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Clinal variation in *PHY* (PAS domain) and *CRY* (CCT domain)—Signs of local adaptation to light quality in Norway spruce

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Abstract

Detection of the genomic basis of local adaptation to environmental conditions is challenging in forest trees. Phytochromes (*PHY*) and cryptochromes (*CRY*) perceive the red (R)/far-red (FR) and blue light respectively, thus playing a fundamental role in regulating plant growth and development. PHYO and PHYP from conifers are the equivalents of PHYA/PHYC and PHYB in angiosperms, respectively. Norway spruce shows an adaptive latitudinal cline for shade (low R:FR or FR-enriched light) tolerance and requirement of FR light for its growth. We analyzed the exome capture data that included a uniquely large data set of 1654 Norway spruce trees sampled across many latitudes in Sweden to capture the natural clines for photoperiod and FR light exposure during the growth season. Statistically significant clinal variation was detected in allele and genotype frequencies of missense mutations in coding regions belonging to well-defined functional domains of *PHYO* (PAS-B), *PHYP2* (PAS fold-2), *CRY1* (CCT1) and *CRY2* (CCT2) that strongly correlates with the latitudinal gradient in response to variable light quality in Norway spruce. The missense SNP in *PHYO* resulting in Asn835Ser, displayed the steepest cline among all other polymorphisms. We propose that these variations in the photoreceptors represent signs of local adaptation to light quality.

KEYWORDS
cline, cryptochrome, missense mutation, photoreceptor, phytochrome, polymorphism, SNP

1 | INTRODUCTION

Light plays a vital role in the regulation of plant growth and development. Phytochrome (*PHY*) and cryptochrome (*CRY*) code for the key photoreceptors that perceive the red (R)/far-red (FR) and blue light respectively and, play fundamental roles in regulating the developmental processes throughout the plant life cycle by sensing the light quality, photoperiod and modulating the light signaling pathway (Casal, 2000). *PHY* and *CRY* mutants have been described in plant model systems, for example, *Arabidopsis thaliana* (*Arabidopsis*) that are sensitive to R/FR wavelength (Franklin & Quail, 2010; Whitelam & Devlin, 1997) and blue light (Yu et al., 2010). Unlike the angiosperm model plant *Arabidopsis*, conifer *PHY*s and *CRY*s are not well characterized for the molecular mechanisms regarding their mode of action regulating different light sensing pathways. Five *PHY*s have been characterized in *Arabidopsis*—*PHYA*, *PHYB*, *PHYC*, *PHYD* and *PHYE* (Casal, 2000). There are only two major *PHY*s denoted as *PHYO* and *PHYP* in conifers, while *PHYN* is a subtype of *PHYO* (Mathews, 2010). *PHYO* phylogenetically diverged leading to *PHYA* and *PHYC* in angiosperms; likewise, *PHYP* phylogenetically diverged

leading to *PHYB*, *PHYD* and *PHYE* in angiosperms (Schneider-Poetsch et al., 1998). Similarly, the three *CRY*s are well studied in *Arabidopsis*—*CRY1*, *CRY2* and *CRY3* (Ponnu & Hoecker, 2022), whereas in conifers only *CRY1* and *CRY2* have been reported so far (Pashkovskiy et al., 2021; Ranade & García-Gil, 2013; Ranade et al., 2019a).

The R:FR ratio under sunlight is 1.2 (Smith, 1994; Warrington et al., 1989). Under the vegetative shade, there is a decrease in the R:FR ratio (0.2–0.8) as R is absorbed by the chlorophyll and other leaf pigments, whereas the FR is reflected (Ballare et al., 1987). Plants perceive shade as a decrease in the R:FR ratio, where there is a higher FR than R. Species that become established and compete well in fully shaded conditions are termed shade tolerant (Grebner et al., 2021). Shade conditions are similar to astronomic shade or twilight, which is also characterized by a low R:FR ratio (Nilsen, 1985) or the FR-enriched light. The geographic location of Sweden leads to a pronounced latitudinal difference in the duration of twilight when the northern latitudes receive longer daily exposure to FR-enriched light (twilight) as compared with the southern latitudes during the growing season (Supporting Information: Figure S1 and references therein).

Local adaption in plants tends to render higher mean fitness to the local populations in their native environment. Detection of the genomic basis of local adaptation to environmental conditions is challenging in forest trees, especially in the long-lived and ancient conifers with large genome sizes, such as Norway spruce (*Picea abies* [L.] H. Harst). Norway spruce is one of the most important conifer species of the Boreal forest that has high economical value. Norway spruce is shade tolerant and it shows a requirement for far-red light to maintain the growth that follows a latitudinal gradient (Clapham et al., 1998; Molmann et al., 2006); however, the underlying mechanism or any genetic correlation with the phenomenon has not been demonstrated. This latitudinal gradient is called as a cline, which is thus defined as gradual change or gradient or a trend in a particular trait/character that varies across a geographical (or latitudinal) range in a species. It is also evident from our previous study that Norway spruce shows the presence of an adaptive cline for shade or FR-enriched light (low R:FR ratio) (Ranade & García-Gil, 2021). Norway spruce seedlings from latitudes across Sweden show significant variation in hypocotyl length when grown under different R:FR ratios (Supporting Information: Figure S2 and references therein). Norway spruce is shade tolerant but the expression analyses did not find *PHY*s or *CRY*s to be involved in shade tolerance in the species (Ranade et al., 2019b). In addition, neither *PHY*s nor *CRY*s were detected to be differentially regulated under shade in the two populations from different latitudes in Norway spruce (Ranade & García-Gil, 2021). Recently, enhanced lignin synthesis and ecotypic variation in defense-related gene expression were reported in Norway spruce in response to shade; the study discussed the local adaptation to extended FR-enriched light as a potential reason for the differential defense-related gene expression (Ranade & Seipel, Gorzsás, et al., 2022). Involvement of photoreceptors in perceiving the light quality motivated our extensive genomic analyses which aimed to reveal possible associations between natural variation in photoreceptors and the latitudinal cline for daily exposure to FR light in Norway spruce across Sweden.

2 | MATERIALS AND METHODS

2.1 | PHYs and CRYs from Norway spruce

Norway spruce homologs for the PHYs and CRYs were retrieved from PlantGenIE (https://plantgenie.org, Norway spruce genome v.1.0) by performing Blastp with the corresponding *Arabidopsis* members from TAIR (https://www.arabidopsis.org/index.jsp). PHYs and CRYs from other species were retrieved from PlantGenIE (https://plantgenie.org) and NCBI (https://www.ncbi.nlm.nih.gov/). The details of all the sequences included in this study are presented in Supporting Information: Table S1. The domain regions of PHYs and CRYs from Norway spruce (Supporting Information: Figures S5–S13) were confirmed by referring to its best match in *Arabidopsis* and by performing searches in the Conserved Domain Database (CDD) (Marchler-Bauer et al., 2015) and UniProt (Bateman et al., 2021), referring to the literature (He et al., 2015; Klar et al., 2007; Li et al., 2011) and by aligning them with *Arabidopsis* sequences using MUSCLE (Edgar, 2004). With the availability of the complete genome of Norway spruce (Nystedt et al., 2013), we revisited the classification of the PHYs and CRYs in this species and analyzed their phylogeny in this work. Phylogenetic trees of protein sequences of PHYs and CRYs from Norway spruce along with the PHYs and CRYs from a few model plants (e.g., *Arabidopsis*, soybean, eucalyptus and poplar) were constructed for further validation, using phylogeny.fr in the 'one click mode' using default settings (https://www.PHYlogeny.fr/) (Dereeper et al., 2008). In brief, the alignment was done with MUSCLE (Edgar, 2004), phylogeny was done using PHYML (Guindon et al., 2010) which is based on the maximum-likelihood principle and the phylogenetic tree was prepared using TreeDyn (Chevenet et al., 2006).

2.2 | Plant material, DNA extraction and sequencing

A total of 1654 individuals (unrelated parents) originating from different latitudes across Sweden, were included in this study. The 1654 individuals were sampled from half-sib families originated from mother trees selected from natural forests from different latitudes (provenances). Only one tree per progeny was sampled and the mothers of each progeny were unrelated. These individuals are a subset of the data set from an earlier analysis by Chen et al. (2021) that has reported the structure of the Swedish populations of Norway Spruce, which shows that there are two distinct groups—one includes central and southern populations in Sweden, while the other includes the populations from northern Sweden. Earlier studies in Norway spruce and Scots pine have reported adaptive latitudinal cline in response to light that steepens from latitude 61°N across Sweden (Clapham et al., 1998; Ranade & García-Gil, 2013). Since we aimed to explore the basis for this cline, therefore we divided the populations by latitudes. For the current analysis, trees were divided into six populations, S1–S6. S1 comprised 245 trees from latitudes

55–57, S2—213 trees from latitude 58, S3—187 trees from latitudes 59–60, S4—213 trees from latitudes 61–62, S5—573 trees from latitudes 63–64 and S6—223 trees from latitudes 65–67. This grouping did not follow any strategy. However, we considered that all the different groups of populations are continuous populations allowing free pollen exchange. In addition, we also considered the fact that the quality of light differs latitude-wise from south to north in Sweden; during the growing season (summer), the northern latitudes receive an extended period of FR light as compared with the southern ones.

Details regarding DNA extraction, exome capture, genotyping and single nucleotide polymorphisms (SNPs) annotation have been previously described in Baison et al. (2019). In short, total genomic DNA was extracted from 1654 trees, from dormant buds, using the Qiagen Plant DNA extraction (Qiagen) following the manufacturer's protocol and DNA quantification was performed using the Qubit ds DNA Broad Range (BR) Assay Kit. DNA library preparation and exome capture sequencing were performed at the RAPiD Genomics. Sequence capture was performed using the 40, 018 diploid probes designed and evaluated for *P. abies* (Vidalis et al., 2018). Sequencing was performed using an Illumina HiSeq. 2500 instrument on the 2 × 100 bp sequencing mode.

2.3 | Determination of nucleotide variation in PHYs and CRYs

Raw reads were mapped against the Norway spruce reference genome v.1.0 and variant calling was performed using GATK HAPLOTYPECALLER v.3.6 (Van der Auwera et al., 2013). The resultant SNPs were annotated using default parameters for SNPEFF 4 (Cingolani et al., 2012). The vcf file was filtered using settings; --min-alleles 2 --max-alleles 2 --maf 0.01 --remove-indels --minQ 10 --max-missing 0.9. *PHYO*, *PHYN*, *PHYP1*, *PHYP2*, *CRY1*, *CRY2* and *CRY3* were analyzed for the presence of SNPs and possible clines associated with those SNPs. Only bi-allelic SNPs were included in this study. The vcf file containing data from the exome sequencing results of 1654 trees that includes the photoreceptors from the current analysis, is deposited in Zenodo, which is the open-access repository developed under the European OpenAIRE program and operated by CERN (https://doi.org/10.5281/zenodo.7065024) (Ranade & García-Gil, 2022). Allele and genotype frequencies were determined using SNPassoc statistical package (González et al., 2007). Analysis of variance and Tukey's posthoc tests (Bonferroni *p* values) were applied to determine the statistical significance of the difference in the allele and genotype frequencies across the populations included in the study. Genetic diversity among the six different populations (pairwise F_{ST} estimates) was estimated using DnaSP 6 (Rozas et al., 2017) including both the synonymous + missense SNPs detected within *PHYO*, *PHYP2*, *CRY1* and *CRY2*. Allele frequencies in each population regarding these four genes were calculated and then regressed on population latitude. R^2 of the linear regression was computed as the proportion of total variance of latitude explained by the frequency of each marker (Berry & Kreitman, 1993). R^2 is the goodness-of-fit of the linear regression model.

3 | RESULTS

3.1 | Phylogeny of PHYs and CRYs

The phylogenetic analysis of phytochromes is in accordance with the previous work in this area (Clapham et al., 1999; Garcia-Gil, 2008; Mathews, 2006, 2010; Mathews, Clements, et al., 2010). The phylogenetic tree of phytochromes (Supporting Information: Figure S3) shows that there are three PHYs (PHYP, PHYN and PHYO) in Norway spruce as described earlier. The tree shows two PHYPs (PHYP1 and PHYP2) in Norway spruce similar to poplar where two PHYBs (PHYB1 and PHYB2) were identified (Howe et al., 1998). Cryptochromes have not been described extensively in conifers in the literature, however, the phylogeny of CRYs have been described in other plant species (Cao et al., 2020; Cashmore et al., 1999). The CRY phylogenetic tree (Supporting Information: Figure S4) reveals the presence of CRY1, CRY2 and two CRY3s in Norway spruce. Details of the sequences used in the construction of the phylogenetic trees are included in the Supporting Information: Table S1. Supporting Information: Figures S5–S13 represent the alignments of Norway spruce PHYs and CRYs with the respective members in *Arabidopsis*, which shows the different domains of the photoreceptors are well conserved, except for PHYP2 and CRY3 (only partial sequences of CRY3 were detected).

Full-length sequences were detected for all the photoreceptors except for both the CRY3 sequences that were found to be partial. In the case of PHYO, PlantGenIE searches retrieved three partial sequences—MA_6809p0010, MA_6809p0020, and MA_6809p0030, which were annotated as PHYA in PlantGenIE. However, these three sequences were combined to form the full-length PHYO protein, which is well-conserved with *Arabidopsis* PHYA (Supporting Information: Figure S6). This combined sequence is denoted as MA_6809 in the current work.

3.2 | Detection of SNPs in PHYs and CRYs

Details of the position and type of SNP along with sequence information and allele frequencies for all four genes are represented in Supporting Information: Table S2. The current work was focused on the missense SNPs in the coding regions, as these polymorphisms cause a change in the protein sequence, which may contribute to modify the protein conformation and a possible alteration in their mode of action. The five missense SNPs showing changes in their allele and genotype frequencies along a latitudinal cline belong to the conserved domains of the respective photoreceptors with well-defined functions namely PAS domain of PHY and the CCT/CCE domain of CRY. PAS is a short form from the names of the proteins in which imperfect repeat sequences were first recognized: *Drosophila* period clock protein (PER), vertebrate aryl hydrocarbon receptor nuclear translocator (ARNT) and *Drosophila* single-minded protein (SIM) (Nambu et al., 1991). The CRY1 and CRY2 contain the CRY1 C-terminal domain (CCT1) or CRY1 C-terminal extension (CCE1) and

CRY2 C-terminal domain (CCT2) or CRY2 C-terminal extension (CCE2), respectively.

SNPs were detected in the coding regions of *PHYO*, *PHYP2*, *CRY1* and *CRY2*, whereas no SNPs were found in *PHYN*, *PHYP1* and *CRY3*. The allele and genotype frequencies of these SNPs showed a gradient across the latitudes from south to north. In other words, these SNPs followed a latitudinal cline. One missense (Asn835Ser, PAS-B) and one synonymous mutation (Leu806Leu, PAS-B), both following a latitudinal cline, were detected in *PHYO*. In *PHYP2*, two missense (Met120Ile, PAS fold-2; Met120Val, PAS fold-2) were found to follow a cline, while two missense (Ile41Val, Thr71Ile) and two synonymous mutations (Ser45Ser; Val107Val, PAS fold-2) did not associate with the cline. Regarding *CRY*, one missense following a cline was detected in *CRY1* (Gly589Val, CCT1/CCE1), while in *CRY2*, one missense (His418Asn, CCT2/CCE2) varied along a latitudinal cline and one synonymous did not (Leu420Leu, CCT2/CCE2). The mutations without cline confirm that only specific SNPs display the cline. In addition, our earlier work with the same Norway spruce populations reported presence of cline in differentially expressed genes in response to light quality (Ranade & García-Gil, 2021). The same study reported a few other genes that were randomly chosen, which were not differentially expressed and did not show any cline in SNPs; these genes were discussed as the control genes. A total of 30 SNPs were detected in these control genes, 19 were missense mutations and 11 were synonymous mutations; none of these SNPs showed the cline.

Three missense polymorphisms in the photoreceptors following a latitudinal cline resulted in alteration of the amino acid with similar chemical properties (Supporting Information: Table S2). Asn835Ser (PHYO) involves a change in the amino acid with a similar chemical property; polar, uncharged Asn gets altered to Ser which is also polar and uncharged. Similarly, Met120Ile and Met120Val in PHYP2 resulted in a change of an amino acid that is nonpolar and hydrophobic to another amino acid with the same chemical property. Two missense SNPs were detected that involved the alteration of an amino acid to another amino acid with a different chemical property. Gly589Val (CRY1) resulted in the alteration of a polar, uncharged amino acid to a nonpolar, hydrophobic and His418Asn (CRY2) resulted in a change from polar, basic amino acid to a polar, uncharged one. The alteration of an amino acid to another amino acid with a different chemical property would modify the mode of action of the protein largely as compared with the alteration of the amino acid to another amino acid with a similar chemical property.

3.3 | Latitudinal clines in the allele and genotype frequencies of detected SNPs

The missense SNP in *PHYO*—Asn835Ser, displayed a statistically significant latitudinal cline in the allele and genotype frequencies (Figure 1) which is the steepest cline compared with the other missense SNPs in *PHYP2*, *CRY1* and *CRY2* that showed moderate clines (Supporting Information: Tables S2–S4, Figures S14–S16). This is also evident from the highest F_{ST} value along with higher and less dispersed R^2 values of *PHYO* as compared with the other photoreceptors (Figure 2). The pair-wise F_{ST} estimates for six populations of Norway spruce across Sweden are shown in Table 1, which includes seven missense and four synonymous SNPs from four photoreceptors. In accordance with the literature on Norway spruce (Chen et al., 2016) F_{ST} values are low but increase with the distance due to lower gene (pollen) exchange. A low F_{ST} value suggests low population genetic differentiation and it is typical of outcrossing conifer species.

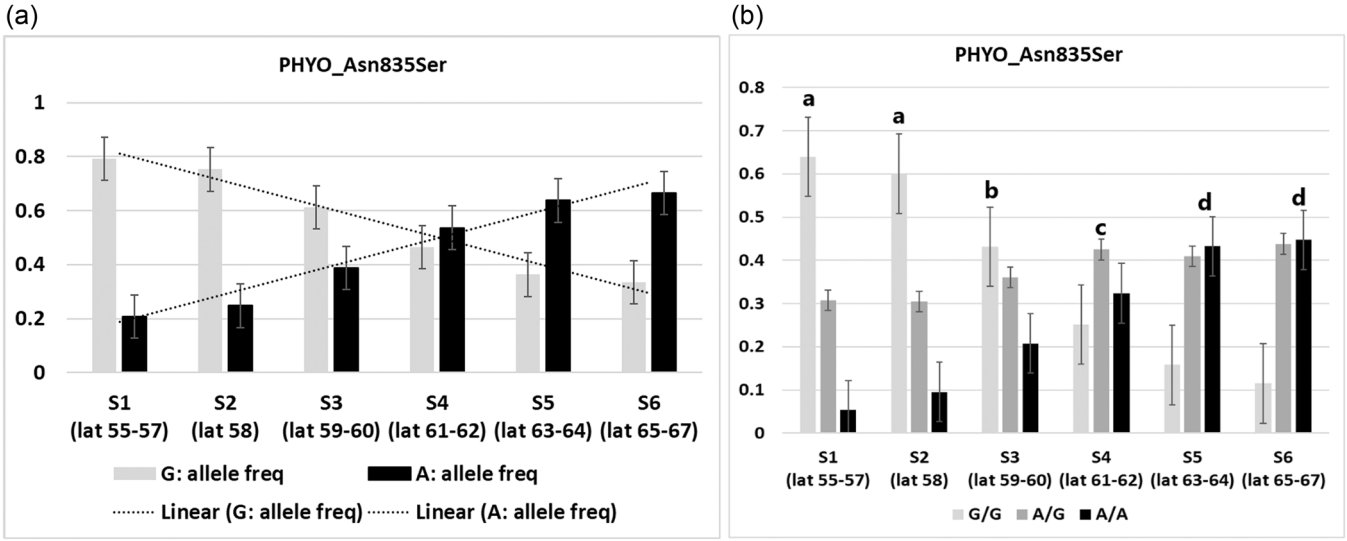

FIGURE 1 Cline regarding variation in allele and genotype frequencies of single nucleotide polymorphisms in the *PHYO* gene in Norway spruce populations across Sweden. (a) Allele frequencies of Asn835Ser. (b) Genotype frequencies of Asn835Ser. One-way analysis of variance and Tukey's posthoc test was performed with the genotype frequencies. Tukey's posthoc categorization is indicated above the bars.

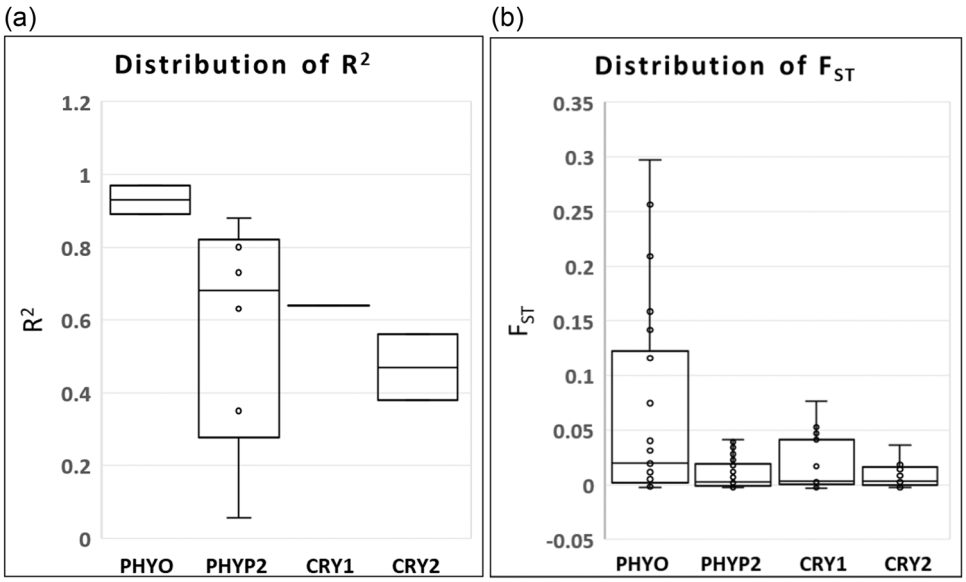

FIGURE 2 R^2 and F_{ST} were calculated considering the missense and the synonymous single nucleotide polymorphisms detected in the particular candidate gene. (a) The distribution of R^2 with reference to allele frequencies across the four photoreceptors showing clines in allele frequencies in the Norway spruce populations in Sweden. (b) The distribution of F_{ST} across the four photoreceptors showing clines in allele frequencies in the Norway spruce populations in Sweden.

TABLE 1 Pairwise F_{ST} estimates for six populations of Norway spruce across Sweden involved in the analysis for detection of latitudinal clines in allele frequencies of the SNPs of phytochromes and cryptochromes.

Population	S2	S3	S4	S5	S6
S1	0.00	0.005661	0.006079	n.a.	0.008693
S2		0.00221	0.002378	n.a.	0.003764
S3			0.00	n.a.	0.00
S4				n.a.	0.00039
S5					n.a.

Abbreviations: n.a., not available; SNP, single nucleotide polymorphism.

4 | DISCUSSION

The individuals included in this study are the subset of an earlier investigation (Chen et al., 2021) that discussed the structure of the Swedish populations of Norway Spruce; it reports two distinct groups—one includes central and southern populations in Sweden, while the other includes the populations from northern Sweden. The low F_{ST} estimates reported by the current analysis (Table 1) are supported by earlier research. A study by Wang et al. (2020) reported lower genetic diversity of Norway Spruce across several populations in Central-Europe, Sweden–Norway and Finland. Several other studies reveal a very low population genetic structure (low F_{ST}) in Norway spruce based on the highly polymorphic microsatellite markers (Androsiuk et al., 2013; Stojnić et al., 2019). However, the high population differentiation for adaptive traits such as the timing of budburst or timing of bud-set contrast with the low F_{ST} values (Notivol et al., 2007; Thomas, 2009).

From the phylogenetic analysis (Supporting Information: Figure S3), the alignments (Supporting Information: Figures S5–S9) and based on previous investigations in phytochromes (Mathews, 2010; Schneider-Poetsch et al., 1998), it can be proposed that PHYO and PHYP from conifers are the equivalents of PHYA/PHYC and PHYB in angiosperms, respectively. Duplication of *PHYA* resulted in the formation of *PHYC* and this duplication appears to have occurred before the diversification of angiosperms (Mathews & Sharrock, 1997). The alignments (Supporting Information: Figures S5–S9) show that the different domains in PHYs are well conserved in conifers. The PAS fold-2 domain of PHY is located in the N-terminal region that acts as a light sensing region, while PAS-A and PAS-B domains belong to the C-terminal region which is the regulatory region that is essential for dimerization, nuclear translocation and for modulating phytochrome signaling (Montgomery & Lagarias, 2002; Rockwell et al., 2006). As the PAS domains are critical for light sensing and signaling, mutations in them lead to impaired/altered response to R/FR light (Neff et al., 2000; Paul & Kurana, 2008). For example, missense mutations in the PAS fold-2 domain of PHYA from *Arabidopsis* showed impaired responses to R/FR light (Wang et al., 2011; Yanovsky et al., 2002) and Ile143Leu polymorphism in PAS fold-2 domain of PHYB from *Arabidopsis* was associated with variation in R light response (Filiault et al., 2008). Likewise, the CCT domain from *CRY* is involved in the blue light signal output (Wang et al., 2015; Yang et al., 2000). In the current study, missense SNPs detected in the coding regions of the respective domains may have an impact on the structure of the proteins. This can cause an alteration in the mode of their action. Considering the essential role of photoreceptors in light quality response, the latitudinal variation in the mentioned SNPs may explain the differential response of the Norway spruce populations to light quality (Clapham et al., 1998; Molmann et al., 2006; Ranade & García-Gil, 2021). The mutations

causing a change of an amino acid into another with different chemical properties, for example, Thr71Ile in PHYP2 (Supporting Information: Table S2), will alter the protein function largely, modifying its sensitivity to light quality. However, further investigation is required to validate the mechanism of action of the altered PHY/CRY under variable light-quality treatments in Norway spruce.

The cline in the missense mutations in *PHY*s, especially the cline detected in *PHYO* (Asn835Ser) which is the steepest cline among all SNPs included in this analysis shows a strong correlation with the cline regarding the response to FR-enriched light reported by earlier investigations (Clapham et al., 1998; Ranade & García-Gil, 2021) (Figure 3, Supporting Information: Figure S2). Previously, a significant excess of nucleotide diversity was reported in photoreceptor genes such as *PHY* and *CRY* in Norway spruce, however, this study did not report any specific cline associated with the variation (Källman et al., 2014). Latitudinal cline was reported in few genes such as MYB DOMAIN PROTEIN 3 and SCREAM 2, that were differentially regulated under shade in different Norway spruce populations (Ranade & García-Gil, 2021). In the same study, low F_{ST} values were also reported, suggesting low population genetic differentiation, similar to the current analysis. Neither *PHY* nor *CRY* was found to be differentially regulated in Norway spruce in response to the shade while comparing the northern and southern populations (Ranade & García-Gil, 2021). Norway spruce is well known for displaying a latitudinal cline in response to FR light (Clapham et al., 1998; Molmann et al., 2006; Ranade & García-Gil, 2021).

PHYO from conifers is the equivalent of PHYA/PHYC in angiosperms as mentioned before; PHYO may be involved in dual functions performed by PHYA and PHYC with reference to flowering. PHYA mediates FR light promotion of flowering with modes of action similar to CRY2; both show a diurnal rhythm in short-day plants acting as day sensors (Mockler et al., 2003). PHYC perceives R/FR light and plays a major role in photomorphogenesis (Li et al., 2019) and is essential in photoperiod depended flowering, particularly under long-day photoperiod (Chen et al., 2014; Woods et al., 2014). Natural variation in *PHYC* is associated with variation in flowering and growth responses in angiosperms (Balasubramanian et al., 2006; Saïdou et al., 2009). *Flowering locus T* (*FT*) plays a central role in the induction of flowering that is regulated by photoreceptors (Böhlenius et al., 2006; Mockler et al., 2003) and the photoreceptors in turn perceive and respond to light wavelength. An *FT* gene was found to be involved in the photoperiodic control of bud-set in a tree species—poplar (Böhlenius et al., 2006). A homolog of *FT* shows a significant correlation between its expression and bud-set or growth cessation in Norway spruce (Gyllenstrand et al., 2007; Karlgren et al., 2013). In addition, clinal variation was observed in *FT* expression levels that increased with latitude in Norway spruce (Chen et al., 2012). In this context, the cline with the missense polymorphism in *PHYO* in Norway spruce can be correlated with the latitudinal gradient in the expression levels of the *FT* gene along with the cline in response to variation in FR light, which needs further molecular validation.

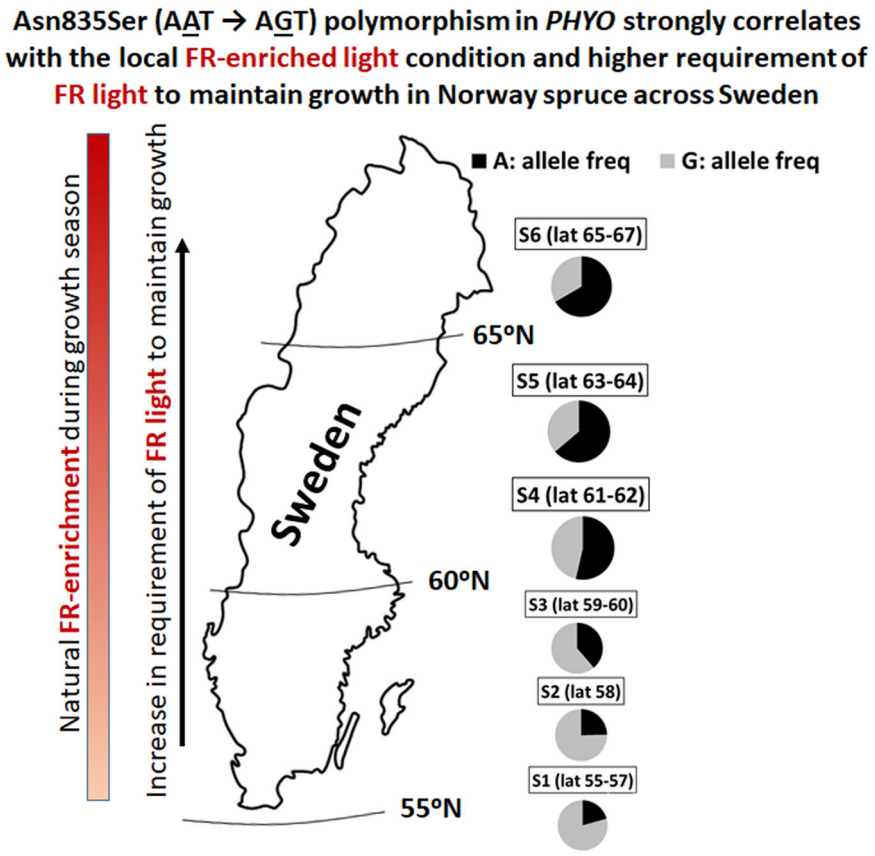

FIGURE 3 Cline regarding the requirement of FR light strongly correlates with the cline in Asn835Ser (AAT to AGT) polymorphism in *PHYO* in the Norway spruce populations in Sweden. The pie charts in the figure represent the allele frequency of the A to G polymorphism in the six Norway spruce populations included in the study.

Natural variation in *PHYs* associated with sensitivity to R/FR light has been identified in *Arabidopsis*, for example, polymorphism in *PHYA* (Maloof et al., 2001) associated with FR and polymorphism in *PHYB* associated with R (Filiault et al., 2008). This further supports the current finding of natural variation in *PHYs* being linked with the earlier reported cline to the requirement for FR and clinal variation in response to low R:FR in Norway spruce as discussed before. PHYA and PHYB appear to have complementary functions in the processes related to seedling development and flowering (Reed et al., 1994). Clinal variation in *PHYB2* in *Populus tremula* was associated with growth cessation and bud-set (Ingvarsson et al., 2006), while control of growth cessation and bud-set by *PHYA* was demonstrated in hybrid aspen (Olsen et al., 1997). *Arabidopsis* mutants in *CRY1/CRY2* are linked to differential response to photoperiod. A *CRY1* point mutation leads to early flowering under short-day conditions and is hypersensitive to blue, R and FR light, in hypocotyl growth inhibition in *Arabidopsis* (Exner et al., 2010). Likewise, a variation in *CRY2* showed a difference in flowering response to photoperiod (El-Din el-Assal et al., 2001; Olsen et al., 2004). Particularly in *Picea*, genes harboring SNP associations with bud-set were found to be similar to *PHYA* and *CRY* in *Picea sitchensis* (Holliday et al., 2010). Latitudinal clines in response to light quality and the processes related to the response to photoperiods such as growth-cessation and bud-set/bud-burst have been described in Norway spruce populations across Sweden (Clapham et al., 1998; Sogaard et al., 2008), yet their association with SNPs in photoreceptors is not mentioned. The clinal variation in the allele/genotype frequencies in photoreceptors observed in the current analysis may be a probable explanation for the clines previously described for growth-cessation and bud-set in Norway spruce.

5 | CONCLUSIONS

Light signaling regulates plant development throughout the plant life cycle. Light responses are mediated by the photoreceptors that play a central role in regulating the light pathways. The genetic diversity of SNPs in the *PHYs* detected in the current work correlates with the latitudinal cline for exposure to a low R:FR ratio in natural conditions. This is the first report so far in conifers, where precise and statistically significant latitudinal clines for SNP variation have been observed in *PHYs*. We propose that Norway spruce populations have adapted to the latitudinal variation in exposure to low R/FR ratio by encompassing their growth rhythms (bud-set/bud-burst) with the local natural light conditions. The knowledge gained by this analysis could be applied to designing strategies in breeding programs for Norway spruce.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The vcf file containing data from the exome sequencing results of 1654 trees that includes the photoreceptors from the current analysis, is deposited in Zenodo (https://doi.org/10.5281/zenodo.7065024). All other data are included in the supplementary information.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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