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Novel genetic markers for tracking pH-responsive genes in *Pinus sylvestris* (L.)

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In ecosystems dominated by acidic soils, species are forced to adapt to unfavorable and stressful substrate conditions and have evolved various strategies. These include a range of physiological and chemical mechanisms and signaling pathways that trigger molecular responses, which has led, in turn, to structural and functional changes in responsive genes. As a result of these adaptations, molecular approaches have enabled considerable progress in explaining the mechanisms and detection of genes responsible for acidic pH tolerance. The development of gene-specific molecular markers offers more options for the marker-assisted detection of substrate-specific populations. Scots pine (*Pinus sylvestris* L.) is a major forest forming conifer species of Eurasia capable of growing on substrates with different pH values. In this study, based on the annotation of pH-responsive genes, novel SNP markers were identified and tested using Scots pine samples originating from habitats with substrates of varying pH. By testing the primers, 33 markers from 27 gene regions were successfully amplified using PCR. Following the sequencing of the PCR products, gene regions were analyzed, focusing specifically on polymorphic sites and synonymous/non-synonymous SNPs. From a total of 15426 sites, it was possible to identify 8 synonymous and 5 non-synonymous SNPs. The genetic variation revealed by the newly designed markers, affecting species' soil pH tolerance help to identify individuals adapted to divergent soil types. In such cases, the markers may serve as valuable genetic tools for studies of adaptive genetic variation in Scots pine, a species used in both reforestation and forest plantations.

KEYWORDS

Scots pine, soil pH tolerance, primer design, SNP, adaptation

1 Introduction

A high degree of genetic diversity in populations is essential to ecosystem stability and resilience to environmental change. Within ecosystems the distribution of plant populations is determined by habitat characteristics and biotope quality. Adaptive behavior of a population provided on the basis of high genetic diversity as a response to habitat characteristics is an important prerequisite for species survival. The morphological and phenological trait responses of individuals are often influenced by extreme environments, and this may induce population-level adaptations (Gray and Brady, 2016; Dussarrat et al., 2021; Everingham et al., 2023; Nacakci and Gülcü, 2025). The threat posed to natural ecosystems by climate change is the focus of many studies aiming to uncover the genetic background of the stress-response traits of various populations inhabiting divergent habitats. Genetic information is, moreover, crucial to the development of conservation strategies, and the sustainable use of genetic resources is therefore becoming ever more important (Hoffmann and Sgrò, 2011).

Forest tree species are key components of the forest ecosystems and as they have long life spans, the shift in the genetic background responsible for adaptation will require a much longer period of time (Davis and Shaw, 2001; Hamrick, 2004; Savolainen et al., 2004; Jump and Peñuelas, 2005). The accumulation of a greater degree of genetic diversity and population variability provides a good basis for the promotion of the selection of genes responsible to mitigate the effects of rapid environmental changes. SNPs in these genes represent an inexhaustible source of polymorphic markers. Since its advent, development in the use of DNA molecular markers has already provided important resources for the study of forest tree species. The identification of microsatellites and SNPs through population genetic analyses has already been widely used to demonstrate the degree of genetic polymorphism in populations. Moreover, the detection of SNPs within genes involved in adaptation has rapidly increased due to improvements of high-throughput DNA sequencing methods (Kuhner et al., 2000; Garvin et al., 2010; Rellstab et al., 2017; Price et al., 2020).

A frequent corollary of climate hazards, long-lasting droughts and extreme environmental events is the changes in soil properties, including shifts in pH. The reaction of forest-forming tree species to changes in soil pH and the resulting effects on their physiological properties are important in the modulation of their adaptive potential. Soil pH can be highly variable and one factor in this is changes in soil water content, which may substantially modify the pH while plants are then forced to react and respond, making changes in their physiological parameters to ensure their continued ability to acquire nutrients and regulate their water acquisition and exchange (Jing et al., 2023). Changes in soil pH may therefore have a strong impact on the plant rhizosphere, affecting respiration, the solubility of nutrients or even modifying a series of biotic interactions (Felle, 2001; Koyama et al., 2001; Gao et al., 2004). The impact of external pH on gene expression modulation has been noted in several previous studies. Gene discovery associated with tolerance to soil carbonate was detected in some genotypes of *Arabidopsis thaliana* having contrasting behavior. A bulk-segregant-analysis sequencing (BSA-Seq) was performed, and transcriptomics revealed 208 root and 2503 leaf differentially expressed genes under bicarbonate stress (Busoms et al., 2023). In the study altogether 69 genes were associated with carbonate tolerance and final set of 18 genes involved in bicarbonate stress responses that had relevant roles in soil carbonate tolerance and the modifications had an impact to the sugar, lipid and protein metabolism of the tolerant genotype. Changes in external pH act as signals. Plants should modify responses auxin signaling, or make changes in cell wall plasticity and extensibility, as well as in the salicylic acid signaling involved in pathogen responses (Lager et al., 2010; Bailey et al., 2023).

As one of the most important forest-forming tree species and due to its capacity to persist in different, even extreme ecological conditions, Scots pine is considered one of the most promising conifer species in the time of climate change. Among the conifers, Scots pine has one of the widest distributions, forming taiga forests across boreal Eurasia. With its broad ecological tolerance, Scots pine can inhabit many types of habitats (Kremenetski et al., 2000; Mátyás et al., 2004; Hallingbäck et al., 2021). It has been documented in Europe in ecologically marginal environments from its northernmost occurrence in Scandinavia and Russia to the southernmost regions of the Mediterranean, including Turkey, Spain or Central Europe (Tóth et al., 2017a, 2021; Wachowiak et al., 2022). Naturally, given this geographic spread, populations can be found on different substrates with great variation in their pH values. Scots pine is even known to grow on rocky limestone cliffs and volcanic outcrops, forming stable populations on both alkaline and acidic sandy soils, and it is also typical of low pH boreal peat bogs. Historical populations of Scots pine are known to have survived the glacial cycles of the Pleistocene, in which they faced large fluctuations of the climate (Birks and Willis, 2008; Tóth et al., 2019).

Despite the ecological and economic importance of various conifer species, achieving complete genome assemblies remains a challenge due to their large genome size and the highly repetitive content of the genomes. Technological advances have, however, enabled the sequencing of several conifer species (De La Torre et al., 2014; Neale and Wheeler, 2019; Scott et al., 2020; Xiong et al., 2021; Neale et al., 2022; Lo et al., 2024; Chen et al., 2025), allowing the study of genes potentially involved in ecological adaptation and the development of specific markers targeting SNP variation (Prunier et al., 2015). Given that Scots pine is adapted to multiple habitat types and grows on substrates with varying pH values, it can be considered a model species for identifying pH-responsive gene candidates within the genome. This, in turn, makes it possible to develop markers aimed at detecting variation among populations growing on substrates with different pH levels. In population genetic studies, such markers would enable the identification of substrate-specific populations and allow for directed and controlled forest management.

2 Materials and methods

2.1 Plant material

The plant material used was obtained from four Central European Scots pine populations previously sampled for a study by Tóth et al. (2017b), while one population was collected later. These populations represent different habitats, each with substrates of significantly divergent pH values, namely peat bogs with low pH (RPO, RMH), Ca-rich rocky outcrops (SKV, SLO), and an alkaline sandy substrate (HFE) (see Supplementary Table S1). Five specimens were selected randomly from each population.

2.2 Bioinformatics data mining

To develop markers for tracking pH-responsive genes or their respective functional sites, it was first necessary to perform a library preparation and annotation of functional regions. In the interests of clarity, we focused on genes displaying significant dissimilarities in gene expression patterns in response to changes in external pH levels. On the basis of work by Lager et al. (2010), a total of 1036 genes were selected and were denoted as pH-responsive if a significant difference (adjusted $P < 0.05$) was observed, plus at least a twofold change at pH 4.5, with the control value being pH 6.0. The "rentrez" package (Winter, 2017) was used, implemented in R (R Core Team, 2013), to select and download EST and protein repository records accurately for each gene from the NCBI database (https://www.ncbi.nlm.nih.gov/). The *Pinus sylvestris* draft genome (NCBI Accession ID: PRJEB1898) was employed which has a sequencing coverage of 12.5×, an assembly size of 6.795 Gbp, approximately 16.1 million contigs, and an NG50 of 447 bp (Nystedt et al., 2013). To map the unannotated Scots pine genome, which consists of 881,136 contigs > 1Kb in length, a total of 2,569 EST and 16,769 protein sequences were downloaded and used as queries. We used the NCBI BLAST+ 'makeblastdb' tool to create a BLAST database from the genome and then conducted BLASTN and TBLASTN searches to identify each locus on the open-source web-based platform Galaxy (https://usegalaxy.org/). Sequences with the highest BLAST hit were selected for further processing. Only sequences with an e-value ≤0.001, sequence identity >98% and alignment length >100 bp were retained. For each locus, redundant sequences were separately aligned using the ClustalW (Thompson et al., 2003) multiple sequence alignment tool in BioEdit (Hall et al., 2011) to create a consensus sequence capable of characterizing the maximal length of each locus. A total of 48 different partial gene sequences could be identified. Following alignment of the sequences in the *P. sylvestris* genome, the corresponding contigs were extracted and used for marker development. The complete workflow is shown in a flowchart, Supplementary Figure S1.

2.3 Primer design

The online software Primer 3.0 (http://primer3.ut.ee/) was used to Design primer pairs. The parameters for this were: (1) primer length, between 18 bp and 23 bp, with 20 bp as the optimum; (2) PCR product size from 90 to 800 bp for each gene; (3) a melting temperature of 57°–62°C, with 59°C as the optimum; and (4) a GC content of 30–70%, with 50% as the optimum. The PCR product size for each gene was kept below 800 bp to ensure efficient Sanger sequencing.

2.4 DNA extraction and PCR amplification

Total DNA was isolated from silica-dried needles using the E.Z.N.A.® SP plant DNA kit. (Omega Bio-tek, Inc., Norcross, GA, USA) following the manufacturer's protocol. In the assessment of the DNA concentration and quality a NanoDrop spectrophotometer (BioScience, Budapest, Hungary) was employed, and the result visually checked on a 1% agarose gel.

PCR amplification was conducted in a total volume of 24.2 µl, of which 1 µl was genomic DNA (about 20 ng), 0.18 µl DreamTaq DNA polymerase (Fermentas, Szeged, Hungary), with, 4.5 µl (10×) Dream Taq Green PCR buffer (ThermoFisher, Waltham, MA, USA), 3 mM MgCl2, 200 µM of dNTPs, 1% BSA, 0.2 mM of each primer and Milli-Q ultrapure water (Merckmillipore, Billerica, MA, USA). The PCR protocol was as follow: (1) pre-denaturation at 94°C for 3 min; (2) 32 cycles at 94°C for 30s; (3) annealing at 58 or 60°C for 45s; and (4) extension at 72°C for 1.2 min, and a final elongation at (5) 72°C for 7 min. For markers with multiple bands, further optimization was achieved via an increase up to 60°C in the annealing temperature. The PCR products were analyzed on a 1.5% (w/v) ethidium bromide-stained agarose gel in 1xTBE buffer to verify the amplification.

2.5 Genotyping by SNP marker candidates

Products appearing as a single band of the expected size (in *P. sylvestris*) were used to genotype samples of different origins using Sanger sequencing. For this, the purification of the products after PCR was achieved in one step via the hydrolysis of the excess primers and dephosphorylated unincorporated dNTPs using ExoSAP-IT™ Express PCR Product Clean-up Reagent, (ThermoFisher Scientific, Carlsbad, CA, USA), according to the manufacturer's protocol. The products of the ExoSAP-IT process were then sequenced on the basis of the forward and reverse primers, in both directions, at Biomi Ltd., Hungary. The amplified sequences were then edited, visualized and aligned using BioEdit Sequence Alignment Editor, software, version 7.0.9.0 (Hall et al., 2011). Following sequence alignment, the

genotyping and final validation of all potential SNPs was performed visually on chromatograms with the aid of a CodonCode Aligner 8.0.2 (Richterich, 2004). The number of polymorphic sites and of insertions/deletions, plus the character of SNPs (i.e. synonymous or nonsynonymous) was calculated using DNA Sequence Polymorphism 6.10.01 (DNASP) software (Rosas and Rosas 1995) and validated with a visual check in CodonCode Aligner 8.0.2.

3 Results

3.1 Primer testing

A total of 40 primers were designed and PCR amplified, and of these 33 yielded high-quality PCR products. Of the total, six primers were not successful, while one was excluded as it yielded only a low-quality sequence. The size range of the amplified sequences fell between 238 and 633bp. The newly designed and successfully tested primers, together with their marker codes and expected PCR products lengths are found in Table 1.

3.2 Evaluation of sequence variation

The newly designed molecular markers relate to 27 genes or partial gene regions that could be annotated as playing a role in trees' adaptation to soil pH. The genes and their functions annotated in Arabidopsis are presented in Supplementary Table S2. Genetic characteristics, including the number of polymorphic sites (S), haplotypes (h), the degree of nucleotide diversity (Pi), and counts of synonymous and non-synonymous sites, were evaluated based on the sequence variation of 5 (or in some cases 4) Scots pine genotypes. Polymorphic site values ranged between 1 and 7, while the number of haplotypes of the newly developed markers ranged between 1 and 4. In nine of the cases, haplotype diversity was above zero. Further, eight synonymous and five non-synonymous sites were detected, and these are listed in Table 2, while the PCR products of the five non-synonymous sites are presented in Supplementary Figure S2.

4 Discussion

Soil pH is known to be a major environmental variable affecting plant life (Bloom et al., 2006; Neina, 2019). Soil pH plays a major role in environmental adaptation via its influence on a series of physiological-chemical reactions, acting as a signaling system that initiates responses at the molecular level, manifested firstly by the expression or alteration of responsive genes (Lager et al., 2010). Our genetic markers are based on DNA sequence variation of these genes. The newly designed primers yielded high-quality PCR products, and of the nine marker sites all turned out to be polymorphic. Our study also represents an SNP discovery test on genes presumed to be responsive to adaptation to soil pH, annotated by comparative genomics in the *P. sylvestris* genome. In the literature, cases are found in which SNP markers from sequences annotated by comparative genomics have been developed and successfully used on diverse plant species (Hall et al., 2002; Fulton et al., 2002; Köbölkuti et al., 2020; Vuruputoor et al., 2023). Translation of the annotated *P. sylvestris* genes followed by similarity searches in protein databases revealed that the corresponding DNA sequences or their encoded proteins have already been identified in other conifers or angiosperm tree species (Table), most likely because these genes occur in evolutionarily conserved genomic regions.

In the course of the sequence analysis, five non-synonymous SNPs were found in five different gene fragments. The biological functions of the proteins encoded by these genes are indicative: cytochrome P450 confers salt tolerance (Werck-Reichhart et al., 2002; Wang et al., 2016) acireductone dioxygenase has been demonstrated to have role in root growth behavior (Ramanathan et al., 2018) and tolerance to drought (Liang et al., 2019); and cystatins are universally present in plant taxa, suggesting a vital protease regulatory role for these proteins in plants in general (Benchabane et al., 2010) via the activation of protective metabolic cascades under abiotic stress conditions (Balbinott and Margis, 2022). Studies have reported an up-regulation of cystatin mRNA transcripts in leaves subjected to conditions adverse to growth, such as drought, salinity or low temperatures (Pernas et al., 1998); L-Asparaginase is a key enzyme for asparagine utilization by plants, and this has an important role in the nitrogen metabolism of developing plant tissues (Sieciechowicz et al., 1988); elevated levels of asparaginase activity are detected in developing tissues like leaves and roots (Michalska et al., 2006), and in late embryogenesis; abundant hydroxyproline-rich glycoprotein is involved in plant stress responses (Johnson et al., 2017).

The polymorphism observed in the sequences should be considered of particular importance, as the relevant genes seem to be essential to adaptation to substrate conditions. It may be presumed that the five non-synonymous SNPs detected influence the expression of some genes and modulate protein structures and function. The assignment of target sequences to genes and the coding sites responsible for the synthesis of the aforementioned proteins result in phenotypic changes of individuals in relation to adaptation.

The small number of samples used (4 and 5 per population) and the limited genotyped panels are methodological constraints that may have influenced our results, potentially leading to the omission of some relevant alleles. On the other hand, this allowed us to detect some putatively large effect loci. However, this sample size still affords the opportunity to further examine the connection between the selection and the soil/substrate properties of the species.

Having knowledge of the genetic background of the tree species in forest ecosystems is increasingly vital because climate change is expected to alter the species composition of forests globally. The decline of certain species opens up an ecological niche to others and leads to their expansion. Scots pine, with its tolerance of widely differing ecological conditions, and high adaptive capacity to extreme habitat sites, is a promising species in terms of its survival in forests and in parks and green spaces. Its presence on various, sometimes extreme substrates, and in conditions of widely varying soil pH suggests, however, that individuals have adapted differently over time. Knowledge of this adaptation is essential to

TABLE 1 List of selected genes sequences with NCBI GenBank sequence accession numbers, marker names, and forward and reverse primer sequences for pH responsive genes in *P. sylvestris*.

Marker name	NCBI GeneBank sequence accession number	Arabidopsis locus ID	Primer sequences (5′–3′) Fw	Primer sequences (3′–5′) Rev	Expected size (bp)	Tm (°C) Fw	Tm (°C) Rev	Ta (°C)
PS01	PV070003-PV070007	AT1G12740	TCTCCAATCAGTGGCCAACC	GCGTTGACTATCCATTCCAGG	437	59.96	58.79	58
PS02	PV070008-PV070012	AT1G17620	TGGAACTGATCCGCATAGCC	GCCCTGGACAAAAGCTGGAA	750	59.89	60.83	58.9
PS033	PV070013-PV070017	AT1G22510	ATAGGGCTGCACTCTAGCCA	AGATGCCTCCCTTTGGTGTG	691	60.40	59.96	59.5
PS04a	PV070018-PV070022	AT1G30690	CTGGGGAGTTCCTCTGCTTC	AAGTACCACCAAGGCACGTT	623	59.75	59.82	59.5
PS04b	PV070023-PV070027	AT1G30690	ACCAAGCAGGCTTTGGATCT	CTCTGTAGCTGAAAGAATTGGGA	599	59.59	58.16	58
PS05	PV070028-PV070032	AT1G32940	GCCGATTAGGAACGTAGCCA	CGTGGACTCCTGTTTCACCA	699	59.90	59.89	58
PS06	PV070033-PV070037	AT1G41830	GATGTTGGTCCTCCCTCCAA	TTGCCGATTTGCATCCCTGA	703	59.01	60.32	58
PS07	PV070038-PV070042	AT1G48320	ACCAAAAATGTGGGTCCAAACA	GATCGACTCGGCGAATGTGA	749	59.16	60.25	59.5
PS09	PV070043-PV070047	AT1G60730	TTGGGTCTCGATGCTGTGAT	TGAACAGCTGTAATGGGATGT	561	59.10	57.28	58
PS10	PV070048-PV070051	AT1G64640	CTTGTCCACAGGAGGAACCC	AAGAGGCTTGTTCTGCCCTG	744	59.96	60.25	59.5
PS11	PV070052-PV070056	AT1G68410	GCCAGACGGATCTAACACCA	AACAAGCAATACCGCTGGGA	628	59.46	59.96	58
PS12	PV070057-PV070061	AT1G71050	GGGAAGTAGGCTCCAAGCTG	CTAGCAGATGCCCAATGCCT	527	60.11	60.18	60
PS13	PV070062-PV070066	AT1G75780	GCCCACCTGACTCAAATCCC	AAAACGCCGATAAGCCAAGC	643	60.68	59.83	59.5
PS14a	PV070067-PV070071	AT1G80160	CAGATTTCACGTCCCTCCCC	TCACGAACAGGCCTTCATCC	523	60.11	60.04	59.5
PS14b	PV070072-PV070076	AT1G80160	ACGAGAATGTCCTCGGCTTT	GCTGATCCACGAACAATCCC	746	59.39	58.98	59.5
PS15	PV070077-PV070081	AT1G80820	AGAACTAGGGCTCTGGGCTT	GGTGGAGCCAGCTGTGAAT	571	60.25	60.00	59.5
PS16a	PV070082-PV070086	AT2G26400	AGCACTAACTGGTGCTCATGTA	TTTGATTTTTCTGAATGGCATCATC	364	59.43	57.16	58

(Continued)

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TABLE 1 Continued

Marker name	NCBI GeneBank sequence accession number	Arabidopsis locus ID	Primer sequences (5′–3′) Fw	Primer sequences (3′–5′) Rev	Expected size (bp)	Tm (°C) Fw	Tm (°C) Rev	Ta (°C)
PS16c	PV070087-PV070090	AT2G26400	GTTCACCTTGGAAGAGCCTCA	CGCCATCAAAAGGTCACTGC	735	59.93	60.11	60
PS17	PV070091-PV070095	AT2G39450	TCTTGCAACCTCTTCACGCT	TGACTGGGTCTAACTGGGGT	625	59.89	59.81	58.4
PS18	PV070096-PV070100	AT2G40880	CATCGCGTAAACCTCCGAGA	TGGACACAGGGTGGTCAAAC	380	59.90	60.11	58
PS20	PV070101-PV070104	AT3G16150	TCAGCATCCCAAAACACATCA	TATGCAGGCTGCCTTACAGC	746	58.13	60.46	58
PS21	PV070105-PV070107	AT3G19430	CAGCAATGTTGTCTCCCTGC	TCGGCGGAATGAGAGTGATG	519	59.48	59.90	59.5
PS22	PV070108-PV070112	AT3G22200	GCAACAGCACATGCTACAGG	TTTCACAGGTGCAAGCAGTT	711	59.83	58.53	58
PS23	PV070113-PV070117	AT3G56230	GAGGTTTCAAACTCCGGCCT	ATGAGTTCCAAAGGGACGGG	535	60.25	59.67	58
PS24a	PV070118-PV070121	AT3G59350	ATGAGCCCGGATCCCCAATA	TGTGTGCTTCTTTGGACACTG	692	60.48	58.98	58
PS24c	PV070122-PV070126	AT3G59350	TCCCAGCTCCACTTCCAGAT	TCTCTGGTTTGACATGGATTGGT	486	60.25	59.93	59.5
PS25b	PV070127-PV070131	AT4G02280	CTGTCGCCACTATCTCCTCG	GTGCGGAATGGTGAGCTGTA	652	59.69	60.39	58
PS26	PV070132-PV070136	AT4G17460	TCTCTCGCCATCTGCACATAC	AGACACGCAAACTCTCCCTC	683	59.93	59.68	58
PS27a	PV070137-PV070141	AT4G22010	AATTTGGCGGGAGTTTTGGC	AAACTCAGATTCCCAGCCCG	573	59.97	60.04	58
PS27c	PV070142-PV070146	AT4G22010	GGTATTCCACAGACAGCGCA	TCCCCAATGCTTGACGACG	475	60.39	60.38	59.2
PS28a	PV070147-PV070151	AT4G23180	TTGCTGTGTGGAAGGACCAG	GCCAACAAAGCAAACCCCAA	557	60.18	60.11	58
PS28b	PV070152-PV070156	AT4G23180	GGGAGGCTTTGGCTCTGTAT	GGCCACGAGCTATTCCCATT	514	59.45	60.18	59.5
PS29	PV070157-PV070161	AT4G24160	CAGGTCTTCAAGCAACGAGC	TCGAACACGAACACAAACCC	540	59.49	58.99	58.4

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TABLE 2 Genetic characteristics of 33 newly developed markers of pH responsive genes in Scots pine genotypes, N: Number of sequences; Ns: Number of sites (alignment length); Ts: Total number of sites (excluding sites with gaps/missing data); Pi: Nucleotide diversity; h: Number of haplotypes; S: Number of polymorphic (segregating) sites; syn: synonymous mutations, nsyn: non-synonymous mutations.

Marker name	NCBI GeneBank sequence accession number	Arabidopsis locus ID	N	Ns	Ts	Pi	h	S	syn	nsyn
PS01	PV070003-PV070007	AT1G12740	5	404	400	0.00100	2	1	0	1
PS02	PV070008-PV070012	AT1G17620	5	672	662	0.00121	3	2	1	0
PS03	PV070013-PV070017	AT1G22510	5	627	614	0.00261	3	3	1	0
PS04a	PV070018-PV070022	AT1G30690	5	600	594	0.00000	1	0	0	0
PS04b	PV070023-PV070027	AT1G30690	5	556	552	0.00000	1	0	0	0
PS05	PV070028-PV070032	AT1G32940	5	626	617	0.00065	2	1	0	0
PS06	PV070033-PV070037	AT1G41830	5	616	614	0.00326	3	5	0	0
PS07	PV070038-PV070042	AT1G48320	5	623	610	0.00197	2	3	0	0
PS09	PV070043-PV070047	AT1G60730	5	446	434	0.00092	2	1	0	0
PS10	PV070048-PV070051	AT1G64640	4	349	320	0.00156	2	1	1	0
PS11	PV070052-PV070056	AT1G68410	5	561	549	0.00073	2	1	0	0
PS12	PV070057-PV070061	AT1G71050	5	483	469	0.00085	2	1	0	0
PS13	PV070062-PV070066	AT1G75780	5	490	488	0.00082	2	1	1	0
PS14a	PV070067-PV070071	AT1G80160	5	404	367	0.00000	1	0	0	0
PS14b	PV070072-PV070076	AT1G80160	5	706	706	0.00000	1	0	0	0
PS15	PV070077-PV070081	AT1G80820	5	485	478	0.00120	2	1	0	0
PS16a	PV070082-PV070086	AT2G26400	5	254	244	0.00328	2	2	0	0
PS16c	PV070087-PV070090	AT2G26400	4	297	284	0.01291	3	7	0	1
PS17	PV070091-PV070095	AT2G39450	5	439	426	0.00094	2	1	0	0
PS18	PV070096-PV070100	AT2G40880	5	256	238	0.00504	4	2	0	1
PS20	PV070101-PV070104	AT3G16150	4	268	241	0.01037	3	5	1	1
PS21	PV070105-PV070107	AT3G19430	5	331	309	0.00216	2	1	0	1
PS22	PV070108-PV070112	AT3G22200	5	689	669	0.00060	2	1	0	0
PS23	PV070113-PV070117	AT3G56230	5	340	335	0.00119	2	1	0	0
PS24a	PV070118-PV070121	AT3G59350	4	640	633	0.00000	1	0	0	0
PS24c	PV070122-PV070126	AT3G59350	5	296	218	0.00459	3	2	0	0
PS25b	PV070127-PV070131	AT4G02280	5	633	624	0.00000	1	0	0	0
PS26	PV070132-PV070136	AT4G17460	5	604	589	0.00000	1	0	0	0
PS27a	PV070137-PV070141	AT4G22010	5	560	548	0.00146	3	2	1	0
PS27c	PV070142-PV070146	AT4G22010	5	451	451	0.00355	2	4	1	0
PS28a	PV070147-PV070151	AT4G23180	5	411	400	0.00000	1	0	0	0
PS28b	PV070152-PV070156	AT4G23180	5	462	448	0.00000	1	0	0	0
PS29	PV070157-PV070161	AT4G24160	5	309	295	0.00136	2	1	1	0
		Total		**15888**	**15426**				**8**	**5**

Bold values indicate the total row.

the management and maintenance of future forests, landscapes and green spaces. Uncovering its genetic background, including the diversity of genes responsible for phenotypic variation, is an important prerequisite to our understanding of species' ability to adapt to different or changing ecological conditions. The newly developed markers and the characterization of SNPs within the parts of the genome examined can contribute in an essential way to the investigation of the genetic basis of adaptive variation in Scots pine populations, making it possible to identify substrate-specific populations.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Author contributions

MH: Conceptualization, Funding acquisition, Data curation, Investigation, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. EGT: Conceptualization, Data curation, Investigation, Methodology, Visualization, Validation, Writing – original draft, Writing – review & editing. EIM: Methodology, Investigation, Validation, Writing – review & editing. JS: Methodology, Validation, Writing – review & editing. ZK: Methodology, Investigation, Validation, Writing – review & editing. ZAK: Conceptualization, Data curation, Investigation, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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