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Multivariate models improve accuracy of genomic prediction for spring frost tolerance in Norway spruce

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Abstract

Warming spring temperatures increase the risk of frost damage to emerging Norway spruce (*Picea abies*) buds by advancing their spring phenology and increasing the frost event frequency. We present a field-based electrolyte leakage assay to assess basal frost tolerance in newly emerging buds. Using this assay, we estimated genetic parameters and tested the effectiveness of multivariate genomic selection (GS) models integrating frost tolerance, bud burst phenology, and height growth. Multivariate models significantly improved frost tolerance prediction accuracy, particularly when incorporating bud burst data, due to a strong genetic correlation between traits ($r \approx -0.63$) and high heritability of bud burst ($h^2 \approx 0.60$). The observed genetic correlations suggest that early-flushing genotypes exhibit higher tolerance to spring frost. Our findings underscore the importance of basal frost tolerance as a complementary trait to traditional phenological frost-avoidance strategies. Additionally, we emphasize that early-stage bud burst assessments in controlled environments can accelerate genomic predictions, overcoming the limitations imposed by long growth cycles. Integrating multi-trait genomic prediction models optimized with bud burst as an assisting trait and optimized model parameters enhances prediction accuracy

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Abbreviations: AD, additive and dominance; AIC, Akaike information criterion; BB, bud burst; EBV, estimated breeding value; EL, electrolyte leakage; FD, frost damage; FT, frost tolerance; GBLUP, genomic-based best linear unbiased prediction; GS, genomic selection; MVGBLUP, multivariate genomic-based best linear unbiased prediction; MVPBLUP, multivariate pedigree-based best linear unbiased prediction; PA, predictive ability; PACC, prediction accuracy; PBLUP, pedigree-based best linear unbiased prediction; REML, restricted maximum likelihood; SNP, single nucleotide polymorphisms; TS, training set; VS, validation set.

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of spring frost tolerance and supports the development of climate-resilient breeding strategies in Norway spruce.

Plain Language Summary

Global warming is advancing the timing of bud burst of Norway spruce (*Picea abies*) by increasing early spring temperatures. In the northern hemisphere, advancing bud burst is making the early developing shoots vulnerable to frost, which can lead to damaged trees and reduced forest health. We developed a laboratory test to measure frost tolerance in developing shoots and examined how this trait is linked to the timing of bud burst and tree height. Using multivariate genomic models, we tested whether including data on the timing of bud burst could improve the prediction of frost tolerance. Our results show that trees with earlier bud development have a better natural frost resistance and that including the timing of bud burst in the multivariate models improved the ability to predict frost tolerance. This improvement is due to the strong genetic link between bud burst and frost tolerance. These findings can help improve breeding strategies to handle spring frost as the climate changes.

1 | INTRODUCTION

The sixth assessment report by the Intergovernmental Panel on Climate Change projects an average global temperature increase of up to 4˚C by the end of the 21st century (Pörtner et al., 2021). Warming is expected to be even more pronounced at higher latitudes, with Arctic temperature increases anticipated to exceed the global average (Pörtner et al., 2021; Price et al., 2013). Rising global temperatures are likely to intensify climate-related abiotic disturbances and lead to an earlier onset of the thermal growing season, particularly in northern ecosystems (Gauthier et al., 2015; Pan et al., 2023; Park et al., 2019). In Northern Europe, climate models predict that the timing of spring leaf development is advancing more rapidly than the last occurrence of frost, primarily driven by warmer spring temperatures (Scheifinger et al., 2003; Schwartz et al., 2006). This shift forces trees to initiate deacclimation and budburst before the risk of frost has subsided, increasing their vulnerability to frost-induced damage (Aalto et al., 2022; Barichivich et al., 2012; Chang et al., 2021; Liu & Zhou, 2018). The boreal forest, which stores approximately 30% of the world's forest carbon (Pan et al., 2013), is predominantly composed of two evergreen coniferous tree species in Northern Europe, Scots pine (*Pinus sylvestris*) and Norway (Schmidt-Vogt, 1977). Understanding the impacts of early spring warming on the growth and carbon sequestration of these dominant species is crucial for assessing the long-term resilience of the boreal biome to climate change (Aalto et al., 2022; Barichivich et al., 2012).

In spring, changes in daylength and temperature initiate the deacclimation process in conifers, leading to a reduction in freezing hardiness and the emergence of new growth. In Norway spruce, rising spring temperatures trigger this deacclimation process, with the rate of tissue dehardening accelerating in response to increasing temperature (Aronsson, 1975; Taulavuori et al., 2004). Although the precise regulatory mechanisms behind these events remain largely unknown, cellular and tissue-level deacclimation begins before visible changes occur in the plant organs (Repo, 1992). Interestingly, the minimum freezing hardiness of conifer buds does not coincide with budbreak (Bigras et al., 2004); instead, both Norway spruce and Scots pine are particularly susceptible to frost damage (FD) during the shoot elongation phase (Christersson, 1971; Langvall et al., 2001; Repo, 1992). Spring frost events can significantly reduce the growth of Norway spruce seedlings, particularly by damaging the photosynthetic tissues of newly emerged shoots, ultimately leading to reduced biomass production (Langvall & Örlander, 2001; Lundmark, 1996). Moreover, frost events during the growing season have been identified as a more critical predictor of annual height increment than mean annual or summer temperatures (Marquis et al., 2020).

In Norway spruce, the timing of bud burst (BB) is largely determined by strong genetic control, balancing the need to maximize the growth period while minimizing the risk of FD (Chen et al., 2018, 2019; Zhou et al., 2020). This genetic control is evident in the temperature sum requirements for BB, which correlates with the latitude of origin—southern populations generally require a higher temperature sum than northern populations (Beuker, 1994; Hannerz, 1994a). In

Norway spruce breeding programs in Sweden, this genetic control over BB is leveraged to reduce the risk of FD from spring backlashes (Chen et al., 2018; Jansson et al., 2013). Seedlings from southern populations, relocated to the northern region, exhibit delayed BB and prolonged growth cessation compared to local provenances, offering an advantage in frost-prone environments (Hannerz, 1996; Rostad, 1988).

Temperature is the primary selective force shaping tree populations in Northern Europe, with frost serving as a strong agent of natural selection, particularly in Norway spruce (Eriksson, 1998; Frank et al., 2017; Howe et al., 2003). Frost backlashes occurring at the onset of the growing seasons are stochastic events, driven more by local conditions than regional climates (Hannerz, 1994a). This localized and unpredictable nature of frost may allow a portion of the population to avoid selective pressure for low-temperature adaptation, thereby preserving genetic variation in frost tolerance (FT) within the population (Howe et al., 2003). Genetic variation in spring FT in trees has primarily been studied in species like apricot (*Prunus armeniaca* L.) (Guerriero et al., 2006) and sweet cherry (*Prunus avium* L.), focusing on the frost sensitivity of different stages of flower bud development (Kaya & Kose, 2022). In Swedish conifer populations, significant variation in spring FT has also been observed, indicating that different trees respond differently to frost events (Hannerz, 1994a; Karlsson & Högberg, 1998; Mullin & Lee, 2013). The evolutionary importance of these frost events for the survival of northern tree species highlights the need for further investigation into FT in actively growing tissues, known as basal FT. However, this area of research has received relatively little attention to date. Nevertheless, substantial population variation in basal freezing tolerance has been observed in non-acclimated tissues of other species, such as *Rhododendron* (Lim et al., 1998) and balsam poplar (*Populus balsamifera* L.) (Menon et al., 2015). Evaluating spring FT by assessing the susceptibility of non-acclimated tissue to freezing temperatures can provide more precise phenotyping data on FT. Currently, Norway spruce breeding programs incorporate frost primarily by evaluating trees that have already experienced FD in the field, limiting experimental planning and complicating long-term studies (Chen et al., 2023, 2021). This approach indirectly estimates FT and does not isolate the basal FT of the tissue, because FT is correlated with phenological traits such as the timing of BB (Chang et al., 2021). Collecting precise phenotypic data on basal FT could facilitate the use of genomic prediction methods in Norway spruce. This would not only enhance our understanding of the genetic basis of spring FT but also inform breeding strategies to improve the resilience of tree populations.

Genomic selection (GS), also known as genomic prediction, is a genome-assisted approach used to estimate breeding values by utilizing a realized relationship matrix instead of recorded pedigrees. This technique increases genetic gains by improving prediction accuracy (PACC) and facilitates early selection, which is particularly valuable for coniferous species with long life cycles. GS modeling involves using phenotypic data to train models that predict an individual's breeding values based on their genotypic information (Meuwissen et al., 2001). Recently, GS has been proposed to shorten breeding cycles in broadleaved tree species such as eucalyptus (*Eucalyptus* spp.) (Estopa et al., 2023; Tan et al., 2017), and conifers like pine (*Pinus* spp.) (Bartholomé et al., 2016; Calleja-Rodriguez et al., 2020; Resende et al., 2012; Ukrainetz & Mansfield, 2020) and spruce (*Picea* spp.) (Fuentes-Utrilla et al., 2017; Gamal El-Dien et al., 2015; Lenz et al., 2017; Resende et al., 2012). In Norway spruce, GS has been explored for traits such as tree height, wood quality (Chen et al., 2018, 2019; Zhou et al., 2020), timing of budburst (Chen et al., 2023), drought tolerance (Laverdière et al., 2022), and biotic disease resistance (Lenz et al., 2020). Abiotic and biotic stress traits are often difficult to measure and require non-standardized and time-consuming methods, making them challenging for traditional breeding programs. To address these challenges, multivariate models, long established in animal breeding and increasingly applied in plants and trees (Henderson & Quaas, 1976; Jia & Jannink, 2012; Lee & van der Werf, 2016; Matějka, 2017), have been extended to GS. When traits are genetically correlated and measured with sufficient precision, multivariate models can enhance the PACC relative to univariate GS (Covarrubias-Pazaran et al., 2018; Jia & Jannink, 2012). Multivariate models are especially advantageous for predicting traits with low heritability by utilizing correlated traits with higher heritability (Guo et al., 2014; Klápště et al., 2020; Thompson & Meyer, 1986). Although the utility of multivariate GS models has

Core Ideas

- Warming springs advance spring phenology in Norway spruce increasing frost damage risk in newly formed buds.
- Early-flushing Norway spruce genotypes have higher spring frost tolerance compared to late-flushing trees.
- Genomic selection models using bud burst as an assisting trait can substantially improve frost tolerance predictions.
- Basal spring frost tolerance in Norway spruce breeding is an essential trait to decrease spring frost risk.
- Multivariate genomic selection aids breeding climate-resilient Norway spruce.

been explored in forest trees like *Eucalyptus* (Ferreira et al., 2022; Rambolarimanana et al., 2018) and white spruce (*Picea glauca*) (Laverdière et al., 2022; Rashidi-Jouybari et al., 2022) studies on their effectiveness for Norway spruce are still limited. These models hold significant potential for improving the breeding efficiency, particularly for traits that are challenging to measure directly.

The primary objective of this study was to determine whether genetic variance in basal spring FT during bud flushing exists within a Swedish Norway spruce breeding population, contributing to the implementation of GS in Norway spruce breeding programs. Given the strong selective pressures imposed by temperature and extreme climate conditions on boreal flora, we introduced a novel electrolyte leakage (EL) assay to directly quantify basal FT. We hypothesized that variation in FT may be expressed during the phenologically vulnerable stage of BB. The study had two specific objectives: (1) to characterize the genetic basis of spring FT trait and its relationship with BB and tree height, and (2) to assess the predictive performance of univariate and multivariate GS models for spring FT and to evaluate their potential applicability for enhancing Norway spruce breeding programs.

2 | MATERIALS AND METHODS

2.1 | Genetic material

A Norway spruce clonal progeny trial was established in 2011 in Northern Sweden (64° 9′ N and 19° 35′ E) using a randomized incomplete block design. The trial consisted of unimproved forest stand material from the average origin latitude of 65.4° and improved material from breeding population 1–3 with the average origins of 65.9° N latitude and 21° E longitude (Skogforsk, the Forestry Institute of Sweden). Formal identification of tree material used in this study was conducted by Adam Klingberg (Skogforsk, the Forestry Institute of Sweden). The trial had 88 incomplete blocks, each containing 60 trees, with a total of 1586 genotypes from 50 families. Among these, 38 families were control-pollinated, involving 57 unrelated parental genotypes, with 63% (36 out of 57) parents crossed only once and the rest of parents crossed twice. The number of genotypes per family ranged from 5 to 42, with an average of 32 genotypes per family. Each genotype was represented by one to eight ramets, averaging 3.3 trees per genotype. For this study, a subset of 665 genotypes across the 38 control-pollinated families was randomly selected for genotyping and only living trees with height of >50 cm were included in the sample selection. These genotypes were represented by 1939 trees in the progeny trial. Each family contributed 12–20 genotypes, with one to eight ramets per genotype, averaging 2.9 ramets per genotype.

2.2 | Phenotyping

2.2.1 | FD assessment

FT was evaluated using an EL assay to assess FD from freshly sampled Norway spruce shoots. Developing shoots were collected at BB stage 3, the most frost-sensitive stage (Glerum, 1973; Hannerz, 1994b; Repo, 1992). For each tree, three to five samples were tested in separate assays. Due to the rapid progression of BB and limited testing capacity, assessments were conducted over three consecutive springs (2019–2021). Each year, between 360 and 855 samples were tested for EL (Table S1). In total, FD was assessed in 492 unique trees during the 3-year period, with approximately 20% of the trees reevaluated in subsequent years for year-to-year comparisons. A total of 218 genotypes from 38 control-pollinated families were tested, with each family contributing five to 22 genotypes and 2.3 ramets were tested per genotype.

2.2.2 | EL assay

FD in needle tissue was quantified using the EL assay, following the protocol by Sutinen et al. (1992). Eight needles at BB stage 3 (Krutzsch, 1973) were excised from each sample and placed in glass vials containing 1 mL of ultrapure water (Milli-Q), immediately placed on ice. A control for each sample was maintained on ice in a separate vial at +4°C, while the experimental samples underwent a freezing treatment.

The assay used a programmable temperature-controlled bath (Julabo FP45), set initially to +2°C. The temperature was then decreased by −1°C per hour. Ice formation was induced with liquid nitrogen-cooled metal probe when the bath temperature reached −1°C. After the temperature reached −3°C and held for 15 min, samples were incubated on ice at +4°C overnight. Following incubation, 1 mL of Milli-Q water was added to each vial, and all vials were placed on a shaker at room temperature for 24 h. Initial EL (C_b) was measured using a conductivity meter (CDM210 Radiometer). Samples were then flash-frozen in liquid nitrogen, returned to room temperature, and shaken for 12 h before final conductivity (C_a) was recorded.

EL was calculated as $\mathrm{EL} = (R_1 + R_2)/2$, where R_1 represents leakage from undamaged control tissue and R_2 represents leakage after cold exposure. Individual leakage percentages (R_1 or R_2) were determined using the formula $\frac{C_b}{C_a} \times 100$, with C_b as the initial conductivity and C_a as the final conductivity after freezing.

For each tree, we sampled three to five independent subsamples (shoots collected in different places) and within each subsample mixed eight needles as technical replicates. The EL assay was conducted and calculated per subsample, and results were averaged per tree prior to spatial analyses (Section 2.4).

2.2.3 | Tree height and BB

In early June 2020, tree height and BB phenology were measured at 9 years of age. Height was recorded for all living trees, measured from the ground to the terminal bud. BB progression was categorized using an eight-stage scale developed by Krutzsch (1973), where stage 0 indicated a quiescent bud (later BB), stage 3 marked the initiation of BB, and stage 8 represented full bud proliferation and needle dispersion, indicating earlier timing of BB.

2.3 | Genotyping

Fresh needles were sampled from 665 genotypes in the springs of 2020 and 2022. Total genomic DNA was extracted using the Qiagen plant DNA extraction protocol. DNA quality and quantity were assessed using the Qubit dsDNA Broad Range Assay Kit (Qiagen). Genotyping was performed using the Norway spruce *Piab50K* single nucleotide polymorphisms (SNPs) array chip (Bernhardsson et al., 2021). Detailed methodologies for SNP calling, data filtering, quality control, and imputation are provided in Chen et al. (2023). After filtering and imputation, 47,092 SNPs were retained for downstream analyses.

2.4 | Spatial analyses

To account for microenvironmental heterogeneity in the field trial, a mixed linear model incorporating spatially correlated residuals was applied to each trait by using ASReml software v4.1 (Gilmour et al., 2015). The model included year as a fixed effect only for FD (assessed across 2019–2021), modeled incomplete block as a random effect, and specified residuals with a first-order autoregressive process in both row and column directions (an AR1 × AR1 structure). This spatial model effectively controlled for block and spatial trends and follows standard methodologies for field trial analysis (Berlin et al., 2017; Dutkowski et al., 2002). Environment-adjusted individual tree phenotypic values, obtained by removing the estimated block and spatial effects, as well as the year effect for FD, and adding back overall mean, were subsequently used as input data for genetic analyses.

2.5 | Estimation of genetic variance components and heritability in univariate models

To estimate genetic variance components and heritability for each trait, four univariate mixed linear models were applied using either pedigree-based best linear unbiased prediction (PBLUP) or genomic-based best linear unbiased prediction (GBLUP). The models are defined as follows:

1. Additive genetic model (GBLUP-A/PBLUP-A): Incorporates only additive genetic effects: $y = X\beta + Z_a a + e$
2. Additive + dominance model (GBLUP-AD/PBLUP-AD): Includes both AD genetic effects: $y = X\beta + Z_a a + Z_d d + e$
3. Additive + residual genetic model (GBLUP-AR/PBLUP-AR): Accounts for additive effects and an additional random residual genetic term to capture unexplained genetic variance: $y = X\beta + Z_a a + Z_r r + e$
4. Additive + dominance + residual genetic model (GBLUP-ADR/PBLUP-ADR): Combines additive, dominance, and residual genetic effects: $y = X\beta + Z_a a + Z_d d + Z_r r + e$

In these models, y represents the vector of adjusted phenotypic values for a given trait (from the spatial analysis), β is the vector of fixed effects (e.g., the overall mean), and a, d, and r are vectors of additive, dominance, and residual random genetic effects, respectively. The incidence matrices X, Z_a, Z_d, Z_r relate observations to these effects in models where they are included. All random effects were assumed to have zero means; their (co)variance structures are specified above.

In the pedigree-based models (PBLUP; Henderson and Quaas, 1976), additive (a) and dominance (d) effects are assumed to follow $a \sim \mathrm{N}(0, A\sigma_a^2)$ and (if included) $d \sim \mathrm{N}(0, D\sigma_d^2)$, where A and D are the pedigree-derived additive and the dominance numerator relationship matrices (Henderson & Quaas, 1976), and σ_a^2 and σ_d^2 are the additive and dominant variance components, respectively. Minor pedigree inconsistencies identified with genomic data were corrected prior to rebuilding A and D (i.e., pedigree reconciliation guided by G). In the genomic models (GBLUP), the pedigree-based matrices A and D were replaced by genomic relationship matrices: the additive genomic relationship matrix G_a (constructed following Vanraden [2008]) and the dominance genomic relationship matrix G_d (constructed following Vitezica et al. [2013]). All relationship matrices—additive (A), dominance (D), genomic additive (G_a), and genomic dominance (G_d)—were constructed using the AGHmatrix package (Amadeu et al., 2023) within the R computational framework. The residual genetic effect r (included in the AR and ADR models) was modeled as: $r \sim \mathrm{N}(0, I\sigma_r^2)$ with its variance σ_r^2 estimated by restricted maximum likelihood (REML) and I is an identity matrix, assuming this term is independent for each genotype. This r term captures additional genetic variance not explained by additive or dominance effects, potentially due to epistatic interactions or other nonadditive influences at the clonal level. All models included a residual error term e, assumed to follow: $e \sim \mathrm{N}(0, I\sigma_e^2)$, where I is the identity matrix, and σ_e^2 is the residual variance. Variance components for all random effects were estimated for each trait using REML in ASReml (Gilmour et al., 2015).

Heritability estimates were derived from the variance component estimates for each trait. Narrow-sense heritability (h^2) the ratio of additive genetic variance to phenotypic variance (Falconer & Mackay, 1996). In the simplest additive model (A), h^2 was calculated as: $h^2 = \widehat{\sigma_a^2}/(\widehat{\sigma_a^2} + \widehat{\sigma_e^2})$, where $\widehat{\sigma_a^2}$ represents the estimated additive genetic variance and $\widehat{\sigma_e^2}$ the estimated residual variance; in the additive + dominance model (AD): $h^2 = \widehat{\sigma_a^2}/(\widehat{\sigma_a^2} + \widehat{\sigma_d^2} + \widehat{\sigma_e^2})$, where $\widehat{\sigma_d^2}$ is the estimated dominance genetic variance; in the additive + residual genetic model (AR): $h^2 = \widehat{\sigma_a^2}/(\widehat{\sigma_a^2} + \widehat{\sigma_r^2} + \widehat{\sigma_e^2})$, where $\widehat{\sigma_r^2}$ is the estimated residual genetic variance; and in the additive + dominance + residual genetic model (ADR): $h^2 = \widehat{\sigma_a^2}/(\widehat{\sigma_a^2} + \widehat{\sigma_d^2} + \widehat{\sigma_r^2} + \widehat{\sigma_e^2})$. Broad-sense heritability (H^2) was defined as the proportion of total phenotypic variance explained by total genetic variance, including both additive and nonadditive components. In the AD, AR, and ADR models, broad-sense heritability was calculated as follows: (1) AD model: $H^2 = (\widehat{\sigma_a^2} + \widehat{\sigma_d^2})/(\widehat{\sigma_a^2} + \widehat{\sigma_d^2} + \widehat{\sigma_e^2})$; (2) AR model: $H^2 = (\widehat{\sigma_a^2} + \widehat{\sigma_r^2})/(\widehat{\sigma_a^2} + \widehat{\sigma_r^2} + \widehat{\sigma_e^2})$; and (3) ADR model: $H^2 = (\widehat{\sigma_a^2} + \widehat{\sigma_d^2} + \widehat{\sigma_r^2})/(\widehat{\sigma_a^2} + \widehat{\sigma_d^2} + \widehat{\sigma_r^2} + \sigma_e^2)$.

2.6 | Genetic correlation between traits estimated in multivariate models

To estimate genetic correlations among FD, BB, and height (HT), a multivariate mixed linear model was applied by using data from 218 clones with FD, BB, and HT jointly available, because FD was assessed on this subset. A trivariate analysis was conducted using either pedigree-based or genomic relationship matrices, corresponding to multivariate pedigree-based best linear unbiased prediction (MVPBLUP) and multivariate genomic-based best linear unbiased prediction (MVGBLUP), respectively. The multivariate model was formulated as follows:

$$y = X\beta + Z_a u + e \tag{5}$$

where y is the vector of adjusted phenotypic values for the three traits combined, β is the vector of fixed effect (including the overall mean), and u is the vector of random genetic effects (breeding values) for the three traits. The random effects were assumed to follow a multivariate normal distribution: $a \sim \mathrm{N}(0, G \otimes A)$ (for pedigree-based analysis) or $a \sim \mathrm{N}(0, G \otimes G_a)$ (for genomic analysis), where G is the additive genetic variance-covariance matrix: $G = \begin{bmatrix} \sigma_{a_1}^2 & \sigma_{a_1a_2} & \sigma_{a_1a_3} \\ \sigma_{a_1a_2} & \sigma_{a_2}^2 & \sigma_{a_2a_3} \\ \sigma_{a_1a_3} & \sigma_{a_2a_3} & \sigma_{a_3}^2 \end{bmatrix}$. Here $\sigma_{a_1}^2$, $\sigma_{a_2}^2$, and $\sigma_{a_3}^2$ represent the additive genetic variances for the FD, BB, and HT, respectively, while the off-diagonal elements ($\sigma_{a_1a_2}$, $\sigma_{a_1a_3}$, and $\sigma_{a_2a_3}$) represent the additive genetic covariances between each trait pair. The symbol $\otimes$ denotes the Kronecker product, and A or G_a corresponds to the pedigree-based or genomic-based additive relationship matrix, respectively. The residuals were assumed to follow: $e \sim \mathrm{N}(0, R \otimes I)$, where R is the residual variance-covariance matrix $R = \begin{bmatrix} \sigma_{e_1}^2 & \sigma_{e_1e_2} & \sigma_{e_1e_3} \\ \sigma_{e_1e_2} & \sigma_{e_2}^2 & \sigma_{e_2e_3} \\ \sigma_{e_1e_3} & \sigma_{e_2e_3} & \sigma_{e_3}^2 \end{bmatrix}$, where $\sigma_{e_1}^2$, $\sigma_{e_2}^2$, and $\sigma_{e_3}^2$ represent the residual variances for FD, BB, and HT, respectively, and the off-diagonal terms ($\sigma_{e_1e_2}$, $\sigma_{e_1e_3}$, and $\sigma_{e_2e_3}$) represent the corresponding residual covariances between each trait pair. The matrix I is an identity matrix for the clones (Hallingbäck et al., 2018; Wei & Borralho, 1998). All other notation (X, Z_a) follows the definitions used in the univariate model, appropriately expanded for multiple traits. The multivariate model was fitted in with ASReml, yielding REML estimates of all required variance and covariance components.

From the multivariate analysis, additive genetic covariances between each pair of traits were estimated. These were used to calculate genetic correlations (r_g) between traits i and j, following standard multivariate genetic theory (Mardia et al., 2024): $r_g = \frac{\widehat{\sigma_{a_ia_j}}}{\sqrt{\widehat{\sigma_{a_i}^2}\widehat{\sigma_{a_j}^2}}}$, where $\widehat{\sigma_{a_ia_j}}$ is the estimated additive genetic covariance between traits i and j, and $\widehat{\sigma_{a_i}^2}$ and $\widehat{\sigma_{a_j}^2}$ are the estimated additive genetic variances for traits i and j, respectively.

2.7 | Cross-validation test on the GS accuracy evaluation

The accuracy of genomic prediction for FD was evaluated using a cross-validation approach. For each prediction model (e.g., PBLUP-A and GBLUP-A), a 10-fold cross-validation procedure was conducted, repeated over 100 iterations. In each iteration, averaged clonal data were randomly partitioned into 10 equal subsets (folds). Of these, nine folds (90%) containing both genomic and average phenotypic clonal data were assigned to the training set (TS), while the remaining one-fold (10%) of genotypes was designated as the validation set (VS). The models were trained on the TS, and predictions were subsequently generated for clones in the VS. The predictive ability (PA) of each model was quantified as the Pearson correlation coefficient between the estimated breeding values (EBVs) and the corresponding adjusted average phenotypic clonal values in the VS (Chen et al., 2018). Further, PACC was defined as the PA adjusted for trait heritability: $\mathrm{PACC} = \frac{r(\mathrm{EBV,\ phenotype})}{\sqrt{h_{\mathrm{clone}}^2}}$ where $r(\mathrm{EBV,\ phenotype})$ is the Pearson correlation coefficient described above, and h_{clone}^2 is the narrow-sense heritability of the trait based on clone mean data, calculated from the dataset averaged for each clone (Dekkers, 2007; Resende et al., 2012). In addition to PACC, we report PA (raw Pearson correlations) for all models and relatedness scenarios in Table S2. All analyses were conducted using ASReml v4.1 (Gilmour et al., 2015) and R software. The mixed model analyses were implemented in ASReml, and custom R scripts were used for cross-validation and statistical tests.

2.8 | Impact of relatedness between training and VSs in the GS model

The impact of genetic relatedness between TS and VS on PACC was assessed by evaluating three partitioning scenarios with varying degrees of relatedness: (1) Close relatedness: Individuals from the same family were distributed across both TS and VS sets to maximize genetic relatedness between the sets. (2) Random relatedness: Individuals were randomly assigned to either the TS or VS without consideration of genetic relatedness. (3) Distant relatedness: To minimize genetic relatedness, we implemented a leave-family-out method, in which entire families present in VS were absent from TS. For GBLUP/PBLUP analysis, we verified these contrasts by examining the distribution of off-diagonal TS-VS entries in the additive relationship matrix (G/A), which were centered near zero under the distant scenario. Each scenario was implemented by partitioning the data into folds accordingly before performing the 10-fold cross-validation, as described above. The prediction accuracies of the GBLUP-A and PBLUP-A models were then compared across these scenarios. To statistically evaluate the impact of genetic relatedness on model performance, a nonparametric Kruskal–Wallis H test was conducted using the kruskal.test function (Acar & Sun, 2013). When significant differences were detected ($p \leq 0.05$), post hoc pairwise comparisons with Bonferroni adjustment were performed to identify which relatedness groups differ significantly, using the pairwise.wilcox.test function. All statistical tests were conducted in the R 4.1.3 framework.

2.9 | Impact of related traits on FD prediction in multivariate GS models

We hypothesized that incorporating correlated traits could improve PACC for FD (Henderson & Quaas, 1976; Jia & Jannink, 2012). To test this hypothesis, three multivariate models were constructed using different trait combinations: (1) FD + HT: A bivariate model combining FD and height (HT). (2) FD + BB: A bivariate model combining FD and BB. (3) FD + BB + HT: A trivariate model including FD, BB, and height (HT). In each scenario, only FD data were hidden (treated as missing) in the VS, while data for the correlated traits (BB and/or height) remained available. This setup reflects a practical application where FT would be predicted for individuals with measurements for earlier or more easily assessed traits (e.g., BB, height) but without direct FD evaluations. To assess the effectiveness of the multivariate models, the same 10-fold cross-validation procedure (100 replicates across the three relatedness partitions) was applied to each model. PACC for FD was then computed as described above. To determine whether the multivariate approach provided a significant advantage, PACC values from each multivariate model were compared against the corresponding univariate model. Specifically, for each relatedness scenario and relationship matrix (pedigree-based or genomic), statistical comparisons were conducted using paired Wilcoxon signed-rank tests (pairwise.wilcox.test function). Significant differences were reported where applicable ($p \leq 0.05$).

2.10 | Impact of dataset of helping trait on FD prediction in multivariate GS models

To evaluate the impact of helping trait availability (BB) on the PACC of FD, we conducted bivariate FD+BB analyses using three distinct datasets, as summarized in Table S3: (1) 218NoData: a dataset comprising 218 clones, with 22 clones in the VS for which no phenotypic data were available. (2) 218BB: the same 218-clone dataset, but with 22 clones in VS where FD data were hidden, while BB data remained available. (3) 665BB: an expanded dataset containing 665 clones, with 22 clones in VS where FD data were hidden but BB data were available. This design allowed us to assess how increasing the dataset size and providing helpful trait information (BB) for additional individuals—including those in VS—affects the PA of FD. For each dataset, we compared the PACC of FD obtained from the multivariate model against that from the corresponding univariate model under the same relatedness scenario. As in previous analyses, paired Wilcoxon signed-rank tests (pairwise.wilcox.test function) were used to identify significant differences between multivariate and univariate predictions. All multivariate analyses were conducted in ASReml and statistical tests were conducted using R.

3 | RESULTS

3.1 | Multi-year measurements of spring FD

FD was phenotyped using EL assay conducted over three consecutive spring seasons in newly emerged Norway spruce shoots. EL measurements were generally consistent across

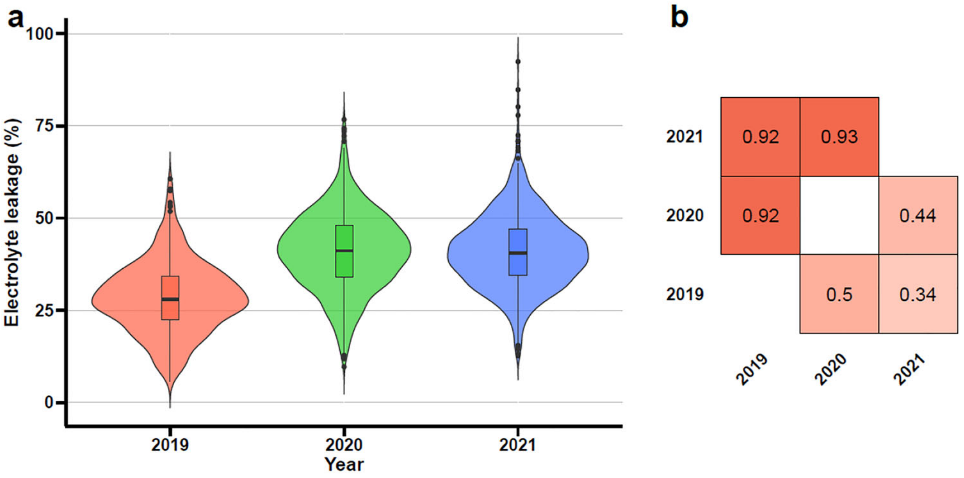

FIGURE 1 Summary of electrolyte leakage measurements for 2019, 2020, and 2021. Figure (a) shows a violin plot overlaid with boxplots representing the percentage of electrolyte leakage for the years 2019, 2020, and 2021. Figure (b) is a matrix showing both phenotypic (lower right panel) and genetic (upper left panel) correlations of electrolyte leakage between the years 2019, 2020, and 2021.

TABLE 1 Descriptive statistics of three traits measured and analyzed for the Norway spruce.

Trait	No. of trees	No. of clones	Min	Max	Mean	SD	CV	Unit
Frost damage	492	218	9.12	52.22	28.56	6.96	24.36%	%
Height	1939	665	0.29	28.18	11.02	3.95	35.84%	dm
Bud burst	1917	665	0	6	2.12	0.82	38.57%	score

Note: Frost damage data have been adjusted to remove the average age difference.
Abbreviations: CV, coefficient of variation; SD, standard deviation.

years, except for 2019, where average leakage was lower (Figure 1a).

Aside from this offset in level, the year-to-year trends were similar. To ensure robust comparisons across years, 20% of the trees were resampled in subsequent years, with representation maintained across all families. This approach provided a consistent dataset over time. The phenotypic correlation of FD between years ranged from 0.34 to 0.50, while the genetic correlation between years was high, approximately 0.92 (Figure 1b). The strong genetic correlation indicates that despite moderated phenotypic repeatability, the EL assay captures a consistent genetic signal, which supports the aggregation of data from all 3 years, enabling FD to be treated as a unified trait in subsequent genetic parameter estimations and genomic prediction analyses.

3.2 | Estimates of genetic variance components and heritability

FD was first measured in 2019, followed by tree height and BB assessments in 2020, to evaluate the impact of FD on growth and phenology. A detailed statistical summary of each trait is provided in Table 1. Genetic parameters for each trait were evaluated using tree-level data. Height and BB resulted in higher coefficient of variance—35% for height and 38% for BB—compared to 24% for FD. Genetic parameters for each trait were assessed using eight genetic models, incorporating either conventional corrected pedigree-based data (PBLUP) or genomic-based pedigree data (GBLUP). These models estimated additive effects (A), AD effects, additive and genetic residual effects (AR), and ADR effects. The genetic results, including Akaike information criterion (AIC) values, genetic variance components, and heritability estimations, are presented in Table 2 and Table S4. Across all traits, the GBLUP-AR model consistently showed the lowest AIC values, indicating the best fit compared to other models. Dominance models (PBLUP-AD, GBLUP-AD, PBLUP-ADR, and GBLUP-ADR) did not outperform AR models, likely due to the limited number of families generated by each parent in the crossing scheme. Notably, AIC values were slightly lower for GBLUP models compared to PBLUP models, suggesting marginally better performance of genomic-based models.

Narrow-sense and broad-sense heritability estimates were consistent across all three traits. For FD, narrow-sense heritability (h^2) was approximately 0.24 in additive-only models (PBLUP-A and GBLUP-A) and about 0.19 in AR models (PBLUP-AR and GBLUP-AR), while broad-sense heritability (H^2) in AR models was about 0.24. BB exhibited the

TABLE 2 Summary of Akaike information criterion (AIC), genetic variance components, and heritability for all models by accounting for genetic matrices.

Trait	Model	AIC	$\widehat{\sigma_a^2}$	$\widehat{\sigma_r^2}$	$\widehat{\sigma_e^2}$	h^2	H^2
Frost damage	PBLUP-A	2381.49	11.83(3.44)[b]		37.76(3.00)	0.24(0.06)	
	PBLUP-AR	2382.15	9.48(4.60)	2.42(3.97)	37.19(3.11)	0.19(0.09)	0.24(0.06)
	GBLUP-A	2381.73	11.35(3.31)		37.92(3.01)	0.23(0.06)	
	GBLUP-AR[a]	2380.22	8.69(4.32)	2.90(3.97)	37.20(3.11)	0.18(0.08)	0.24(0.06)
Height	PBLUP-A	7164.97	3.36(0.61)		12.81(0.49)	0.21(0.03)	
	PBLUP-AR	7164.56	2.15(0.72)	0.95(0.55)	12.64(0.49)	0.14(0.04)	0.20(0.03)
	GBLUP-A	7163.67	2.67(0.53)		13.12(0.49)	0.17(0.03)	
	GBLUP-AR	7162.69	1.55(0.49)	1.28(0.47)	12.64(0.49)	0.10(0.03)	0.18(0.03)
Bud burst	PBLUP-A	180.81	0.4(0.03)		0.26(0.01)	0.61(0.02)	
	PBLUP-AR	180.81	0.40(0.03)	0(0)	0.26(0.01)	0.61(0.02)	0.61(0.02)
	GBLUP-A	179.92	0.4(0.03)		0.26(0.01)	0.6(0.02)	
	GBLUP-AR	179.23	0.32(0.05)	0.04(0.03)	0.26(0.01)	0.51(0.06)	0.58(0.03)

[a] Letter in bold represents the best model with the smallest Akaike Information Criterion (AIC) value compared to all models.

[b] Standard error (SE) is represented in parentheses.

highest heritability, with h^2 ranging from 0.51 to 0.61 and $H^2 = 0.58 - 0.61$, while height (HT) displayed lower heritability ($h^2 = 0.10 - 0.21$, $H^2 = 0.18 - 0.21$) (Table 2). For both FD and height, nonadditive genetic variance ($\widehat{\sigma_r^2}$) estimates were significant, exceeding 20% of the estimated additive genetic variance ($\widehat{\sigma_a^2}$) in both PBLUP-AR and GBLUP-AR models. In contrast, no nonadditive genetic variance was observed for BB in the PBLUP-AR model, with only minimal nonadditive variance detected in the GBLUP-AR model.

Comparing heritability estimates (h^2 or H^2) between GBLUP and PBLUP models, GBLUP captured between approximately 81% ($h^2 = 0.17$ in GBLUP-A vs. $h^2 = 0.21$ in PBLUP-A for height) and approximately 100% of the genetic variances estimated by PBLUP ($H^2 = 0.24$ in both GBLUP-AR and PBLUP-AR for FD). Because heritability is directly derived from model-based variance components, these comparisons indicate that the two methods partition genetic variance in closely similar ways in our data. In addition, pedigree-based models tend to slightly overestimate additive genetic components compared to marker-based models (Calleja-Rodriguez et al., 2021). Accordingly, both PBLUP and GBLUP models performed comparably in estimating heritability across traits. Additionally, standard error in heritability estimates for height and BB were lower than those for FD (Table 2).

In the AR models, the estimated additive genetic variance ($\widehat{\sigma_a^2}$) was generally lower compared to the A models for both PBLUP and GBLUP approaches. This reduction in additive variance led to h^2 estimates in the AR models (Table 2). However, the estimated residual genetic variance ($\widehat{\sigma_r^2}$) partially compensated for the reduced additive variance, resulting in H^2 estimates in the AR models that were comparable to h^2 of the A models (Table 2). These findings suggest that models estimating only additive variance (PBLUP-A or GBLUP-A) may overestimate additive effect estimates by inadvertently capturing nonadditive effects.

3.3 | Effects of relatedness between training and VSs on PACC

A cross-validation study was conducted to assess PACC, focusing on both PA and PACC at the clone level for the conventional breeding model (PBLUP-A) and the GS model (GBLUP-A). This analysis considered three levels of relatedness between TS and VS across three traits: FD, height, and BB. For FD, differences between the GBLUP-A and PBLUP-A models were minimal (Figure 2), though PBLUP-A showed a slight improvement when TS and VS were distantly related compared to GBLUP-A. For height and BB, the GBLUP-A model outperformed PBLUP-A across relatedness levels. GBLUP-A demonstrated a significant improvement over PBLUP-A, with an average PACC of 0.48 versus 0.38 for height and 0.60 versus 0.51 for BB. While differences were marginal for FD (0.35 vs. 0.36) (Table S2).

PA values displayed similar trends to PACC (Figure S1; Table S2). PACC was calculated as PA divided by the square root of clone mean heritability (h_c shown in Table S5), which evaluates the theoretical accuracy of breeding values used in the selection programs. PA values aligned with the clone mean heritability of each trait, while PACC values were independent of such heritability. For example, despite height exhibiting the lowest clone mean heritability, its PACC values were generally higher than those for FD (Figure 2).

The study revealed significant differences in PACC based on the level of relatedness between TS and VS. Models

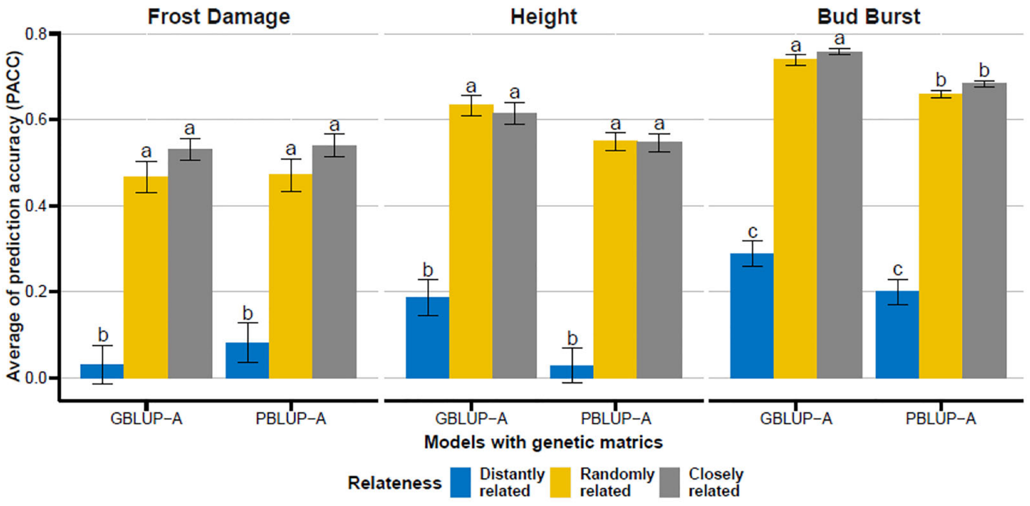

FIGURE 2 Average prediction accuracy (PACC) estimates for two prediction models (GBLUP-A and PBLUP-A) under three relatedness scenarios: distantly related, randomly related, and closely related. Black error bar represents the standard error calculated from 100 repetitions. Different letters above the bars indicate statistically significant differences between the prediction models and relatedness scenarios, as determined by the Kruskal–Wallis *H* test at a significance level of $p \leq 0.05$.

constructed with closely related TS and VS (i.e., originating from the same family) had the highest PACC for all traits, with average accuracy ranging from 0.53 to 0.76, depending on the model and trait (Figure 2; Table S2). In contrast, models with distantly related clones between TS and VS (i.e., originating from unrelated families) showed a substantial decline in PACC for both GBLUP-A and PBLUP-A models. For FD in the GBLUP-A model, the average PACC value decreased from 0.53 to 0.03. Similarly, for height, PACC decreased from 0.63 to 0.19, and for BB, from 0.76 to 0.29 (Figure 2; Table S2). Random assignment of clones into TS or VS resulted in slightly lower PACC values than those observed with closely related sets.

3.4 | Impact of traits in multivariate models on FD prediction

To enhance the PACC for FD, multivariate models were evaluated by incorporating traits with significant genetic correlation to FD, such as height and BB (Figure 3). A moderate positive correlation was observed between FD and height, with values of 0.36 in the MVGBLUP model and 0.45 in the MVPBLUP model, and a weaker negative correlation between BB and height, with values of −0.24 and −0.31, respectively. The analysis revealed a strong negative genetic correlation between FD and BB, with values of −0.63 in the MVPBLUP model and −0.70 in the MVPBLUP model. These genetic correlations suggest that incorporating these traits into multivariate models has the potential to improve the PACC for FD, particularly when the assisting trait is strongly correlated and well measured.

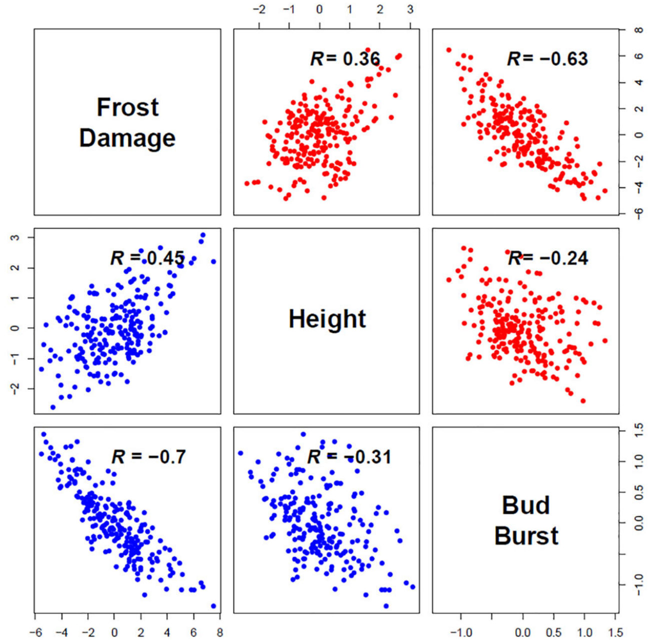

FIGURE 3 Genetic correlations among frost damage, height, and bud burst. The lower left panel represents the breeding values estimated using the pedigree-based multivariate model (MVPBLUP) for these three traits, while the upper right panel shows the breeding values estimated from the genomic-based multivariate model (MVGBLUP) for the same traits. The *x*- and *y*-axes in each panel represent breeding values of the respective traits (in their original measurement units: frost damage in % electrolyte leakage, bud burst in score units 0–8, and height in diameter). The *R* values in each figure indicate the genetic correlation between each pair of traits under these two different model estimations.

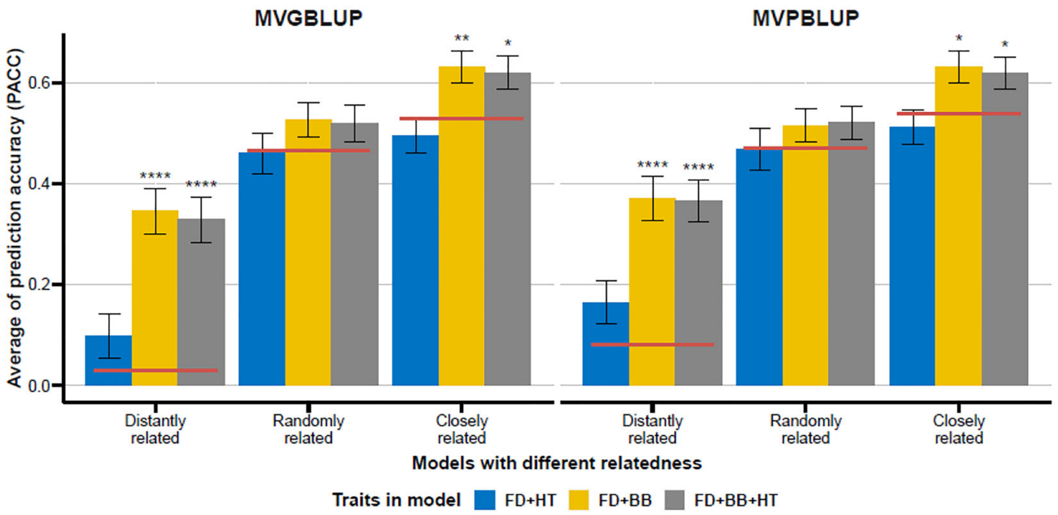

FIGURE 4 Average prediction accuracy (PACC) of frost damage in univariate and multivariate models with different trait combinations across levels of relatedness. Each colored bar plot shows the average PACC for frost damage prediction in multivariate models that include combinations of traits: frost damage and height (FD + HT), frost damage and bud burst (FD + BB), and all three traits (FD + BB + HT). The red lines represent the average PACC values for frost damage from each of a univariate model for comparison. The *x*-axis represents three levels of relatedness between training set and validation set. Each scenario was conducted using two types of models: pedigree-based multivariate model (MVPBLUP) and genomic-based multivariate model (MVGBLUP). Error bars denote the standard error (SE) calculated from 100 repetitions. Asterisks (*) indicate the level of significance compared to the univariate model based on a Wilcoxon test, $*p < 0.05$; $**p < 0.01$; $****p < 0.0001$.

Multivariate models, particularly those incorporating FD + BB, significantly outperformed the univariate models in predicting FD, especially when TS and VS were distantly related. In the MVGBLUP model, the average PACC increased from 0.03 to 0.35, while the MVPBLUP model showed more than a fourfold increase, from 0.08 to 0.37 (Figure 4). Even in cases of closely related TS and VS, a notable improvement of approximately 19% was observed, with PACC increasing from 0.53 to 0.63. However, for randomly related TS and VS, improvements were minimal, with PACC rising only slightly from 0.47 to 0.52. The multivariate model incorporating FD and height (FD + HT) yielded only marginal improvements in PACC under distantly related TS and VS, with no significant changes or slight declines observed at other relatedness levels (Figure 4). Incorporating all three traits (FD + BB + HT) did not result in significant improvements over the FT + BB model, and the performance of the three-trait model was often slightly worse than that of the FT + BB model. Moreover, MVGBLUP models generally performed comparably to the MVPBLUP models.

3.5 | Impact of dataset in multivariate models on FD prediction

Three dataset scenarios were compared to univariate models across three levels of relatedness between TS and VS to understand how the effect of dataset size and inclusion of BB data influences prediction outcomes. In the first scenario, 218 clones were included in the analysis, with 22 clones in the VS but completely devoid of any phenotypic data for individuals in the VS (218NoData). In comparison to the univariate model, this scenario showed a slight improvement in PACC for the MVPBLUP models, with an increase of 0.03 for both distantly and closely related TS and VS (Figure 5). However, other models showed minimal or no improvement, indicating that the presence of BB data only in the TS had limited impact on enhancing PACC in the multivariate framework. In the second scenario, the same number of clones (218) were included in the analysis, again with 22 clones in the VS, but in this case, BB data were available for individuals in the VS (218BB). This scenario, the same as FD + BB, resulted in a considerable improvement in PACC, particularly for distantly related TS and VS. PACC increased from 0.03 to 0.35 in the MVGBLUP model and from 0.08 to 0.37 in the MVPBLUP model. For closely related TS and VS, PACC also improved significantly, with an increase of 0.1 in both models (Figure 5). These findings indicate that the availability of BB data in the VS substantially enhances FD prediction, especially when the genetic relatedness between TS and VS is distant. In the third scenario, the total number of clones increased to 665, with 22 clones still in the VS, and BB data available both in TS and VS (665BB). When comparing this scenario to the 218BB scenario, a higher increase in PACC was observed in both distantly related and randomly related multivariate models. Notably, for distantly related TS and VS, the PACC values in both the MVGBLUP and MVPBLUP models approached the levels observed in univariate models under randomly

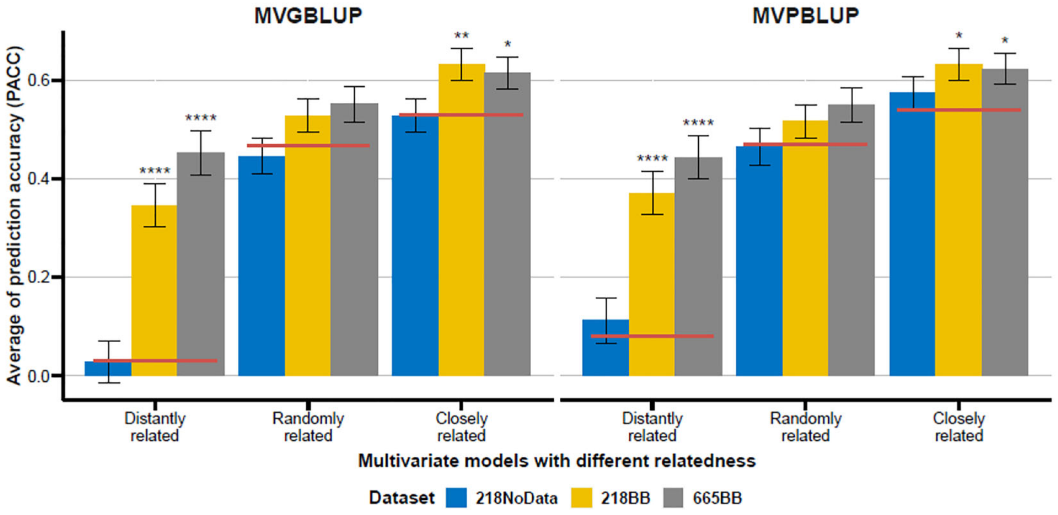

FIGURE 5 Average prediction accuracy (PACC) of frost damage in univariate and multivariate models with different bud burst dataset across levels of relatedness. The colored bar plots represent three dataset scenarios: 218 clones used in bud burst, without data in the validation set (218NoData), 218 clones used in bud burst, with data in the validation set (218BB), 665 clones used in bud burst, with data in the validation set (665BB). The red lines represent the average PACC values for frost damage from each of a univariate model for comparison. The x-axis represents three levels of relatedness between training set and validation set: distantly related, randomly related, and closely related. Each scenario was evaluated using two models: MVPBLUP and MVGBLUP. Error bars denote the standard error calculated from 100 repetitions. Asterisks (*) indicate the level of significance compared to the univariate model based on a Wilcoxon test, $^*p < 0.05$; $^{**}p < 0.01$; $^{****}p < 0.0001$.

related conditions, approximately 0.45 (Figure 5). However, for closely related TS and VS, the PACC in the 665BB scenario was slightly lower than in the 218BB scenario, with a decrease of about 0.02. These findings suggest that increasing the number of clones in the TS can significantly improve the PACC for FD, especially when predicting less related genotypes between TS and VS scenarios. However, the benefits of increasing the TS size are less pronounced for closely related TS and VS, where performance gains tend to plateau or slightly decrease.

4 | DISCUSSION

4.1 | When multivariate BLUP models can enhance PACC of FT

Incorporating genetically correlated assisting traits can enhance the accuracy of predicting a target trait compared to univariate models, provided the assisting trait is strongly correlated and well measured. Forest tree breeders collect data on multiple traits and make selection decisions based on a combination of these traits, making multivariate selection a practical method to guide selection decisions. In the present study, multivariate models exhibited either significant or marginal improvements in PACC for spring FT when used in conjunction with other traits, such as BB and/or height, compared to univariate models (Figure 4). The advantage of multivariate models is trait-dependent (Cappa et al., 2022; Lenz et al., 2020; Rambolarimanana et al., 2018). Specifically, PACC was higher when BB was included as a correlated trait, whereas accuracy estimations decreased when FT was predicted in conjunction with height. These results are explained by the differences in genetic correlation between FD and BB/height (Figure 3), and the differing heritability estimates of these traits (Table 2). BB has a heritability >0.6 and a strong genetic correlation with FT ($r = -0.7$), which together contribute to improved PACC. Previous simulation and empirical studies have demonstrated that PACC increases significantly when the genetic correlation between traits exceeds 0.5 (Calus & Veerkamp, 2011; Cappa et al., 2022; Montesinos-López et al., 2016). Moreover, simulation studies also show that the predictability of traits with low heritability can benefit from the inclusion of a high heritability of assisting traits (Jia & Jannink, 2012; Neyhart et al., 2019). Additionally, adding a third trait did not improve the performance of the two-trait models, which aligns with observations in previous studies (Cappa et al., 2022; Lenz et al., 2020; Rashidi-Jouybari et al., 2022).

These findings highlight the importance of selecting assisting traits with strong genetic correlations and higher heritability to the target trait in multivariate models. In Norway spruce, BB serves as a cost-effective and time-efficient predictor for FT, as it is routinely assessed in breeding programs and exhibits a strong genetic correlation between juvenile and mature growth stages (Danusevičius et al., 2024; Ekberg et al., 1994; Hannerz et al., 1999). This suggests that early-stage BB observations—such as those conducted at the seedling or sapling stage in nursery—can serve as a reliable proxy for

FT, allowing selection decisions to be made early and thereby accelerating the breeding cycle.

4.2 | Impact of relatedness between training and VSs on PACC

Increasing the genetic similarity between TSs and VSs in genomic prediction models has a positive impact on PACC (Chen et al., 2018; Covarrubias-Pazaran et al., 2018; Tan et al., 2017). Our results show that the degree of genetic relatedness between TS and VS critically impacts the PACC of FT in both univariate (Figure 2) and multivariate models (Figures 4 and 5). The models constructed using distantly related populations consistently exhibited lower prediction estimates compared to those using randomly selected or closely related populations, regardless of the model employed. Interestingly, incorporating BB as an assisting trait in multivariate models substantially enhanced PACC for FT, especially when TS and VS were distantly related (Figure 4). This improvement is probably due to the high correlation between BB and FT ($r \approx -0.63$ in our study) as supported by previous studies (Arojju et al., 2020; Guo et al., 2014; Jia & Jannink, 2012). The multivariate approach mitigated the effects of low genetic relatedness, increasing the potential to include more genetically diverse individuals and capture broader genetic variation in breeding programs. Notably, significant gains in PACC were also observed in closely related populations, indicating that multivariate models can provide benefits without necessarily expanding the genetic pool. This finding highlights the population-specific nature of prediction models and the necessity of accounting for population structure to avoid inflated prediction estimates of accuracy (Werner et al., 2020). Therefore, when constructing training populations for genomic prediction, it is important to include individuals that represent the genetic background of the target population to maximize accuracy. Conversely, if predictions are intended to be applied across very diverse or unrelated populations, breeders may need to accept lower PACC or develop population-specific models, unless assisting traits like BB are used to help bridge the genetic gaps.

4.3 | Optimization of multivariate GS strategies

Highly genetically correlated traits can enhance multivariate genomic prediction and offer cost- and time-efficient benefits. However, the availability of trait information can vary. Previous work in crop species has explored optimization of multivariate GS when certain trait data are missing on some individuals, demonstrating the beneficial effects of indirect and assisted multi-trait GS on PACC (Arojju et al., 2020; Fernandes et al., 2018). In this study, we evaluated different strategies to optimize multivariate GS models for predicting FT using BB as an assisting trait (Figure 5). The first strategy involved including BB phenotypic data in the VS, combining genotypic data with phenotypic information of assisting trait (218BB scenario). This approach significantly improved PACC compared to univariate models, particularly in distantly related populations. Including BB data in the VS is practical because BB is routinely collected and can be easily and reliably measured even from young Norway spruce seedlings in a nursery setting. Similar benefits of incorporating assisting trait data in both TS and VS have been widely reported in crops like wheat (Rutkoski et al., 2016; Shahi et al., 2022; Winn et al., 2023), rice (Muvunyi et al., 2022), and sugarcane (Islam et al., 2022). However, before using BB data as an assisted trait from nursery or early field assessments in multivariate GS models, the existing correlation between BB and FD measured in the field should be further validated. The second strategy assessed models where phenotypic data of the assisting trait were omitted from the VS, relying solely on genotypic information (218NoData scenario). This approach is attractive for species with long rotation times, like coniferous trees, since genotyping can be conducted on young seedlings or embryos. However, our results indicated that the multivariate models under such conditions did not outperform univariate models regardless of the relatedness between TS and VS. Earlier studies also reported insignificant differences in prediction accuracies between multi-trait and single-trait models under similar conditions (Bhatta et al., 2020; Calus & Veerkamp, 2011; Muvunyi et al., 2022; Shahi et al., 2022). These results imply that when phenotypic data for the assisting trait are not utilized for the selection candidates, multivariate GS offers no advantage over a univariate approach. In such cases, traditional indirect selection using the correlated trait itself may be equally or more effective. For instance, given the high genetic correlation between BB and FT (~−0.63) and the moderate heritability of FT (~0.24), selecting individuals based on BB alone would be expected to achieve a correlated genetic gain in FT comparable to (or even exceeding) the accuracy of our multivariate GS models. Thus, if BB data on candidates are readily available, a breeder could rely on indirect selection for FT via BB without requiring a genomic model. The third strategy involved increasing the number of clones with BB phenotypic data in the TS (665BB scenario), which improved prediction estimates of FT, particularly in distantly related population. This indicates that expanding the TS with individuals characterized for highly heritable and strongly correlated assisting traits can enhance PACC by mitigating noise and bias in the genomic relationship matrix associated with low relatedness (Lund et al., 2016; Norman et al., 2018). However, the benefits plateaued when TS and VS were closely related, suggesting a threshold beyond which further increases in TS size or additional BB data do not yield significant improvements in PACC. These findings are particularly applicable to the Swedish forest breeding program, where distant relatedness between TS and VS is common due to a crossing strategy in which each parent is used only one to three times. Utilizing assisting traits with high genetic correlation like BB can significantly improve PACC when relatedness is low. Moreover, phenotyping cost and labor-intensive traits, such as FT, may limit the number of individuals included in the breeding strategy, and this can be compensated by including highly correlated traits in selecting models. In practice, these outcomes encourage expanding the training population with additional families or clones—especially those phenotyped for key traits like BB and FT—to improve genomic prediction models when applying GS to less-related selection candidates.

4.4 | Comparison of pedigree-based and genomic-based prediction models

We compared pedigree-based BLUP (PBLUP) and genomic BLUP (GBLUP) models to elucidate additive (A) and nonadditive (R) genetic effects on FD, BB, and height (HT) traits (Table 1). Heritability estimates obtained from both PBLUP and GBLUP were largely comparable across traits, suggesting that either method can provide reliable heritability estimation. Differences in accuracy across traits likely reflect variation in the number of ramets per clone, with higher replication (as in height and BB) improving the precision of estimates relative to FD, which had fewer ramets per clone. Narrow-sense heritability (h^2) estimates were slightly higher in PBLUP models than in GBLUP models, consistent with prior findings (Chen et al., 2023). However, GBLUP models provided more reliable estimates by capturing Mendelian segregation patterns not discernible in pedigree data (Lee et al., 2010; Muñoz et al., 2014; Visscher et al., 2006). Among all models, the GBLUP-AR model exhibited the lowest AIC values across the three traits, indicating superior performance in separating additive and nonadditive variances and outperforming pedigree-based models. Notably, we identified significant nonadditive effect in FD contrasting with previous findings by Chen et al. (2023), which may be due to differences in sampling methodologies, differences in FD assessment methods, and different environmental conditions (Velazco et al., 2019). Recognizing this nonadditive effect is essential for preventing overestimation of additive genetic variance, thereby improving the accuracy of breeding value estimation in tree breeding (Calleja-Rodriguez et al., 2021).

In this study, GBLUP models demonstrated slightly higher PACC for HT and BB (Figure 2**;** Table S2), likely due to their ability to capture realized genetic relationships, unlike PBLUP models, which rely on recorded pedigrees information. Furthermore, the higher PACC of GBLUP models was influenced by factors such as number of progenies, levels of co-ancestry, statistical methods, and potential pedigree errors, which may introduce inconsistencies in the relative performance of PBLUP and GBLUP approaches (Suontama et al., 2019; Vela-Avitúa et al., 2015). However, the accuracy from the GBLUP model over PBLUP was limited in our data (Figure 2). A plausible explanation is that we reconciled pedigree discrepancies using genomic data before rebuilding the ***A*** matrix, thereby improving PBLUP performance. In addition, the clonal replicated design increased PBLUP accuracy, further narrowing the benefit of modeling with realized genomic relationships. This is consistent with simulations by Stejskal et al. (2022), which showed that extensive clonal progeny testing can reduce the relative advantage of GBLUP over PBLUP. Finally, the high-quality FD phenotypes (EL measurements averaged per tree prior to analysis) may have further compressed the incremental advantage of GBLUP.

4.5 | Genetic relationship between traits and practical implications for Norway spruce breeding

In this study, we applied a nondestructive phenotyping approach involving artificial freezing treatments and ion leakage assay to quantitatively assess FD during bud flush. This high-throughput EL assay represents a novel phenotyping strategy for large-scale screening of FT in a tree breeding context, enabling controlled evaluation of basal FT outside of sporadic natural frost events. This method evaluated the basal FT of fresh needle tissue by introducing FD at defined developmental stage for each genotype. The results of EL measurements were generally consistent across years, apart from 2019, when average leakage values were lower. This deviation likely reflects differences in sampling design, with more replicates per tree but fewer trees overall in the 2019 dataset (Table S1). Importantly, because FD was always assessed at a comparable BB stage, the impact of environmental variation on EL measurements is expected to have been minimal.

Our results revealed a strong negative genetic correlation between FD and BB (Figure 3), indicating that early-flushing genotypes exhibit greater tolerance to spring frost than later flushing ones. Notably, the greater tolerance (lower FD) of earlier flushing genotypes observed in this northern Swedish material may not generalize across regions. In Eastern Norway (~61°N), Skrøppa and Steffenrem (2016) reported a positive association between earlier BB and higher spring FD, consistent with stronger frost-avoidance benefits of later flushing under frequent frost events. These context-dependent patterns argue for index selection that balances FT with phenology and growth objectives, rather than selecting on BB alone. Practically, this means weighing both FD (or FT) and

BB in a multi-trait index and calibrating weights for the target deployment zone. Taking it together, this contextual evidence aligns with evolutionary expectations that the direction and strength of selection on flushing time depend on local frost regimes. This result aligns with evolutionary theories suggesting that genotypes initiating growth earlier experience stronger selective pressures from frost events, thereby resulting in enhanced basal FT (Agrawal et al., 2004; Fineblum & Rausher, 1995). However, spring frosts are perceived as a less significant challenge in northern regions than in southern Sweden, where more frequent frost events likely exert stronger selective pressure, resulting in a higher correlation between basal FT and BB. To verify this, similar studies in lower latitudes are necessary. Additionally, we observed a positive correlation between FD and tree height, suggesting a potential trade-off between increased basal FT and reduced growth, a pattern observed in other stress-related traits affecting growth (Camarero et al., 2024). However, previous studies indicate that early growth initiation is associated with reduced height growth, potentially reflecting the influence of northern growth conditions, as trees from northern provenances are typically early flushers (Hannerz et al., 1999; Helmersson & Westin, 2019). In this study, the populations originated from northern latitudes (66°–67° N) across different altitudes with differing temperature sums, which complicated the isolation of FT effects on growth within this specific study setting. The hypothesis of reduced height as a trade-off for increased basal FT would warrant further exploration through similar FT studies conducted in field environments recently exposed to FD.

Later initiation of growth in spring is associated with increased height in Norway spruce (Helmersson & Westin, 2019), primarily due to delayed growth cessation in autumn, which allows a longer growth period (Worrall, 1973), and this was also observed in this study (Figure 3). This pattern helps trees with later BB avoid frost events and their subsequent growth loss. Currently, Norway spruce breeding in Sweden mostly relies on a frost-avoidance strategy, with preference given to genotypes with later BB, especially in frost-prone areas of southern Sweden (Hannerz & Langvall, 2000). However, this selection strategy may present future challenges as warming spring temperatures trigger earlier BB, heightening the risk of FD (Hänninen, 1991; Svystun et al., 2021). If breeding programs focus solely on frost avoidance via later BB, reduced basal FT could become problematic as warming causes spring growth initiation to outpace selection for delayed BB, which is primarily temperature regulated (Worrall & Mergen, 1967). In this context frost avoidance strategies may become ineffective, particularly as BB initiation is advancing faster than last spring frost events (Schwartz et al., 2006). Relying solely on late BB selection as a frost-avoidance measure also risks unintended consequences. Specifically, genotypes that break bud later in spring also tend to set buds later in autumn, which could lead to increased vulnerability to autumn frost events as warming temperatures delay growth cessation (Hannerz, 1994c; Skrøppa & Dietrichson, 1986). Our findings highlight the importance of basal FT as an essential trait for consideration in future forest tree breeding programs. From a practical breeding perspective, our results suggest that multivariate GS can be effectively applied in Norway spruce breeding by genotyping young progeny and leveraging easily measurable early traits, such as BB, as proxies for FT. For instance, breeders could assess BB timing in seedlings within a nursery and incorporate these data into a multivariate GS model to predict FT, thereby enabling selection decisions without the need for prolonged field trials or exposure to natural frost events. The successful implementation of such early selection requires a well-structured training population that represents the genetic diversity of the breeding material, along with confidence in the strong genetic correlation between early BB and later FD. While this strategy entails additional genotyping and phenotyping costs, it offers substantial reduction in the breeding cycle for FT. However, potential trade-offs must be carefully managed. Selecting exclusively for early BB to enhance FT may reduce height growth, necessitating balanced breeding objectives. Multivariate GS models offer flexibility to integrate multiple traits, allowing breeders to optimize selection indices that prioritize FT while maintaining desirable growth characteristics. For example, an index could be designed to favor FT while selecting for moderate BB timing, thereby avoiding extremely early or late flushing. Such flexibility is a key benefit of genomic prediction models that integrate multiple traits versus one-trait-at-a-time selection. By doing so, Norway spruce breeding programs can develop planting stock that maintains productivity while improving resilience to spring FD.

This study builds on previous research by applying multivariate GS to an adaptive trait in forest trees, demonstrating the practical benefit of integrating genomic data with high-throughput phenotyping in breeding programs. The strategies identified here provide practical guidance for enhancing basal FT in Norway spruce, including the integration of early BB measurements with EL assays to facilitate GS at the seedling stage, despite the species' long breeding cycle. Moreover, the observed genetic correlation between FT, BB, and height raises questions about the long-term effectiveness of current frost-avoidance strategies in breeding programs, particularly in the context of a warming climate. Our results emphasize the necessity of incorporating basal FT into Norway spruce breeding programs to support climate-resilient forest management.

AUTHOR CONTRIBUTIONS

Tuuli Aro: Conceptualization; data curation; formal analysis; investigation; software; validation; writing—original draft; writing—review and editing. **Biyue Tan**: Formal analysis; investigation; methodology; resources; software; visualization; writing—original draft; writing—review and editing. **Zhi-Qiang Chen**: Data curation; formal analysis; resources; writing—review and editing. **Henrik Hallingbäck**: Methodology; resources; validation; writing—original draft; writing—review and editing. **Mari Suontama**: Funding acquisition; resources; writing—original draft; writing—review and editing. **Johan Westin**: Conceptualization; methodology; resources; writing—review and editing. **Harry Wu**: Funding acquisition; resources; writing—review and editing. **Vaughan Hurry**: Conceptualization; data curation; funding acquisition; methodology; resources; supervision; writing—original draft; writing—review and editing.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The datasets for genetic and phenotypic data created for this study can be found in public repository Scilifelab Figshare [DOI:10.17044/scilifelab.28210955].

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SUPPORTING INFORMATION

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