the presence of *Pa*LAR3B allele did not significantly affect the spread of the fungus through root contacts. No other factor measured (family, distance, stump diameter) appeared to have an impact on the spread of the pathogen.

The experimental setting in this study was not typical of a regenerated forest site as the trees were planted at a very dense spacing. This density most likely supported the spread of *H. parviporum* to neighbouring trees from the inoculation point via root contact (more root contacts due to the short distance) making the setup ideal to test the possible effect *Pa*LAR3 genotypes had on new root infections as differences in fungal growth inside the wood were small. However, we found no evidence suggesting that the heterozygous *Pa*LAR3B allele decreased infection pressure through root contacts. In natural conditions *Heterobasidion* species can spread from stumps to new tree regeneration, and from seedling to seedling forming annually expanding disease centres (Piri et al., 2021). In our setting we observed similar infection rates for *H. parviporum* already after 3 years as previously observed for *H. annosum* s.s. (Fr.) Bref. under normal silvicultural practices after over 10 years (Piri et al., 2021). Even under continuous infection pressure, the natural resistance of trees may cause fungal growth rate in living roots to be reduced by 10–30 cm per year compared to stumps (Bendz-Hellgren et al., 1999), which may explain why 60% of the sample trees were not infected.

Resistance of Norway spruce against *H. annosum* s.l. is a quantitative trait associated with several known defence responses (Lind et al., 2014) with some variation depending on the environment. Lind et al. (2014) mapped four quantitative trait loci for four distinct resistance traits against *H. parviporum* into the *Picea abies* genome. These traits included fungal spread within sapwood and ability to prevent infection establishment (Lind et al., 2014). The *Pa*LAR3 gene was

TABLE 1 Number of *Pa*LAR3 A and B alleles in sampled trees, and *H. parviporum* infection rates observed in the stumps

*Pa*LAR3	H. Parviporum observed		
Allele	No	Yes	SUM
AA	184	130	314
AB	85	54	139
BB	4	2	6
SUM	273	186	459

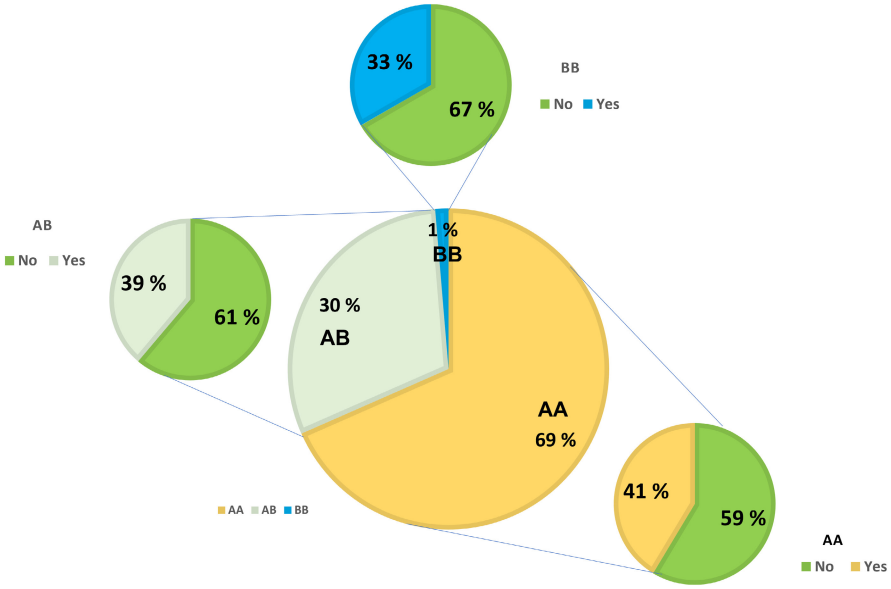

FIGURE 1 Percentages of *Pa*LAR3 genotypes are presented in the middle chart: AA (69%), AB (30%) and BB (1%) from the trees harvested for this study ($N = 459$). The smaller charts present percentage of observed *H. parviporum* in stumps inside the allele (no = *H. parviporum* not observed, yes = *H. parviporum* observed).

previously reported to control fungal growth in sapwood (Nemesio-Gorriz et al., 2016), but our results suggested it has no effect on establishing new infections via root systems.

4 | CONCLUSION

The *Pa*LAR3B allele in Norway spruce genotype does not reduce the frequency of new root-to-root infections by *H. parviporum*.

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DATA AVAILABILITY STATEMENT

Data available from the authors.

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