

GENETIC VARIATION AND INHERITANCE OF BUD FLUSHING IN A NORWAY SPRUCE SEED ORCHARD ESTABLISHED IN ROMANIA

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Abstract: Genetic variation and parameters for bud flushing were estimated in a seed orchard of Norway spruce established in Romania. The experiment contained 28 clones originated from 7 forest districts located in the Eastern Carpathians. Bud flushing was assessed in two consecutive growing seasons (2017 and 2018) by examining 168 trees. Results revealed high genetic differences both at the clone level, at the population and within population level. Bud flushing of the Norway spruce clones exhibited strong clinal variation with the altitude of origin of the plus trees. The clones from lower altitude started earlier their growing in spring compared with those from higher altitude. The clone heritability estimates ranged from 0.85 to 0.94, which means strong genetic control and implies that it can be effectively selected during breeding. The knowledge of the genetic variation of this adaptive trait is extremely important in breeding, but also for management and conservation of forest under climate changes.

Key words: Norway spruce, bud flushing, genetic variation, heritability, correlations.

1. Introduction

Norway spruce is one of the most important forest species in Romania, both for economic and ecological purposes. It covers about 22% of the national forest area and 77 % of the coniferous forest area (approximately 1,450,000 ha) [20]. Since the middle of the 19th century, Norway spruce was the most cultivated

species outside its natural range. Thus, the artificial stands of Norway spruce created outside the natural area reached currently 360,000 ha [24]. Also, 41% of the artificially regenerated area is still planted with Norway spruce (about 17 million seedlings).

In Romania, the tree breeding programme was started in the 1960s, and the Norway spruce was one of the

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prioritized species. Selection of the seed stands followed by the plus trees selection and establishing of seed orchards were the first steps in the breeding programme [5]. Over 1400 plus trees were selected in natural and artificial stands. There have been installed 13 seed orchards (96 ha) of which only 9 seed orchards (72.9 ha) remained by now. They are currently the main sources for producing genetically improved seeds in Romania.

Breeding activities have focused on improving growth, wood quality and resistance to biotic and abiotic factors. Among abiotic factors, late spring frosts often cause injuries to young shoots of Norway spruce if the forest reproductive material is poorly adapted to the climate of the planting site. The frosts spring produce stem forking by killing the terminal shoot, which are considered one of the main shape defect because reduces the timber use value.

Phenological studies have shown that the trees growing season in Europe increased on average by 11 days during 1960 – 1990, and climate change is expected to advance the spring in the future [14]. One of the consequences will be increasing the frost damages due to earlier bud burst. Thus, bud flushing is one the most important traits in breeding but also for successful of regenerations. The phenology of bud flushing is fundamental for survival and growth of the forest species.

At the European level, the first results regarding the geographic variability of bud flushing in Norway spruce come from IUFRO experiments [7, 12]. The climate control and the timing of bud flushing in Norway spruce were studied by Beuker 1994, Leinonen and Hanninen 2002, Sogaard et al. 2007, Vitasse et al. 2009,

Malyshev et al. 2018. However, despite the economic importance of Norway spruce, studies regarding the genetic control of bud flushing are limited as compared with those for growth or wood quality [4, 6, 9-11, 17].

Therefore, knowing the genetic variation and inheritance of the important adaptive traits is extremely important both from a theoretical and practical point of view. Information is essential in the species breeding programme for selection of the genotypes or populations with high adaptability or plasticity. Also, they are useful for the delineation of the provenance regions and the transfer of forest reproductive material, in order to maximize adaptability and wood yield.

To address these problems, the objectives of this study were: 1) to investigate the genetic variation of bud flushing in a seed orchard of Norway spruce; 2) to determine the genetic parameters; 3) to assess the relationships among variation of bud flushing and geographic parameters of the location of parents' origin; and 5) to evaluate the implications for the breeding programme.

2. Material and Methods

2.1. Genetic Material and Assessments

Within 1979 - 1981, 197 plus trees were selected from 7 forest districts, both from natural and artificial seed stands located in the Eastern Carpathians. Further, a clonal seed orchard was established at Fantanele Forest District (Eastern Carpathians) on a surface of 15 ha. The seed orchard is situated far away from other spruce stands, in the oak zone at 46°36' N latitude, 26°50' E longitude at an elevation of 300 m above sea level (a.s.l.)

and currently comprises the vegetative copies of 168 plus trees belonging to 2 provenance regions [22]. In this study, we chose 28 clones originated from all 7 forest districts. The location of the plus trees tested in this experiment is presented in Table 1 and Figure 1.

The assessment of bud flushing (BB) was made in two consecutive growing seasons (2017 and 2018) by examining 168 trees (6 ramets per clone in three replications). Because the trees heights are ranging between 7 to 10 meters, it was not possible to assess the terminal bud of the annual shoot.

Table 1

Location of the plus trees studied in this experiment.
Provenance region by Parnuta et al. (2010)

| Crt. no. | Clone number | Altitude [m] | Lat. N | Long. E | Forest district | Provenance region |
|----------|--------------|--------------|--------|---------|------------------|-------------------|
| 1 | 1-2 | 1000 | 46°89' | 26°14' | Tarcau | A2 |
| 2 | 3-7 | 650 | 46°47' | 26°43' | Moinesti | A2 |
| 3 | 8-12 | 650 | 46°14' | 26°68' | Manastirea Casin | A2 |
| 4 | 13-14, 16-18 | 1100-1200 | 46°49' | 26°22' | Agas | A2 |
| 5 | 15 | 900 | 46°95' | 25°94' | Ceahlau | A2 |
| 6 | 19-25 | 860-1180 | 46°42' | 26°40' | Comanesti | A2 |
| 7 | 26-28 | 420 | 47°25' | 26°71' | Pascani | G1 |

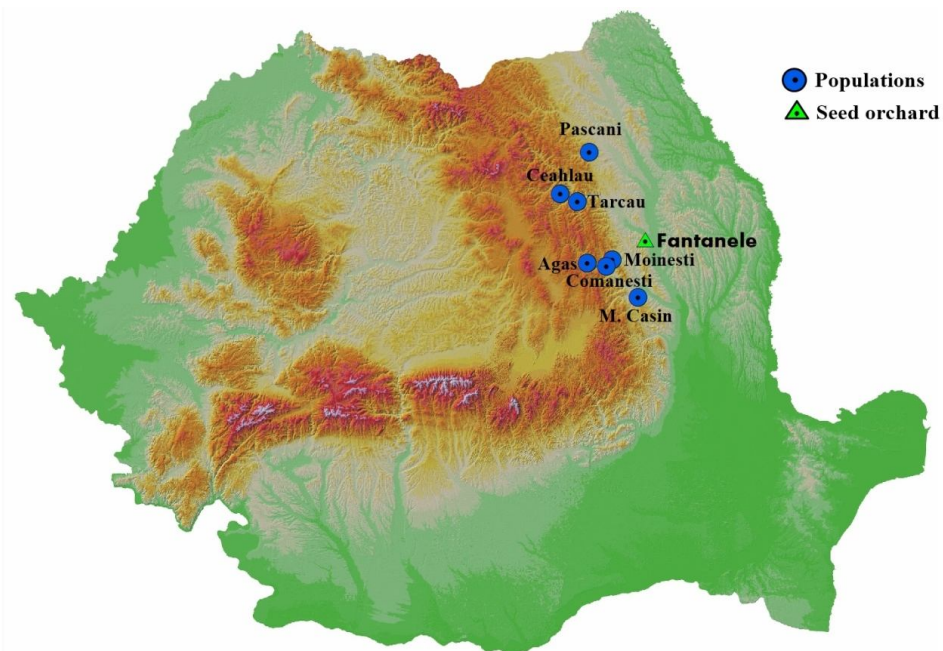


Fig. 1. Location of Norway spruce plus trees and the seed orchard

The bud flushing survey involved recording the terminal bud of a lateral branch situated at the 2 m height and always on the eastern part of the tree crown. Accordingly with Ducci et al. 2012, we have used a 5-point scale starting from 1 = unbroken bud to 5 = needles completely open and fully elongated. This has simplified the interpretation of the data by ranking the clones to a scale of 1 = late to 5 = early. The observations were made in a single day with a periodicity of five days. Thus, three evaluations were performed in each year starting at 26 April in 2017 and 2018 too.

2.2. Statistical Analysis

Individual tree data were analyzed separately using appropriate mathematical model [19] (eqn. 1):

$$Y_{ijkl} = \mu + B_i + P_j + C_l + CB_{li} + e_{ijkl} \quad (1)$$

where:

Y_{ijkl} is the performance of the k^{th} tree in the i^{th} repetition in the l^{th} clone in the j^{th} population;

μ - the overall mean;

B_i - the effect of the i^{th} repetition;

P_j - the effect of the j^{th} population;

C_l - the effect of the l^{th} clone;

CB_{li} - the interaction of the l^{th} clone and i^{th} repetition; and

e_{ijkl} - the random error associated with $ijkl^{\text{th}}$ trees.

The analyses and variance components were performed using the SPSS software program version 19 (Univariate GLM & VARCOMP). The assumptions of the model

were checked by the Shapiro & Wilk test for normality and by Levene's test for homogeneity.

The genotype heritability (h^2_G) and individual heritability (h^2_i) were calculated by the following formulas [19] (eqn. 2):

$$h^2_G = \sigma^2_C / (\sigma^2_C + \sigma^2_E / r) \quad (2)$$

$$h^2_i = \sigma^2_C / (\sigma^2_C + \sigma^2_E) \quad (3)$$

where:

σ^2_C is the variances among clones;

σ^2_E - the error variance;

r - number of repetitions.

Pearson's correlations based on clone means were also calculated to examine relationships between bud flushing and geographic coordinates of the parents' origin (LAT = latitude; LONG = longitude; ALT = elevation, in metres a.s.l.).

3. Results

3.1. Genetic Variation and Variance Components

The analyses of variance for bud flushing in the two years are presented in Tables 2 and 3. Very significant differences among clones were found for all evaluations ($p < 0.001$). The differences among populations were also significant. The differences among the clone within a population were significant only for three populations: Moinesti, Comanesti and Pascani (Table 4).

Table 2

Analysis of variance for bud flushing of Norway spruce in 2017

| Source of variation | D.F. | BB-I-2017 | | BB-II-2017 | | BB-III-2017 | |
|---------------------|------|----------------|-----------|----------------|-----------|----------------|-----------|
| | | s ² | F | s ² | F | s ² | F |
| Population | 6 | 4.157 | 28.319*** | 16.919 | 24.621*** | 6.505 | 14.618*** |
| Clone | 27 | 1.364 | 17.627*** | 5.325 | 11.470*** | 2.283 | 7.103*** |
| Repetition | 2 | 0.077 | 1.000 | 0.613 | 1.321 | 0.363 | 1.130 |
| Error | 126 | 0.077 | | 0.464 | | 0.321 | |

The level of significance is represented as follows: * p < 0.05; ** p < 0.01; *** p < 0.001.

BB-I-2017 = the first evaluation in the year 2017, BB-II-2017 = the second evaluation in the year 2017, etc.

Table 3

Analysis of variance for bud flushing of Norway spruce in 2018

| Source of variation | D.F. | BB-I-2018 | | BB-II-2018 | | BB-III-2018 | |
|---------------------|------|----------------|-----------|----------------|-----------|----------------|-----------|
| | | s ² | F | s ² | F | s ² | F |
| Population | 6 | 7.431 | 21.965*** | 11.174 | 24.608*** | 8.353 | 23.602*** |
| Clone | 27 | 2.268 | 9.526*** | 3.086 | 7.854*** | 2.569 | 8.461*** |
| Repetition | 2 | 0.024 | 0.100 | 0.113 | 0.288 | 0.310 | 1.020 |
| Error | 126 | 0.238 | | 0.393 | | 0.304 | |

The level of significance is represented as follows: * p < 0.05; ** p < 0.01; *** p < 0.001.

BB-I-2018 = the first evaluation in the year 2018, BB-II-2018 = the second evaluation in the year 2018, etc.

Table 4

Analysis of variance for bud flushing within Norway spruce populations in 2017

| Population | BB-I-2017 | | BB-II-2017 | | BB-III-2017 | |
|------------|----------------|-----------|----------------|-----------|----------------|-----------|
| | s ² | F | s ² | F | s ² | F |
| Tarcau | 0.333 | 2.000 | 1.333 | 2.667 | 0.083 | 1.000 |
| Moinesti | 1.583 | 11.875*** | 2.700 | 9.000*** | 1.117 | 11.167*** |
| M. Casin | 0.050 | 0.750 | 1.050 | 1.016 | 0.200 | 0.207 |
| Agas | 0.000 | 0.000 | 1.217 | 2.147 | 0.883 | 2.944 |
| Comanesti | 0.040 | 0.833 | 2.937 | 15.417*** | 2.214 | 7.750*** |
| Pascani | 2.389 | 14.333** | 1.722 | 6.200* | 0.222 | 0.205 |

In 2017, the bud flushing evaluation was started on 26 April when 78 % of trees had their buds unbroken and 22% in the stages 2 and 3. In 2018, evaluation was started on the same date but 38 % of buds were already burst and only 62% were unbroken. The average index has varied between 1.26 (at first evaluation, BB-I-2017) to 3.99 (at the third evaluation, BB-III-2017) in 2017, and between 1.52 (BB-I-2018) to 4.10 (BB-III-2018) in 2018. The

bud flushing was closely related with the air mean temperature of the April month, which was 9.1°C in 2017 and 14.4°C in 2018. The earliest clones were from Pascani (28, 27, 26) and Moinesti (7, 6, 5, 3) forest districts, which already had almost all buds in stage 2 and 3. The latest clones were from Comanesti (20, 21), Agas (17) and Tarcau (1, 2) forest districts, which had the smallest indices at the end of the evaluations (Figures 2 and 3). It can

be observed a stability in the behavior of clones related to bud flushing. In the two years, the same clones started the

growing earlier and the same clones were tardive.

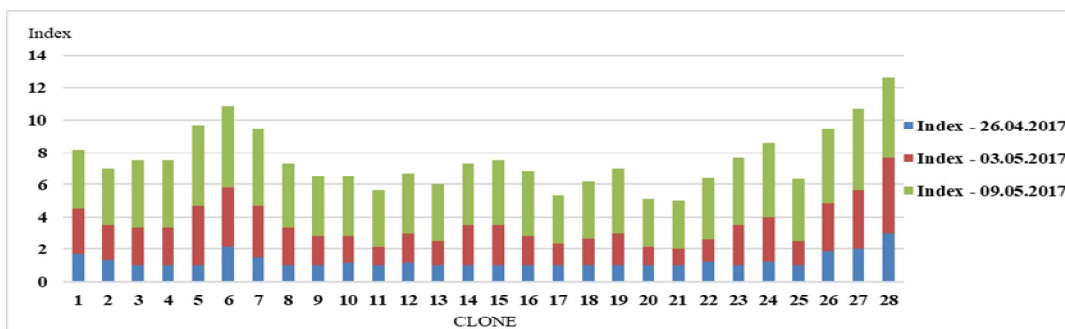


Fig. 2. Variation of Norway spruce bud flushing in the spring of 2017

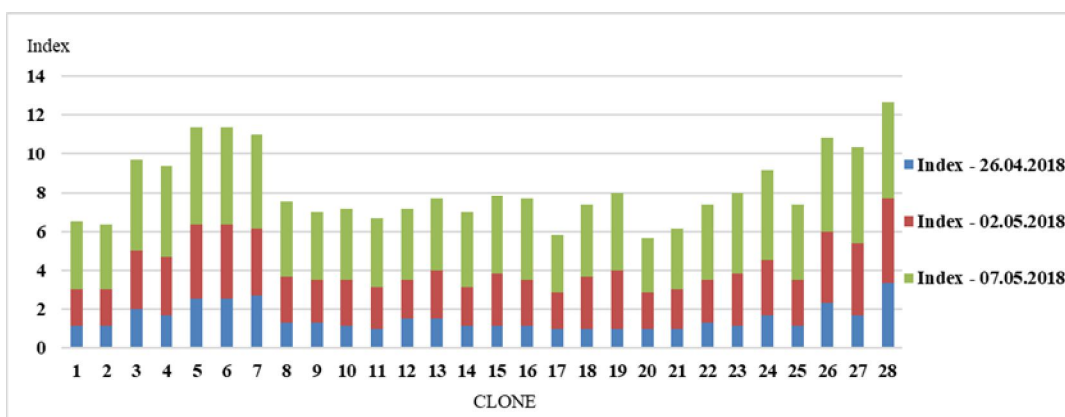


Fig. 3. Variation of Norway spruce bud flushing in the spring of 2018

3.2. Heritability

The values of the heritability coefficients are listed in Table 5. We calculated both the genotype heritability, at the clone level, and the individual heritability, ramets within clone. The clone heritability estimates ranged from 0.85 to 0.94 and was higher than individual heritability. The individual heritability takes into account the microsite variation within the seed orchards but is not so important since we will select clones and not ramets. The highest values of heritabilities were obtained for first evaluation of each year,

which corresponds to beginning of the growing season.

Table 5
Estimates of averages, phenotypic variances and heritability

| Evaluation | Mean | σ^2_{ph} | h^2_i | h^2_G |
|-------------|------|-----------------|---------|---------|
| BB-I-2017 | 1.26 | 0.455 | 0.848 | 0.944 |
| BB-II-2017 | 2.29 | 1.725 | 0.772 | 0.910 |
| BB-III-2017 | 3.99 | 0.747 | 0.666 | 0.857 |
| BB-I-2018 | 1.52 | 0.827 | 0.759 | 0.904 |
| BB-II-2018 | 2.65 | 1.222 | 0.716 | 0.883 |
| BB-III-2018 | 4.03 | 0.854 | 0.712 | 0.881 |

Where σ^2_{ph} = the phenotypic variances, h^2_G = the genotypic heritability

3.3. Phenotypic correlations

Correlations between BB evaluations in 2017 and 2018 are shown in Table 6. Very significant correlations were obtained between evaluations from each year and among years too. Correlations among BB and geographical coordinates of the

location of origin of the plus trees were found for altitude only. The bud flushing was negatively and very significant correlated with altitude of the parents' origin (r between -0.542^{**} to -0.691^{***}). Correlation with latitude and longitude of the plus tree origin were not significant.

Table 6

Trait - trait phenotypic correlations and correlations with altitude of origin of the plus trees

| Evaluation | BB-II-2017 | BB-III-2017 | BB-I-2018 | BB-II-2018 | BB-III-2018 | Altitude |
|-------------|----------------------|----------------------|----------------------|----------------------|----------------------|-----------------------|
| BB-I-2017 | 0.764 ^{***} | 0.608 ^{**} | 0.723 ^{***} | 0.645 ^{***} | 0.533 ^{**} | -0.542 ^{**} |
| BB-II-2017 | - | 0.898 ^{***} | 0.821 ^{***} | 0.824 ^{***} | 0.828 ^{***} | -0.607 ^{***} |
| BB-III-2017 | | - | 0.815 ^{***} | 0.902 ^{***} | 0.968 ^{***} | -0.691 ^{***} |
| BB-I-2018 | | | - | 0.857 ^{***} | 0.807 ^{***} | -0.675 ^{***} |
| BB-II-2018 | | | | - | 0.913 ^{***} | -0.637 ^{***} |
| BB-III-2018 | | | | | - | -0.666 ^{***} |

4. Discussion

Generally, wind-pollinated tree species exhibit high genetic variability within populations and low differentiation among them [8]. Also, moderate to steep genetic clines along climatic gradients for phenology and growth traits are specific in these species [1, 18, 21]. Results of the present study showed that there is high genetic variation among populations and clones for bud flushing in Norway spruce. At the same time, in three out of seven studied populations we found statistically significant within population variability.

Seed orchard is located far outside of the natural range of species, in different climatic conditions from those of the origin stands, but bud phenology shows well-developed adaptive divergence. Thus, bud flushing of the Norway spruce clones exhibited strong clinal variation with the altitude of origin of the plus trees. In the two years, clones from lower altitude

started earlier their growing in spring compared with those from higher altitude.

The earliest clones were from an artificial stand situated at 400 m altitude in Pascani Forest District. The phytoclimatic zone where are situated this stand are specific to oak forests and in no way to spruce forests. It is well known that the natural distribution of Norway spruce in Eastern Carpathians is between 600 – 1200 m altitude [24]. But the clones from this stand started earliest their growing season which means that plus trees were already adapted to this warmer climate condition when the grafts were harvested. This mechanism of adaptation is called epigenetics and is well-known in Norway spruce [23]. The epigenetic way of adaptation implies that the phenotype may change drastically from one generation to the next. Compared with selection, which acts slowly, epigenetic adaptation is a quick process that does not

involve any changes in the DNA sequences.

Bud flushing is a trait under strong genetic control, as evidenced the values of heritability coefficients. This implies that it can be effectively selected during breeding. Thus, to increase the survival of the planting stock the plant material with late growth onset or with high level of phenotypic plasticity should be used.

In addition to genetic determinism, air temperature was an important factor which determined the timing of budburst and results are in agreement with many other study [15].

5. Conclusions

The experiment reveals a large genetic variability within species level for bud flushing which can be used in breeding programme and forest regeneration. Results highlight high genetic control for this trait at the clone level, and certainly large genetic gain can be obtained in future breeding generations. The climatic condition of both planting site and origin location have influenced the starting of growth in Norway spruce. Also, the study revealed an adaptive differentiation, indicating the involvement of epigenetic processes in adaptation of Norway spruce to different environments. The knowledge of the genetic variation of the adaptive traits is extremely important in breeding, but also for management and conservation of forest under climate changes.

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