HABITAT TYPE DIFFERENTIATION IN PERIPHERAL *PINUS SYLVESTRIS* POPULATIONS BASED ON SEED TRAITS AND GERMINATION DATA

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Abstract: Seed morphology and seed germination power can be useful to characterise genotypes in natural populations. Measures of size and shape, their correlation and relationship with the germination capacity may be either the result of developmental programs or the response to a specific environmental condition. At the first instance, the development of the seed is highly influenced by the environment, its size and weight being strongly determined by the genotype and the environmental factors acting on the mother tree. Secondly, the survival of plants and their populations in fragmented landscapes strongly depends on their dispersal potential, seeds having specific morphological adaptations that influence their movement towards suitable germination microsite. Finally, germination and characteristics of juvenile seedlings are also influenced through seed morphology, inducing a successful distribution of the species. In our study we've focused on quantifying variation in seed traits and germination power among and within marginal populations of *Pinus sylvestris* L, considering the type of habitat. Discriminant function analysis showed significant differentiation of populations growing in peat bogs. Seed length, wing length and the percent of germination were the most useful traits to identify seeds of peat bog origin that are most probably adapted to the bog specific environment.

Key words: *Pinus sylvestris,* seed morphology, germination parameters, adaptation, habitat type

INTRODUCTION

Natural selection in plant populations often have as result genotype – environment interactions. In the absence of other forces, selection should cause each local population to evolve traits that provide an advantage under its local environmental conditions. As a result resident genotypes in each local population would have on average a higher relative fitness in their local habitat than genotypes originating from other habitats (Kawecki and Ebert 2004). In this aspect, morphological patterns leading to local adaptation are fundamental in plant life histories, and have profound consequences on many aspects of plant ecology and evolution.

By this way, morphological studies offer a feasible alternative to compare local populations, which have evolved under different conditions, but with the requirement of considering also past historical events (Korona 1996, Travisano and Rainey 2015). Since local adaptation in populations connected by gene flow must be due to recent natural selection, morphological studies and descriptions of plant structures aim to understand the relationships between structure and function in evolution and may contribute to define developmental situations associated with genomic composition and activity (Cervantes *et al.* 2016).

Among morphological surveys, reproductive phenological observations are some of the most sensitive data in identifying how plant species respond to regional climate conditions and to climatic changes. As reproductive organs, seeds are in highly specific interaction with the environment, influencing not only the germination of individuals, but also the distribution. It is important for the storage of life in the context of protection for the embryo, and its shape, size and weight are strongly influenced by the genotype and the environment where the mother tree grows (Castro 1999). The morphology of the seed is in its turn dependent upon the genetic constitution and the modifying effect of the environmental factors acting on seed formation and seed maturity (Andersson and Studies 1963). At the first instance, seeds have specific morphological adaptations that influence seed movement into suitable germination microsites (Chambers and Macmahon 1994). These variations in morphological characters could be due to the fact that the species grows over a wide range of rainfall, temperature and soil type. Soil properties, climate, and disturbance characteristics determine the physical attributes and micro- topography of exposed soils. In turn, these soil attributes influence both the horizontal and vertical movement of seeds.

After seed germination, the new individuals would react to soil, climate and management with a type of growth and development that is optimal under the offered conditions, emphasising the genetic component of performance (Andersson and Studies 1963), but also the existence of ecological factors which influence individual and stand development.

Variations in seed morphology in relation to habitat have been reported in a number of tree species (Kaushik *et al.* 2007). In *Pinus*, variations can mainly be attributed to the influence of the mother tree on the genetic composition of the seed coat and gametophyte, and to environmental conditions during seed development (Surles *et al.* 1993). The size of the seed easily changes, not only with the climatic conditions of the year but even with the difference in cone size, the number of seeds per cone and the position within the cone (Ehrenberg *et al.* 1955). As a broad generalisation, it appears that taxa associated with stressful environments have smaller cones (Richardson and Rundel 1998) and it is

intuitive to expect the largest seeds in the largest cones, but KEELEY and ZEDLER (2000) showed, that correlation between cone size and seed size is poor.

The approximately 110 species of the genus *Pinus* (RICHARDSON and RUNDEL 1998) exhibit one or two seed-dispersal systems. At some *Pinus* species, i.e. *Pinus cembra* or *Pinus pinea* the seeds are enveloped in stone-hard shell, are wingless and disseminated by animals, some are large-seeded pines like Jeffrey pine (*Pinus jeffreyi*) and sugar pine (*Pinus lambertiana*) with wind-dispersed winged seeds, but of which the dispersal can be also animal-mediated (VANDER WALL 2002). The seeds of several diploxylon species, among them also *Pinus sylvestris* L. are dispersed mostly by the wind. Their seeds are typically small with relatively large wings that have the potential to carry them well beyond the canopy of the parent tree. The cones open at seed maturity and shed seeds in the fall (CASTRO 1999).

Scots pine (*Pinus sylvestris*) has the widest geographical range of all pines and is one of the dominant tree species in northern Europe and Asia. Its populations occur on different types of well drained mineral soils, representing a broad range of variation in pH, nutrient availability and sustain on different vegetation types (OHLSON 1999). Consequently, not only the geographical, but also the ecological range is very wide. Scots pine populations are found both in habitats with high ground water table such as peat bogs, or in other extremities like dry rocky substrates or forming dry coniferous forests. The species' occurrence is split up into local populations strictly adapted to various types of soil and climate. During adaptation, certainly there are differences in genetic characters with regard to juvenile growth. To a large extent, however, the differences can be referred to casual characteristics of the seeds, embryo development, endosperm state, seed weight and in maturity in relation to the climate (EHRENBERG *et al.* 1955).

The distance over which the tree disperses seeds depends on the plant traits as well as environmental conditions and varies strongly in time and space. As the phenotypic variation could be a result of different parental environments, the production of polymorphic seeds (differing in size, shape, colour, germinability or dispersability) is thought to broaden the range of conditions under which Scots pine can germinate and, thus, increases the chances of reproducing in an unpredictable environment. In Central Eastern Europe Scots pine is mostly present in isolated, peripheral localities and populations survive under diverse environmental conditions within scattered geographic areas. In these regions the species has experienced large-scale fluctuations in the effective population size and the rate of gene flow that may not be reflected among contemporary populations (HEWITT 2000, PAMILO and SAVOLAINEN 1999, TÓTH *et al.* 2017*a*, *b*). Scots pine peripheral populations, in addition that they possess imprints of historical events also might be distinctive due to adaptations to different habi-

tat extremes. Distinctive adaptation patterns to different habitat extremes in peripheral populations from Central and Northern Europe, the Balkans, Iberia, and Anatolia have been shown in several morphological studies (DZIALUK *et al.* 2009, PYHÄJÄRVI *et al.* 2007, SEMIZ *et al.* 2007).

The objective of the present investigation was to understand the nature, extent and pattern of variation existing in some peripheral populations in respect to seed characters and the degree of correlation between the morphology of seeds and germination. Present study is also a completion of our previous investigation on cone morphology and needle anatomy, which revealed significant differentiation among populations growing in different habitats, the results obtained being evaluated as signs of local adaptation with detectable phenotypic patterns (KÖBÖLKUTI *et al.* 2017). Taking into consideration that the production of seeds with different sizes and shape may be a more effective and evolutionary stable strategy than the production of uniform crop (HAIG 1996), in our hypothesis we presume that local adaptation to extreme ecological sites could have consequences not only on cone and needle morphology but also on the morphological characters and germination of seeds.

MATERIAL AND METHODS

Study area

The materials were collected from natural stands in Central Eastern Europe, in the fall of 2015. Within the natural range of the species, we sampled populations with peripheral distribution and from habitat types like raised bogs, dry rocky outcrops or mixed forests on lower elevation, characterised by specific competition features. The geographic locations and habitat conditions are presented in Table 1 and Figure 1.

Seed data collection

Four healthy trees in each population were randomly selected. 25 to 40, fully ripened, brown coloured, 2 year old cones were collected from each tree. Cones were collected randomly from the crown of the sample tree. Only non-diseased cones were included in the study. Following the collection, cones were retained in room temperature, so that they opened after three weeks, then all seeds were extracted manually with a laboratory lancet and stored in paper bags, from each sample tree being wrapped separately. Following seed extraction, cones were desiccated on 30–40 °C. They were stored in airtight plastic bags for measurements. In reference to all studied traits (40 seed/population), five seed morphological traits

Table 1. List of studied *Pinus sylvestris* populations from the Central and Eastern European peripheral distribution of the species.

No.	Pop. abbrev.	Country	Residential area	Latitude (N)	Longitude (E)	Altitude (m)	Est. size (km²)	Habitat
1	RFE	Romania	Fântâna Brazilor	46.50	16.33	953	0.32	peat bog
2	SME	Slovakia	Medzi bormi	49.16	19.37	813	0.06	peat bog
3	RML	Romania	Ponor	46.33	23.34	925	0.10	peat bog
4	RMO	Romania	Băile Tuşnad–Mohoş	46.13	25.91	1052	0.58	peat bog
5	RPS	Romania	Poiana Stampei	47.30	25.12	878	1.43	peat bog
6	RBE	Romania	Poşaga de sus	46.49	23.36	524	0.84	rocky substrate
7	RCO	Romania	Lacu roşu	46.47	25.47	1507	0.04	rocky substrate
8	CHR	Czech Rep.	Hřensko	50.53	14.23	162	79	rocky substrate
9	HZA	Hungary	Szalafő	46.87	22.37	231	0.08	mixed forest
10	RVR	Romania	Putna-Vrancea	45.55	26.30	906	0.3	mixed forest

were measured, and four ratios were calculated. On each winged seed four morphological characters were measured: seed length (SL); seed width (SW); wing length (WL); wing width (WW). These parameters were measured with an electronic caliper with 0.1 mm accuracy. Ten seeds from every tree were taken to measure seed
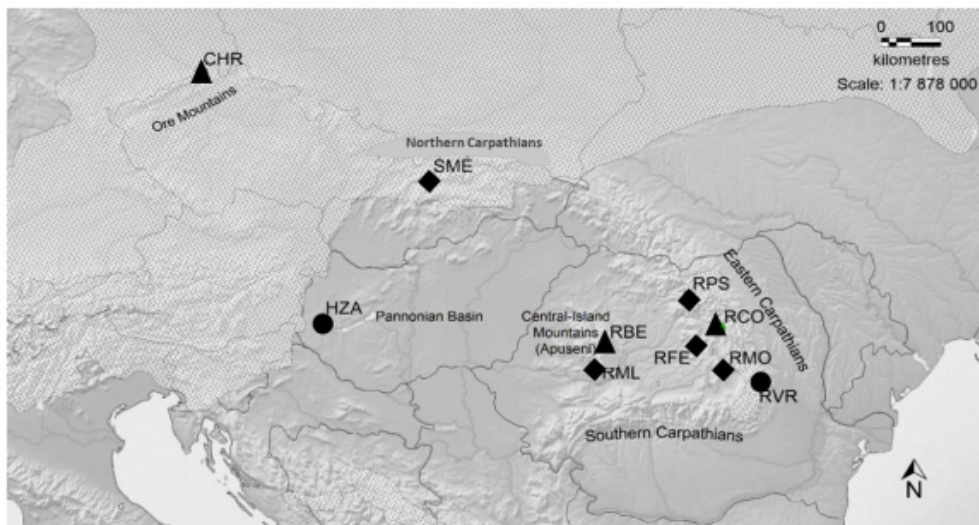

Fig. 1. Sampled populations of *Pinus sylvestris* L. The shape of icons indicates the type of habitat (rectangulars: peat bogs, triangles: rocky substrates, circles: mixed forests) the distribution of the species (based on Euforgen map) is shaded.

weight (g/ten seed) (SWE). Additional characteristics were assessed by calculating four ratios: seed length/seed width (SL/SW); wing length/wing width (WL/WW); seed length/wing length (SL/WL) and seed width/wing width (SW/WW).

Progeny trial; collection of germination data

After the morphological measurements, the extracted seeds were used to establish a common-garden trial located in the Botanical Garden of Soroksár (47° 24'–19° 09') in spring 2016. 15 seeds/mother tree were sampled from four mother trees per population and sown in pots of size 0.5 l (40 : 40 : 20 garden soil for conifers: peat: perlite), 0.5 cm depth on 5 March 2016 under common-garden greenhouse conditions. During germination, seedlings were kept under natural light conditions (in a periodically shaded greenhouse) with watering applied one or two times per week. Germination was scored once weekly between 5th of April and 19th of June 2016.

Germination study

Germination was scored once weekly between 5th of April and 19th of June 2016. We calculated **Seed germination percentage** using the following formula (ISTA 1985):

Germination % = Number of germinated seeds/Total number of seeds × 100

Germination associate parameters were calculated by using:
a. **Speed of germination** (Ginwal *et al.* 2005):

$$GS = n1/d1+n2/d2+n3/d3+.....,$$

where n1, n2, n3 = number of germinated seeds on day 1, 2, 3...; d1, d2, d3... = day 1, 2, 3...

b. **Mean germination time** (MGT) (Roberts and Ellis 1989):

$$MGT = n1 \text{ x } d1 + n2 \text{ x } d2 + n3 \text{ x } d3 + .../ \text{ Total number of days},$$

where n1, n2, n3= number of germinated seeds on day 1, 2, 3; d1, d2, d3 = day 1, 2, 3.

c. **Mean daily germination** (MDG) (Ginwal *et al.* 2005):

$$MDG = \text{Total number of germinated seeds/ Total number of days}$$

d. **Peak value** (PV) (Ginwal *et al.* 2005):

PV = Accumulated number of germinated seeds/Corresponding number of days

e. **Germination value** (GV) (Ginwal *et al.* 2005):

$$GV = PV \times MDG$$

Statistical analysis

Statistical analysis was carried out on 3240 sampled morphological data to investigate the seed traits and the relationship between seed variables and germination associated parameters. Morphological variation was analysed with IBM SPSS 20.0 (IBM Corp.) and Microsoft Excel. One-Way ANOVA, discriminant analysis and the Mantel test was performed. Maximal-minimal values, arithmetical means and standard deviations were calculated and analysed for all populations. One-way analysis of variance (ANOVA) was used to determine significant differences between the means of variables. Bivariate Correlation-analysis was used to detect relationship between each seed variable (parameter) and also between seed variables and germination associate parameters. We applied discriminant-analysis at first only with seed morphological dataset, then with both morphological and germination parameters to predict a categorical dependent variable and to determine whether a set of variables is effective in predicting category membership. The analysis was performed by stepwise method which classify by computing from group sizes within groups with combined groups plots. We sorted the studied populations according to the type of their habitat (peat bog, rocky substrate, and mixed forest) to detect any grouping by traits, which are or not suited to the specific environment. The term "mixed forests" means all forest types growing in tree community including beside the pines also broadleaf species. Mantel test was performed to test relationship between the geographical and morphological multi-character differences among the populations. Euclidean distances and geographical distances between populations were used for the evaluation using GenAlEx 6.5 (Peakall and Smouse 2012) software.

RESULTS

Seed morphological characters

Nine morphological traits and ratios with the average values and standard deviations are summarised in Table 2.

By performing One-Way ANOVA test, seed length (SL), seed width (SW), wing length (WL), wing width (WW) and seed weight (SWE) (Fig. 2) showed significantly higher values in case of RBE population, samples from SME had significantly lower values considering wing length (WL), wing length/wing width

Table 2. Average values with standard deviations of the analysed seed characteristics.

Code	Characters	RFE (peat bog)	SME (peat bog)	RML (peat bog)	RMO (peat bog)	RPS (peat bog)	RBE (rocky substrate)	RCO (rocky substrate)	CHR (rocky substrate)	HZA (mixed forest)	RVR (mixed forest)
SL	Seed length (mm)	3.80 ±0.60	3.66 ±0.50	3.90 ±0.42	4.23 ±0.48	4.06 ±0.60	4.82 ±0.52	4.20 ±0.56	4.18 ±0.78	4.30 ±0.52	4.40 ±0.50
SW	Seed width (mm)	2.10 ±0.26	2.23 ±0.27	2.40 ±0.32	2.50 ±0.29	2.44 ±0.34	2.84 ±0.45	2.46 ±0.45	2.45 ±0.35	2.53 ±0.38	2.80 ±0.60
WL	Wing length (mm)	13.23±2.26	10.00±1.18	15.07±1.61	14.13±1.61	13.14±2.09	15.56±1.59	13.72±1.61	12.38±1.66	14.51 ±1.22	13.04±1.83
WW	Wing width (mm)	4.65 ±0.74	4.35 ±0.49	5.10 ±0.66	4.51 ±0.65	4.57 ±0.80	5.89 ±0.78	4.78 ±0.53	4.81 ±0.87	5.31 ±0.66	5.17 ±0.85
SL/SW	Seed length/ seed width	1.83 ±0.33	1.65 ±0.28	1.64 ±0.23	1.70 ±0.20	1.67 ±0.25	1.72 ±0.21	1.74 ±0.28	1.71 ±0.26	1.72 ±0.22	1.61 ±0.27
WL/WW	Wing length/ wing width	2.89 ±0.54	2.30 ±0.23	2.99 ±0.41	3.16 ±0.36	2.94 ±0.57	2.68 ±0.38	2.89 ±0.37	2.63 ±0.49	2.76 ±0.35	2.60 ±0.59
SL/WL	Seed length/ wing length	1.84 ±0.33	1.66 ±0.28	1.65 ±0.23	1.70 ±0.20	1.67 ±0.25	1.72 ±0.21	1.74 ±0.28	1.71 ±0.27	1.72 ±0.22	1.61 ±0.27
SW/WW	Seed width/ wing width	0.46 ±0.10	0.52 ±0.08	0.48 ±0.08	0.56 ±0.10	0.55 ±0.11	0.49 ±0.10	0.52 ±0.08	0.52 ±0.08	0.49 ±0.11	0.55 ±0.11
SWE	Seed weight (g/ten seed)	0.008	0.010	0.011	0.009	0.008	0.007	0.010	0.010	0.010	0.008

(WL/WW) (Fig. 2B, D) and significantly higher in case of seed length/wing length (SL/WL) ratios (Fig. 2E). A distinct separation could be also observed in one group of SME, RMO, RPS populations, all from peat bogs, defined by low values of wing width (WW) variable (Fig. 2B). Samples from RML showed significantly lower values at seed weight (SWE), seed length/seed width (SL/SW), seed length/wing length (SL/WL), seed width/wing width (SW/WW) traits (Fig. 2C–E).
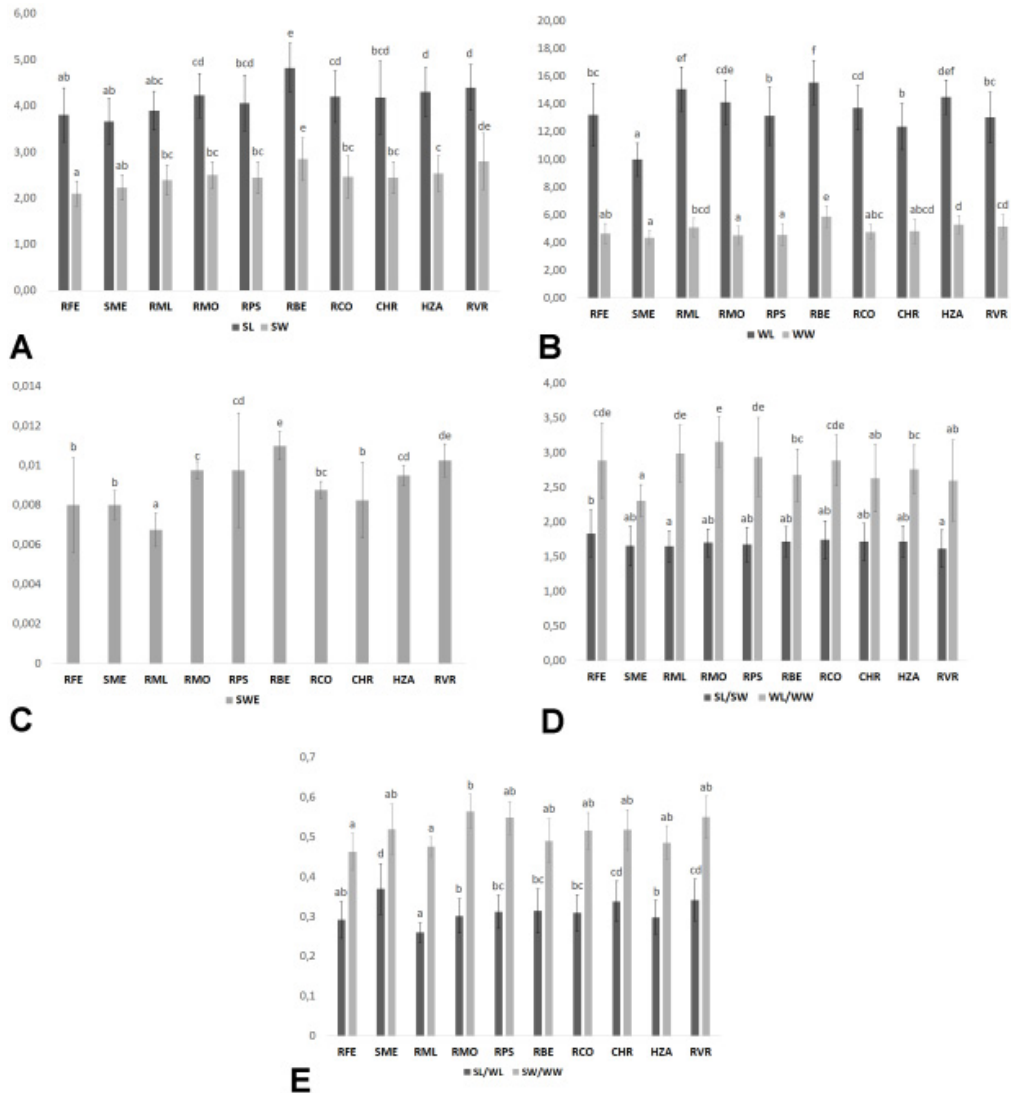

Fig. 2. Statistically significant seed morphological variables of Scots pine among the sampled populations. Unit of the measurement is mm (SL, SW, WL, WW) and g (SWE), except SL/SW, WL / WW, SL/WL and SW/WW.

Before carrying out the discriminant function analysis using Ward Linkage, three groups were formed according to habitat type: peat bog, rocky substrate and mixed forest. Based on the measured morphological datasets, with the first variable (Function 1) responsible for 87.3% of the variation and the second (Function 2) responsible for 12.7% of the variation revealed a slight pattern of populations by the separation into two groups: mixed forest (3) with rocky substrate (2) vs. peat bog (1). The highest level of differences between the populations was defined for function 1 by seed length (SL), wing width (WW) and seed weight (SWE) (Fig. 3).

The hierarchical cluster tree using Ward's linkage is presented in Figure 4. Within the dendrogram, samples from RMO and RPS with similar type of habitat (oligotrophic peat bog) were forming one subcluster, also the RCO and CHR samples (rocky substrate), with a relatively well supported relationship with HZA (mixed forest) samples. Another subcluster comprises RBE and RVR (rocky substrate and mixed forest) samples and a special highlight is needed on SME population, characterised by a completely distant position.

By performing Bivariate Correlation-analysis to detect relationship between each variable and defining correlation as significant at the 0.01 and 0.05 level we obtained the following results, presented in Table 3.

The outcome of Correlation analysis showed strong correlation between most of the variables, with two exceptions: between wing length/wing width–seed width (WL/WW–SW) and seed length/seed width–wing width (SL/SW–WW) variables. We used the Mantel test for the study of relationship between seed morphological divergence and geographic distance. The Mantel correlation was equal to $r_{xy} = -0.100$. $p = 0.410$ ($R^2 = 0.01$). Our results are statistically significant at an alpha (p) of 0.05. The scatterplot between elements in the two matrices showed no linear relationship between the morphological (Y axis) and geographic distances (X axis) (Fig. 5).

Table 3. Correlation between each morphological seed variable (**significant at level 0.01; *significant at level 0.05).

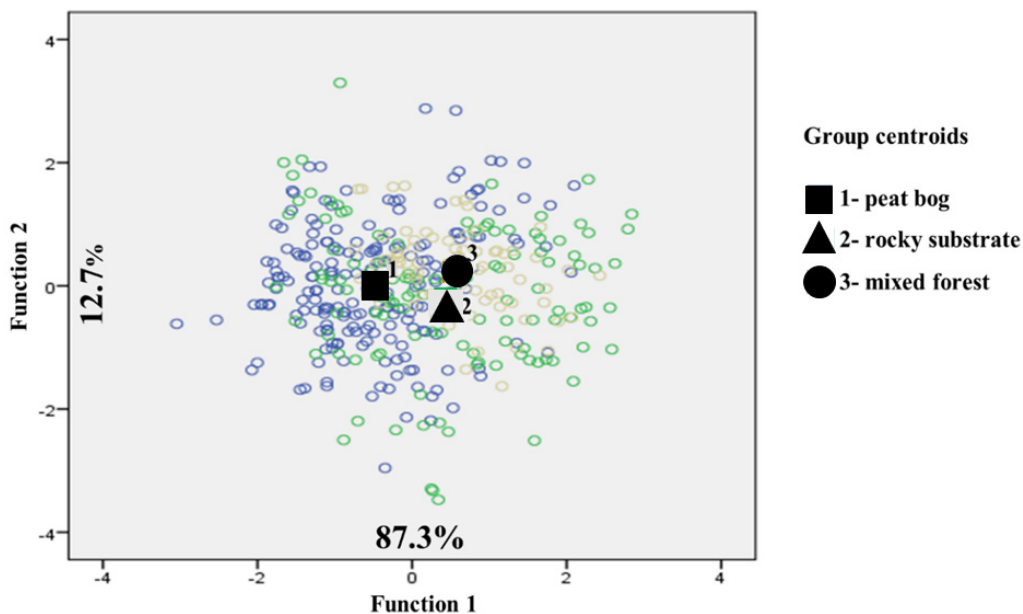|	SL	SW	WL	WW	SL/SW	WL/WW	SL/WL	SW/WW
SL		0.496**	0.467**	0.304**	0.428**	0.171**	0.415**	0.188**
SW	0.496**		0.245**	0.360**	–0.532**	–0.102*	0.172**	0.547**
WL	0.467**	0.245**		0.470**	0.176**	0.494**	–0.589**	–0.190**
WW	0.304**	0.360**	0.470**		–0.096	–0.512**	0.208**	0.556**
SL/SW	0.428**	–0.532**	0.176**	–0.096		0.273**	0.232**	–0.365**
WL/WW	0.171**	–0.102*	0.494**	–0.512**	0.273**		–0.347**	0.394**
SL/WL	0.415**	0.172**	–0.589**	–0.208**	0.232**	–0.347**		0.346**
SW/WW	0.188**	0.547**	–0.190**	–0.556**	–0.365**	0.394**	0.346**	

Fig. 3. Differentiation of *Pinus sylvestris* L. populations by habitat type detected by discriminant function analysis, on the basis of morphological seed characteristics: 1 = peat bogs (RFE, SME, RML, RMO, RPO); 2 = rocky substrates (RBE, RCO, CHR); 3 = mixed forests (HZA, RVR).
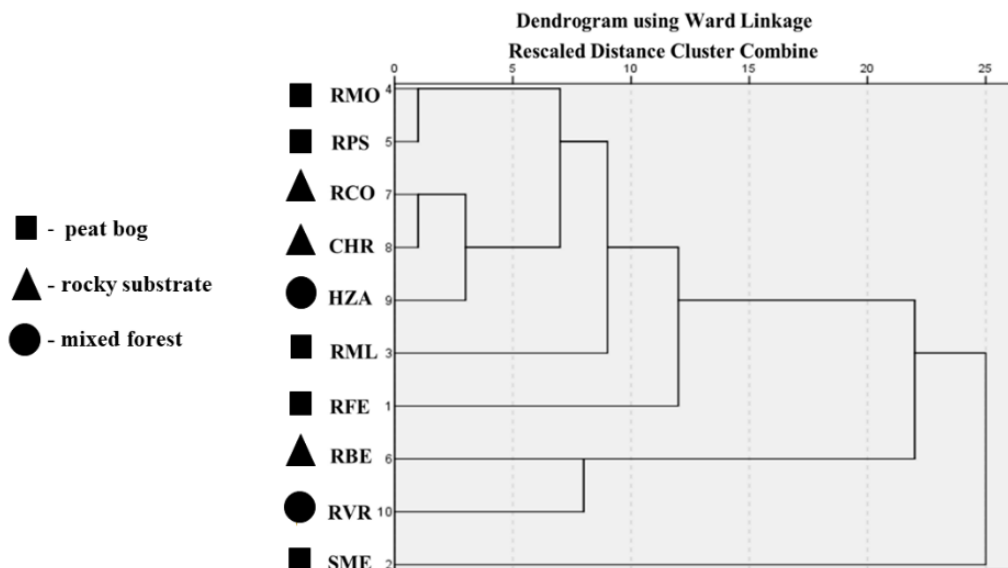

Fig. 4. Dendrogram generated with IBM SPSS 20.0 using Ward Linkage among 10 *Pinus sylvestris* L. populations from different habitats (RMO, RPS, RML, RFE, SME = peat bog; RCO, RBE, CHR = rocky substrate; HZA, RVR = mixed forest).
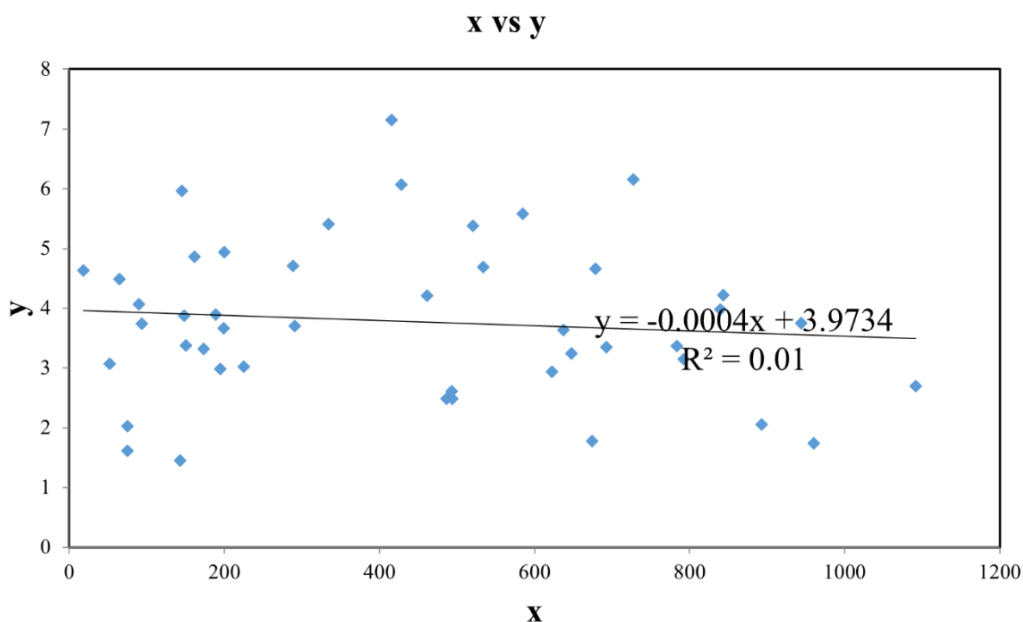
Fig. 5. Relationship between pairwise Euclidean distance and geographic distances (rxy = 0.100; p = 0.410; R2 = 0.01) for the 10 *Pinus sylvestris* populations.

Germination associated parameters

The germination associated parameters for the studied Scots pine populations are presented in Table 4 and Figure 6.

Maximum Germination percentage (PG) (Fig. 6B) has been recorded at RFE (76.67) and CHR (75.00) populations. The other values declined up to the lowest value of 23.33, the Germination percentage (PG) in case of RVR. Similar to seed germination percent, at the Speed of germination (GS) (Fig. 6C) the high-

Table 4. Germination associated parameters for the 10 *Pinus sylvestris* populations.

Pop. abbrev.	RFE	SME	RML	RMO	RPS	RBE	RCO	CHR	HZA	RVR
GV	0.05	0.02	0.02	0.00	0.02	0.02	0.01	0.04	0.02	0.01
PG	76.67	41.67	50.00	43.33	36.67	28.33	31.67	75.00	51.67	23.33
GS	13.50	2.62	6.29	5.96	3.18	5.11	3.83	12.74	8.35	3.78
MGT	138.41	119.77	139.88	149.71	377.94	220.35	197.65	381.29	134.71	242.35
MDG	0.19	0.12	0.14	0.16	0.38	0.25	0.03	0.39	0.21	0.26
PV	0.10	0.06	0.14	0.06	0.12	0.08	0.10	0.12	0.08	0.08

GV = germination value, PG = % of germination, GS = germination speed, MGT = mean germination time, MDG = mean daily germination, PV = peak value

est values were also recorded in the case of the RFE and CHR populations (13.50 and 12.74), the minimum value being recorded at SME samples (2.62). The Mean Germination Time (MGT) (Fig. 6D) also reached its maximum levels in the case of CHR and RPS (381.29 and 377.94), declining up to the lowest level in the case of SME (119.76). Mean daily germination (MDG) (Fig. 6E) has its maximum values (0.39 and 0.38) at the same populations (CHR and RPS), but with the lowest registered data in the case of RCO (0.03). In the case of Peak Value (PV) (Fig. 6A), parameters varied from the minimum of 0.06 (SME and RMO) to the maximum of 0.14 in the case of RML population. Germination Value (GV) (Fig. 6F) was recorded maximum in the case of RFE (0.05) and minimum at RCO and RVR (0.01) samples.
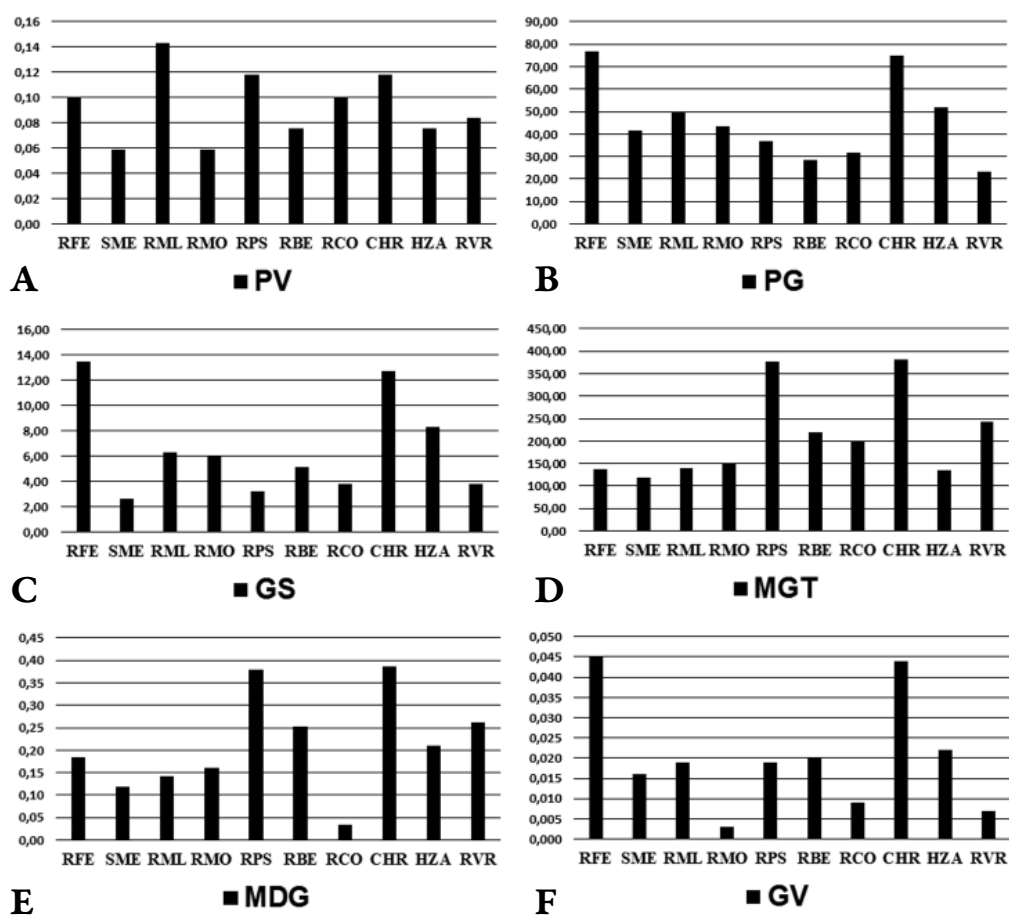

Fig. 6. Parameters associated with germination for the studied Scots pine populations (A = peak value (PV); B = germination % (PG); C = speed of germination (GS); D = mean germination time (MGT); E = mean daily germination (MDG); F = germination value (GV)).

By carrying out the discriminant function analysis using Ward Linkage, with three pre-formed groups according to habitat type (peat bog, rocky substrate and mixed forest), but with germination associate parameters also included in the analysis, with the first variable (Function 1) responsible for 93.3 % of the variation and the second (Function 2) responsible for 6.7 % of the variation revealed likewise in the case of our result based only on morphological data, the separation of populations into two groups: mixed forest (3) with rocky substrate (2) vs. peat bog (1), but with a more stronger pattern of differentiation between these two groups. The highest level of difference between the populations was defined by the following variables; for function 1: PG (Germination %), SL (seed length) and WL (wing length) and for function 2 by GS (Speed of germination), SWE (seed weight), SW (seed width), WW (wing width) and GV (Germination Value) (Fig. 7).

By performing Bivariate Correlation-analysis to detect relationship between seed morphology and germination associated data and defining correlation as significant at the 0.01 and 0.05 level, significant correlation at the 0.05 level between GS with SL/WL and SW/WW likewise between PG with SL, SW and SWE variables have been recorded (Table 5).
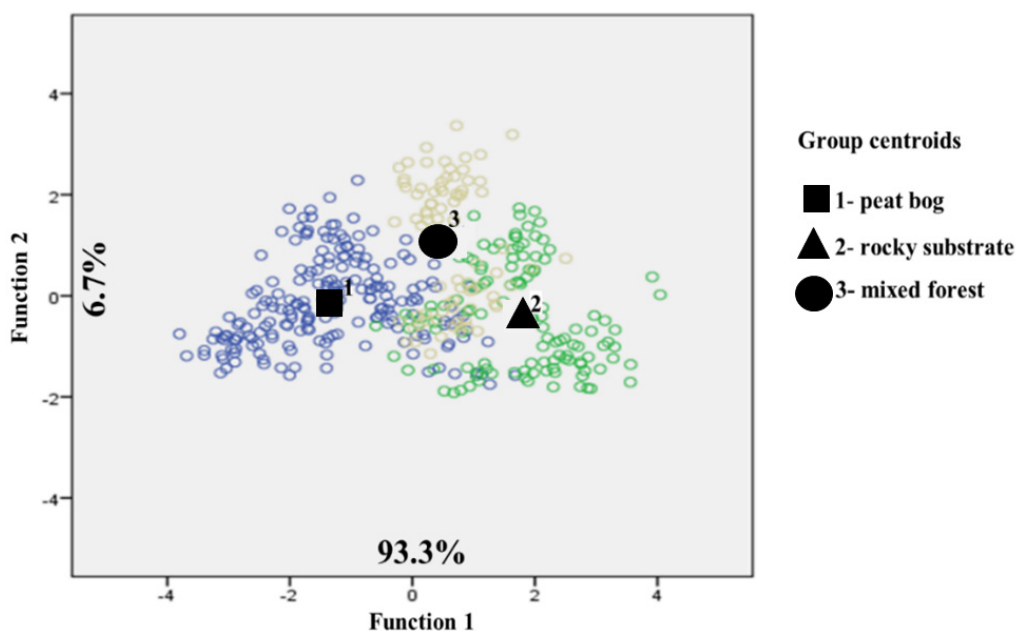

Fig. 7. Differentiation of *Pinus sylvestris* populations by habitat type detected by discriminant function analysis, on the basis of morphological seed characteristics and germination associated parameters:1 = peat bogs (RFE, SME, RML, RMO, RPS), 2 = rocky substrate (RBE, RCO, CHR), 3 = mixed forest (HZA, RVR).

Table 5. Correlation between seed morphology and germination associated variables in the studied 10 Scots pine populations (**significant at level 0.01; *significant at level 0.05).

Variable	SL	SW	WL	WW	SWE	SL/SW	WL/WW	SL/WL	SW/WW	GV	GS	PG
SL		0.496**	0.467**	0.304**	0.473**	0.428**	0.172**	0.413**	0.188**	0.035	0.086	0.178**
SW	0.496**		0.245**	0.360**	0.353**	−0.532**	−0.101*	0.173**	0.548**	0.037	0.116*	0.151**
WL	0.467**	0.245**		0.470**	0.303**	0.177**	0.495**	−0.590**	−0.191**	−0.027	−0.109*	0.001
WW	0.304**	0.360**	0.470**		0.275**	−0.097	−0.512**	−0.208**	−0.555**	0.011	−0.028	0.059
SWE	0.473**	0.353**	0.303**	0.275**		0.068	0.039	0.106*	0.090	0.026	0.094	0.224**
SL/SW	0.428**	−0.532**	0.177**	−0.097	0.068		0.274**	0.228**	−0.365**	−0.023	−0.059	−0.004
WL/WW	0.172**	−0.101*	0.495**	−0.512**	0.039	0.274**		−0.348**	0.391**	0.000	−0.044	−0.015
SL/WL	0.413**	0.173**	−0.590**	−0.208**	0.106*	0.228**	−0.348**		0.348**	0.026	0.152**	0.108*
SW/WW	0.188**	0.548**	−0.191**	−0.555**	0.090	−0.365**	0.391**	0.348**		0.043	0.135**	0.096
GV	0.035	0.037	−0.027	0.011	0.026	−0.023	0.000	0.026	0.043		0.866**	0.867**
GS	0.086	0.116*	−0.109*	−0.028	0.094	−0.059	−0.044	0.152**	0.135**	0.866**		0.917**
PG	0.178**	0.151**	0.001	0.059	0.224**	−0.004	−0.015	0.108*	0.096	0.867**	0.917**	

DISCUSSION

In natural habitats, the resources such as mineral nutrients, water and light necessary for plant growth are heterogeneously distributed in space and time (ZHANG *et al.* 2017). In these habitats the quality of seeds depends on the genetic constitution of the seeds (ANDERSSON and STUDIES 1963) and on the modifying effect of the habitat, the environmental properties of the growing sites of the mother tree (CASTRO 1999). Latter interactions of seeds with their environment also can be highly specific, influencing the germination of individuals and the distribution of species (CHAMBERS and MACMAHON 1994). Different morphological characters, such as various wings, seed shapes and sizes, lead to further differences in seed dispersal patterns that are related to various ecological strategies. Species produce seeds with highly divergent morphology. The level of differences in morphology found in our study is congruent with several earlier described variations regarded to be specific morphological adaptations to different environments (ALÍA *et al.* 2001, BILGEN and KAYA 2007, DZIALUK *et al.* 2009, JASIŃSKA et al. 2014, KINLOCH *et al.* 1986, KÖBÖLKUTI *et al.* 2017, LABRA *et al.* 2006, PRUS-GLOWACKI *et al.* 2003, PYHÄJÄRVI *et al.* 2007, SEMIZ *et al.* 2007, TURNA 2003). Morphological differences of seeds with regard to different environmental conditions are also listed in works of several authors. Seed size (mass) is central to many aspects of plant ecology and evolution (LEUBNER-METZGER *et al.* 2010, MOLES *et al.* 2005). WULFF (1986) suggested that the seed size of a species represents the amount of maternal investment in an individual offspring. NAVIE *et al.* (1998) argued that the less dispersible heavier seeds produced in natural areas would likely form a persistent seed bank. Larger seeds have better survival in dry conditions, and also larger seeds have higher percentage of emergence and survival (LEISHMAN and WESTOBY 1994).

Seed morphological characters

Seed size often increases with increasing dryness (BAKER 1972), presumably because of the need on dry sites for vigorous early seedling development. Latter findings support our observation regarding the significantly higher values after performing One-Way ANOVA test in the case of RBE (dry rocky substrate) population at SL, SW, WL, WW, SWE traits (seeds with significantly higher size and mass). Our samples from RML showed significantly lower values at SWE, SL/SW, SL/WL, SW/WW traits. This peat bog population has seeds with lower mass, longer shape associated with shorter and slimmer wings. In literature, the size and shape of the seed are described as a feature which can easily change, not only with the climatic conditions of the year but even with the difference in cone

size, the number of seeds per cone and the position within the cone (Ehrenberg *et al.* 1955). The number of seeds in each cone sampled was not determined in our study, but our previous results suggested significant differences rather in structure (by higher values of length, width, and thickness of apophysis) than in the size of cones from peat bog populations (Köbölkuti *et al.* 2017). However, due to Keeley and Zedler (2000), correlation between cone size and seed size is poor. Although seed size is one of the least variable trait in plants (Marshall 1986), seeds do show a considerable degree of phenotypic plasticity in response to the environmental conditions under which they develop (Fenner 1992). The lower SWE, SL/SW, SL/WL, SW/WW traits of seeds from RML population may be an inevitable consequence of resource constraints that limits the ability of the parent plant to control individual seed size (Vaughton and Ramsey 1998) and definitely a peat bog can be characterized by specific edaphic conditions. Our significantly low values for WW variable in the case of SME, RMO and RPS peat bog populations are supported by the findings in lodgepole pine populations (McGinley *et al.* 2017).

Based on the measured morphological datasets, discriminant function analysis revealed a slight separation of populations from peat bogs defined by lower values of SL, WW and SWE variables. Nevertheless, these morphological traits are probably influenced by the cone structure (McGinley *et al.* 2017), tree age, general health of the trees, and the specific macro- and micro- habitat of the parent trees (Dangasuk and Panetsos 2004).

Within the hierarchical clustering, some samples (from RMO and RPS both from peat bog) were forming one subcluster, also the RCO and CHR samples (rocky substrate), with a relatively well supported relationship with HZA (mixed forest) samples. Despite of this, our dendrogram showed that populations are not homogeneous in grouping by habitat type regarding seed and wings morphology. The reason of this difference existed among the studied populations could be explained by the different origin of populations. The distant position of SME population most probably is the result of the introgressive hybridisation within hybrid swarm populations of *Pinus sylvestris* and *P. mugo* formerly mentioned by Christensen and Dar (1997) and Wachowiak and Prus-Glowacki (2008).

Our coefficient correlation analysis between each morphological seed variable showed agreement with the general tendency of relationships between most of the characters (Cervantes *et al.* 2016, Chambers and Macmahon 1994, Ehrenberg *et al.* 1955, Greene and Johnson 1993).

The Mantel correlation matrices showed no linear relationship between the morphological and geographic distances. This result may reflect that seed's phenotypic variation is determined by covariance between the genetic and environ-

mental effects (REHFELDT 1991), or could be due to the fact, that our studied Carpathian populations represent only a small geographic range from the species' large distribution. We emphasize also that based on our former genetic research, palaeoclimatic modelling data and fossil evidences, conifers and some broadleaf trees were continuously present throughout LGM in refugial territories around the Pannonian Basin (MITKA *et al.* 2014, RONIKIER 2011, WILLIS and VAN ANDEL 2004).

Germination associated parameters in relation to morphological seed characteristics

Morphology and seed size are usually correlated, but how morphology affects germination and seedling growth is less understood. The chance that a seed will develop into an established seedling is dependent upon the site, which provides the specific conditions for its germination and development (SHELDON 1974). By carrying out the discriminant function analysis with pre- formed three groups according to habitat type (peat bog, rocky substrate and mixed forest), with both seed morphological and germination associated parameters, our results revealed a strong pattern of differentiation. Peat bog populations were defined by lower values of PG, SL and WL for Function 1 and for Function 2 by values of GS, SWE, SW, WW and GV situated between the values of populations from mixed forests and rocky substrate. Our lower SL associated with intermediate SW values means smaller seeds. Although, seed size is one of the least variable traits in plants (MARSHALL 1986) seeds do show a considerable degree of phenotypic plasticity in response to the environmental conditions under which they develop (FENNER 1992), with effect of nutrient supply on seed size. By these authors the size and the number of the seeds produced by the plant are determined by the nutrient status of the mother plant at the time of flower bud initiation, since much of the nutrient content of the seeds must be translocated from the vegetative tissues. As peat bogs develop under oligotrophic conditions, our lower germination percentage and smaller size of the seeds from peat bogs could be explained by these specific conditions of this type of habitat.

The results of Bivariate Correlation analysis were significant at 0.05 level between PG with SL, SW and SWE likewise between GS with SL/WL and SW/WW variables. Early studies showed that seedlings grown from large seeds have higher seedling establishment, growth and survival (BLADÉ and VALLEJO 2008). Our lower PG values also correlate and commensurate with smaller seed size. The GS variable is one of the oldest concept of seedling vigour. The interest in germination speed is based on the theory that only those seeds which germinate rapidly and vigorously under favourable conditions are likely to be capable of

producing vigorous seedling in field conditions (GINWALL *et al.* 2005). The SL/WL and SW/WW variables define the relation between the sizes of the seeds in relation to the wings. As these two variables have higher values, seeds have increased size compared to their wings. The presence of endosperm in mature seeds provides the size of the seed. Stored proteins in endosperm are important source of energy during early germination (ANGELOVICI *et al.* 2011). Higher seed size value in comparison to the wings means increased amount of endosperm and consequently vigorous germination.

CONCLUSIONS

Our study suggests that variation in the seed morphology and germination of *Pinus sylvestris* could be the consequences of local adaptation to different ecological sites. The distribution of variability among populations provided information not only concerning the background of these populations, but also gives some prognosis how they will sustain in the future with respect to regeneration capacity. For example, the seeds collected from the RBE population, originated from a dry, exposed habitat have seeds increased in size and weight, most probably because of the need of early seedling development. Higher seed size value means increased amount of endosperm. The correlation between the seed morphology and germination associated data may define a successful multi-trait selection. As a consequence of their vigorous germination, RFE and CHR populations maybe used as source of superior quality seeds. Seeds collected from peat bogs are smaller, with longer shape. The correlation between these smaller seeds and lower germination percent is helpful in the early evaluation for seed selection. Populations with increased germination percent and germination speed may also show higher seedling growth.

Due to its wide distributional range with varying geographic, climatic and edaphic conditions and its long evolutionary history, variation among *Pinus sylvestris* populations from different sites likely occurs, which may be reflected in the morphology of the generative organs and germination properties of the species. Revealing morphological differences of populations from different habitat types is necessary and allows successful conservation of the species' genetic resources for guided distribution of genetic material among recultivated stands.

* * *

Összefoglaló: Eltérő élőhelyek növényállományai magmorfológia és csírázási paraméterek révén is jellemezhetőek, egyrészt elsősorban annak okán, hogy a magméret, -alak és csírázás úgy genetikailag, mint a környezet által meghatározott. Amíg a magfejlődés folyamata elsősorban környezeti hatásoknak kitett, a mag mérete és súlya az anyanövény genotípusa és a rá ható környezeti tényezők függvénye. Mindemellett az állományok túlélése nagymértékben függ magvaik terjedési képességétől. A magmorfológiában detektálható variabilitás olyan – sajátos környezethez történő – alkalmazkodásra utal, amely révén a magvak nagyobb sikerrel érnek el csírázásukhoz alkalmas talajfelszínt. Végül, de nem utolsósorban a magmorfológia hatással van a leendő fiatal növény csírázására is, terjedést biztosítva a faj számára. Munkánkban néhány perifériális erdeifenyő (*Pinus sylvestris*) populáció magmorfológia és csírázási paraméterek által meghatározott, állományon belüli variabilitásának, illetve populációk közötti differenciálódásának detektálását tűztük ki célul, mindezt az élőhelytípus alapján. A diszkriminancia-analízis a tőzeglápi állományok szignifikáns elkülönülését eredményezte. A maghossz, a szárnyhossz és a csírázási arány azok a változók voltak, amelyek a lápi állományok elkülönülését meghatározták.

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