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ORIGINAL PAPER

Genetic variability of *Pseudotsuga menziesii* (Mirb.) Franco in northern Poland

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ABSTRACT

Among the introduced conifers, *Pseudotsuga menziesii* (Douglas-fir) appears to be one of the best-acclimatised species in its secondary range in Europe. This undeniable success was most likely the result of matching secondary growth conditions to the ecological requirements of the native populations. Nevertheless, our knowledge of the genetic structure of the first-generation is still incomplete, leaving uncertainty whether climate changes have influenced the genetic structure of the studied populations.

The main aim of the study was to determine the genetic diversity of Douglas-fir populations in northern Poland.

Total DNA was isolated from needles collected in five old-growth and one younger stand. Four nuclear microsatellite markers were applied coupled with standard methods of statistical analyses of population molecular genetics.

The high genetic diversity of the five first-generation stands corresponds to the diversity of native populations while the younger stand is characterised by a significantly lower genetic variability and a higher inbreeding index. Genetic distance analysis indicates a distinct grouping into two clusters: young population and the first-generation old populations.

Here, for the first time we present the results of genetic diversity analysis of this species in northern Poland – western part of South Baltic region. Our results suggest higher genetic distance between older tree stands and younger one. Additionally, the younger stand was characterise by inbreeding. Considering the limitations of the dataset low (number of tree stands), we call for a genetic verification of the origin of artificial populations of Douglas-fir in Poland.

KEY WORDS

basic forest material, Douglas-fir, genetic diversity, non-native species, SSR markers

Introduction

Pseudotsuga menziesii (Mirb.) Franco was introduced to Europe from North America in the 19th century (Brus *et al*., 2019). The natural range of this species encompasses the western part of the USA (states of Oregon and Washington) and Canada (Little and Viereck, 1971). Two varieties of *P. menziesii* have been delineated under natural growing conditions: the coastal variety *Pseudotsuga menziesii* var. *menziesii* (Mirbel) Franco, which inhabits along the Pacific coast of Canada and the USA, and the inland variety *Pseudotsuga menziesii* var. *glauca* (Mayr) Franco, which spans the Rocky

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Mountains from Canada to Mexico (Aas, 2008). Originally, the seeds and seedlings of *P. menziesii* were imported to the United Kingdom in 1827, where *P. menziesii* was utilized for scientific research and as an ornamental species (Dimitrova *et al*., 2022). Following the successful demonstration of the breeding value of *P. menziesii* under conditions of the secondary distribution area, it was introduced into forestry on a large scale in numerous European countries. In present-day Poland, *P. menziesii* was first introduced in 1830 (Burzyński, 1990; Kleinschmit and Bastien, 1992; Chylarecki, 2004), with a notable increase in prevalence observed between 1895 and 1945 (Barzdajn, 2013). In the 19th century, seeds of *P. menziesii* were directly sourced from native populations in North America (Eckhart *et al*., 2017). Since the latter half of the 20th century, European forest scientists have initiated a breeding programme aimed at selecting local forest reproductive material (Chałupka, 2014). Selected populations of alien tree species well-acclimatized to European climatic conditions have become particularly important in recent years (Alizoti *et al*., 2022). In Central Europe, *P. menziesii* is considered to be a silvicultural alternative to Norway spruce in both ecological and economic terms (van Loo and Dobrowolska, 2019). However, only a few studies (Mejnartowicz, 1976; Burzyński, 1990; Niemczyk *et al*., 2021) have described the intraspecific variation in adaptation to local climatic conditions in Poland. There is also limited information available regarding seed ecology and their behaviour (dormancy break) under new conditions, especially with regard to snowless winters (Jastrzębowski *et al*., 2021). Strategies for transforming forest stands with native species into stands with alien species should consider not only the adaptive potential of the population but also the risk of their invasion into native habitats (Schmid *et al*., 2014; Lange *et al*., 2022; Wohlgemuth *et al*., 2022), as well as potential distribution changes under future climate conditions (Dyderski *et al*., 2018; Puchałka *et al*., 2023).

Adaptation is the natural process of continuous genetic improvement of populations as a result of natural selection, which increases the probability of survival and reproduction of organisms under given conditions (Hartl and Clark, 2007). As a result of natural selection, there is a noticeable increase in the occurrence of alleles that enhance the chances of population survival in a particular environment (Przybylski *et al*., 2021). Studies on the genetic structure of populations allow us to understand the distribution of gene variants in different geographical regions and the interrelationship among populations of this species (Amarasinghe and Carlson, 2002). Analysing genetic markers such as Simple Sequence Repeats (SSR) allows one to determine whether isolated populations exist and whether seed migration influences the genetic structure of populations (Eckert *et al*., 2008). Studies of the genetic structure of *P. menziesii* in autochthonous populations in the Cascade Mountains region of Oregon by Krutovsky *et al*. (2009) pointed out that trees from different areas had unique genotypes. This result suggests the presence of genetic isolation among certain populations. This study contributed to the understanding of seed migration and dispersal of trees in the region. Attempts to identify the origin of *P. menziesii* populations introduced into different regions of Europe have been made (Berney, 1972; Fontes *et al*., 2003), leading to conclusions regarding genetically diverse plant material. A recent study on the genetic structure of *P. menziesii* (Hintsteiner *et al*., 2018; Neophytou *et al*., 2020; van Loo *et al*., 2015) demonstrated a clear genetic differentiation between 'coastal' and 'interior' varieties. In Europe, most *P. menziesii* populations belong to the 'coastal' variety, which performs better in common garden studies than the 'interior' variety (Nicolescu *et al*., 2023). Hermann and Lavender (1999) concluded that the 'interior' variety is unsuitable for cultivation under European conditions. Conversely, contrasting conclusions can be drawn from genetic experiments conducted in Poland. The superiority of 'coastal' provenance over 'interior' provenance in terms of productivity has been demonstrated in Western Poland (Mejnartowicz, 2007), while Niemczyk *et al*. (2021) reported a predominance

of 'interior' populations over 'coastal' populations at the eastern limit of the secondary range of Douglas-fir in Europe.

Although various models have been developed for strategies to utilize native *P. menziesii* populations in reforestation in different regions of Europe (*e.g.*, genetically-based assisted migration; Isaac-Renton *et al.*, 2014), knowledge about the origin and performance of European populations of this species remains limited, as there are no comprehensive archives documenting the introduction of this species. Therefore, attempts have been made to indirectly determine the origin of European populations by analysing SSR. However, these studies only concerned selected populations or limited regions of the *P. menziesii* secondary range (Neophytou *et al.*, 2020; van Loo *et al.*, 2015), leaving unanswered questions about how well the first-generation populations (established from native seed material) fit into the new ecological niches. Here, we present for the first time the genetic diversity of first-generation *P. menziesii* populations from western part of South Baltic region (specifically northern Poland) – a region of intensive introduction pursued by East Prussian foresters in the nineteenth century. We hypothesize that the first-generation populations are characterized by higher genetic diversity in relation to second, *i.e.*, younger populations.

Materials and methods

Study site and sampling. The plant material (needles) used for the laboratory analyses was collected in winter 2021 from populations: CHO, CZA, LEB, JAM, RAM – the exact age of the stands is given in Table 1. The stands represented an east-west gradient in northern Poland (Fig. 1D). The origin of seeds used to establish the studied stands is not documented, but it is very likely that seeds was imported from British Columbia (Canada) or Washington State (USA) by the German scientist Adam Schwappach in 1879-80 and 1891-95. Currently, four out of six populations studied (CHO, CZA, LEB and JAM) are considered as selected seed stands (Fonder *et al.*, 2007), the RAM population is a nature reserve 'Forest of Warmia' (https://nowe-ramuki.olsztyn.lasy.gov.pl/rezerwaty-przyrody) and the ELK population is a commercial forest stand.

In each stand, needles from 12 to 22 randomly selected trees (Table 1) were collected for genetic analyses. The minimum distance between the sample trees in older tree stands was 25 metres, while in younger one the distance was at least 10 meters due to small area of the tree stand. For the laboratory analyses the plant material was stored in 1.5 ml tubes (Eppendorf Safe-Lock Tubes) at constant temperature of –24°C.

Table 1.

Overview of the genotyped populations in Northern Poland; Population IDs: CHO – Chojna, CZA – Czaplinek, LEB – Lębork, JAM – Jamy, RAM – Nowe Ramuki, ELK – Ełk; Coordinates: WGS84, N – latitude, E – longitude; FRM – Forest material code; FH – Forest habitats: BMśw – fresh mixed coniferous forest; LMśw – fresh mixed broadleaved forest, Lśw – fresh broadleaved forest; No. of trees-number of genotyped adult trees used for the population genetic analysis

Population IDs	N	E	FRM	FH	Age	Area [ha]	No. of trees
CHO	52.962563	14.298080	MP/2/31484/05	LMśw	108	5.77	20
CZA	53.701128	16.430265	MP/2/31547/05	Lśw	118	4.00	12
LEB	54.517101	17.808341	MP/1/24664/05	Lśw	110	1.15	22
JAM	53.462778	19.365556	MP/2/44442/06	LMśw	140	1.0	22
RAM	53.652500	20.524120	reserve stand	Lśw	138	3.03	13
ELK	53.933365	22.198891	–	LMśw	64	1.05	21

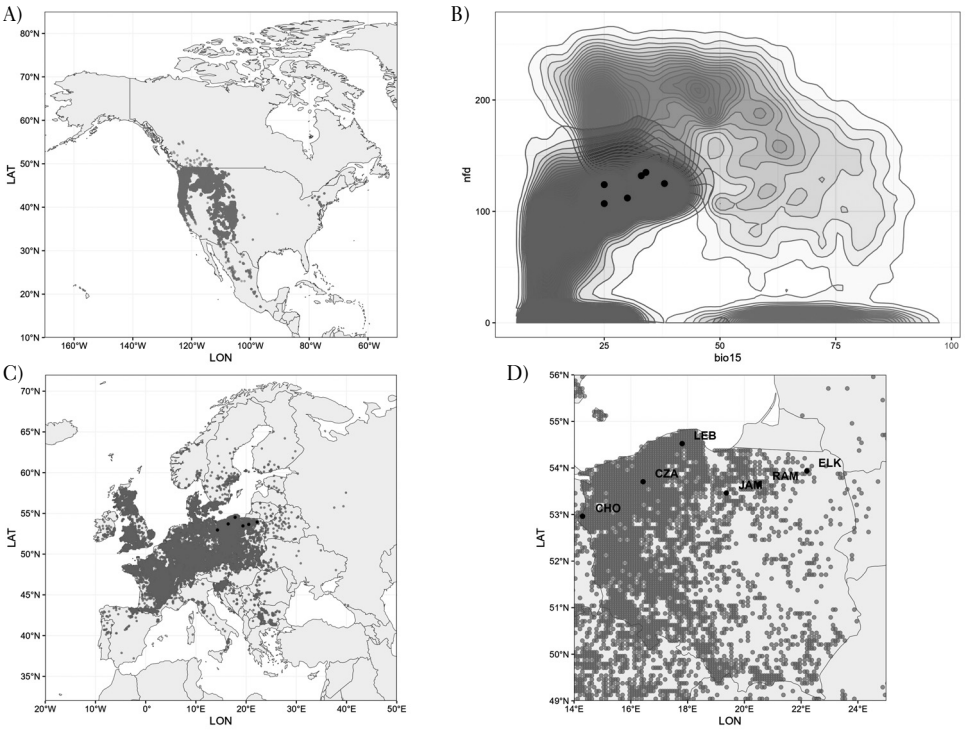

Fig. 1.

Natural distribution of *P. menziesii* in North America (Panel A) and secondary distribution in Europe (Panels C and D). Study sites in Poland (black circles; Panels C and D). Climatic envelope of *P. menziesii* in North America and Europe (Panel B). nfd – number of frost days; bio15 – precipitation seasonality. For population IDs see Table 1. Bioclimatic variables from the Chelsea database (Karger *et al*., 2017). Douglas-fir occurrence data downloaded from the Global Biodiversity Information Facility database (GBIF, www.gbif.org). Distribution range was limited to the natural range of the species using digitized polygon maps of the ranges (Little and Viereck, 1971; Caudullo *et al*., 2017).

SSR ANALYSES. Total genomic DNA was isolated from the collected material using a commercial kit (Macherey-Nagel Gmbh & Co; Germany). The quality of the DNA isolate was controlled using 2% agarose gel and a Quawell spectrophotometer (LabX, 334 King street, Midland, ON Canada), DNA was obtained in the range of 80 ng/µl to 450 ng/µl, with purity at an absorbance ratio of 260/280 varying from 1.6 to 2.1. All samples were diluted to 20-30 ng/µl using deionised water. Molecular analyses were performed using four nuclear microsatellite markers (PmOSU_2G12, PmOSU_4A7, PmOSU_3B2, PmOSU_5A8 – Table 2) selected on the basis of other research work (Slavov *et al*., 2004). The forward primers were fluorescently labelled with the fluorochromes. Amplification was performed through one multiplex reaction. Each PCR reaction was performed in a volume of 10 µl, with the following composition: 5 µl Multiplex buffer (Qiagen, Poland), 0.2 µl (10 µM) of each primer, 1 µl of extracted DNA, and PCR-grade water up to a final volume of 10 µl. The PCR thermal profile was as follows: 95°C for 15 min; followed by 30 cycles at 95°C for 30 s, 54°C for 30 s, and 72°C for 1 min., with a final extension of 60°C for 30 min. Genotyping analysis was performed on an ABI 3500 Genetic Analyzer capillary sequencer (Applied Biosystems, Foster City, CA, USA) and allele length analysis was performed using GeneMapper® version 5 (Thermo Fisher Scientific Inc., Carlsbad, CA, USA).

Table 2.

Primers sequences and general characteristics of the markers analysed (Slavov *et al.*, 2004). Marker name according to GenBank

Locus	Forward primer 5'-3'	Reverse primer 5'-3'	Repeat motif	Annealing temperature [°C]
PmOSU_2 G12	CAAGGACTCATA TGGGAAA	AACATCAGTAA TAACCTTTT	$(AC)_{11}...(AC)_{19}...(GCAC)_5...(GCAC)_4$ $(AC)_7...(AC)_6$	54
PmOSU_4 A7	TTGTAAAAATTC CATGTAT	AAGTGGGGGA GTGTGTAAT	$(TG)_5...(TG)_5...(CG)_7(TG)_4...(TG)_{29}...$ $(ATC)_5$	54
PmOSU_3 B2	CTTTGGAGTTCT TAATATAG	GATAATAGCAC CCCACCATA	$(TG)_{22}(CG)_7$	54
PmOSU_5 A8	CATTTTTGGATT CTGGTTTTG	ATGCCTCAAGC TATGTAATC	$(TG)_{11}...(TG)_{10}$	54

Statistical analyses. The values of the following attributes were calculated using GenALEx 6.5 (Peakall and Smouse, 2006): number of alleles (*Na*), effective number of alleles (*Ne*), observed heterozygosity (*Ho*), expected heterozygosity (*He*), Wright's coefficient (*F*), and inbreeding coefficients (*Fis*). The *p*-value for each locus and population was calculated for the *Fis* and *Rh* factor value using Fstat ver. 2.9.3 (Goudet, 2003). By using Arlequin ver. 3.5 (Excoffier and Lischer, 2010) the *Fst* genetic distance value, including the '*p*-value' for this factor, was calculated between tested populations. The principal coordinates analysis (PCoA) included in the GenALEx 6.5 (Peakall and Smouse, 2006) program was carried out based on the value of *Fst*, the fixation index providing an estimation of the genetic differentiation of the tested populations. The test for the presence of null alleles was performed using Micro-Checker v2.2.3 (http://www.microchecker.hull.ac.uk) (Van Oosterhout *et al.*, 2004). Loci in which null alleles are likely to be present were determined by checking whether the frequency of homozygotes exceeded a threshold value. The correlation between the geographical distance of the populations studied and their genetic distance was verified using the r-Pearson formula:

$$r(x,y) = \frac{cov\ (x,y)}{\sigma x} \cdot \sigma y \tag{1}$$

where:

$r(x, y)$ – the *r*-Pearson correlation coefficient between the variables x and y,
$cov(x, y)$ – covariance between the variables x and y,
σ – standard deviation.

Results

Molecular analyses proved polymorphicity of all analysed loci, with the highest genetic variability at the PmOSU_4A7 *locus* with 35 alleles and the lowest variability at the PmOSU_5A8 *locus* with 8 alleles. On average, 12.2 alleles per marker were obtained for the studied stands, with the most alleles per marker found in CHO and JAM (15.3 and 14.8 respectively) and the least in ELK (6.8). The results indicated that three populations from all the stands studied (JAM, LEB, ELK) were not in Hardy-Weinberg genetic equilibrium, a condition that was not affected by the null alleles (Table 3). The results of the analysis of the effective number of alleles (Ne) highlight the minimum value of the index in the ELK population (3.3), with the average parameter for the stands (8.2) (Table 4). In the group of stands that are not in Hardy-Weinberg genetic equilibrium, the ELK population has the highest inbreeding coefficient (0.31), while the other populations (LEB, JAM) are characterised by lower inbreeding (Table 4).

For the ELK stand, the study showed (Fig. 2) high differences from the other populations in the following parameters: number of alleles (*Na*), effective number of alleles (*Ne*), expected heterozygosity (*He*). No significant differences from the other analysed populations were found in the ELK population for the number of private alleles (Fig. 2). The RAM, JAM, CZA, LEB, CHO stands do not differ significantly from each other in the analysed indices of genetic variability.

The studied stands were characterised by genetic variability (*Fst*) ranging between 0.015 to 0.084, and the values distinguishing the ELK population from the other populations were significant for the criterion evaluated (Table 4). To illustrate the genetic similarity of the populations studied in relation to each other, an analysis was performed using the PCoA method for genetic distance data (Fig. 3), which includes two principal components responsible for 66.35%

Table 3.

Analysis of the occurrence of Null alleles in populations (http://www.microchecker.hull.ac.uk)

Locus	RAM	JAM	CZA	LEB	ELK	CHO
PmOSU_4A7	no	yes	yes	yes	yes	no
PmOSU_5A8	no	no	no	na	yes	no
PmOSU_3B2	yes	yes	no	yes	no	no
PmOSU_2G12	no	yes	no	no	no	yes

Table 4.

Genetic diversity in the analysed populations (means): *Na* – number of alleles, *Ne* – effective number of alleles, *Ho* – observed heterozygosity, *He* – expected heterozygosity, *Rh* – Allelic Richness per locus and population, F_{is} – fixation index; statistical significance: ** p≤0.01; for population IDs see Table 1

Populations	*Na*	*Ne*	*Ho*	*He*	*Rh*	F_{is}
RAM	11.5	8.9	0.77	0.87	10.67	0.156
JAM	14.8	10.0	0.69	0.88	10.93	0.237**
CZA	11.0	8.6	0.80	0.86	10.70	0.108
LEB	13.8	9.2	0.69	0.87	10.44	0.226**
ELK	6.8	3.3	0.49	0.68	5.54	0.306**
CHO	15.3	9.0	0.76	0.87	13.07	0.095
Total	12.2	8.2	0.70	0.84	11.88	0.188

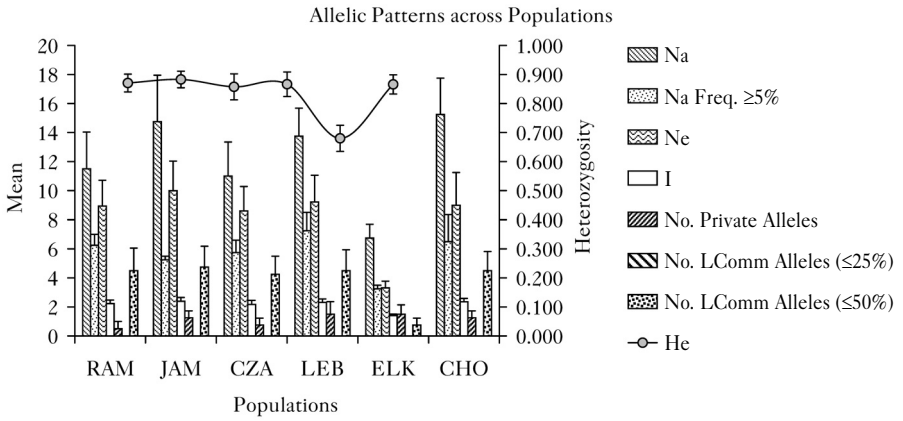

Fig. 2.

Genetic diversity of analysed Douglas-fir populations

Na – number of alleles, He – expected heterozygosity; for population IDs see Table 1

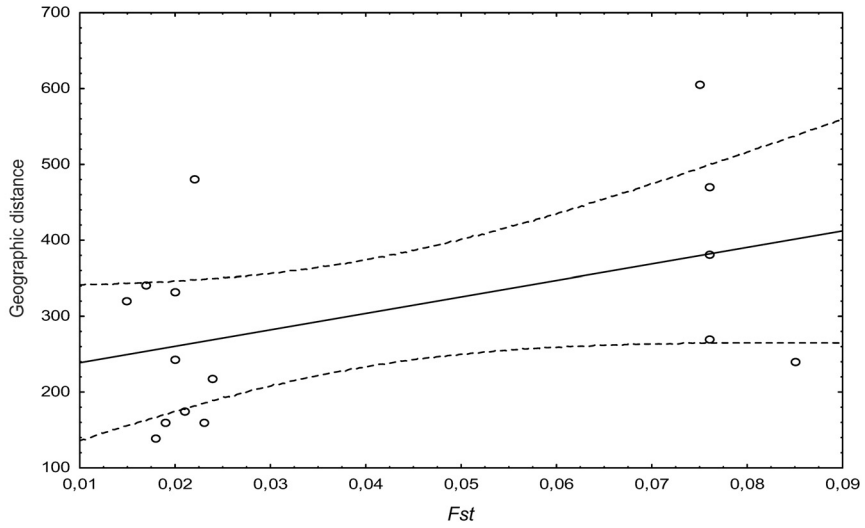

Fig. 3.

The analysis of Pearson's correlation between geographic distance [km] and genetic diversity of populations (*Fst*) yielded a result of r=0.45.

of the total variability. The result obtained shows that the ELK stand is different from the others. The results also illustrate that stands which are geographically distant from each other are genetically quite similar (CHO-RAM) , while other stands that are geographically close to each other show a significant genetic distance (ELK-RAM). The result of the r-Pearson correlation shows an average correlation (r=0.45) of geographical distance with genetic distance.

Discussion

Pseudotsuga menziesii exhibits a high degree of genetic diversity within its natural range, as evidenced by studies utilizing SSR markers (Neophytou *et al*., 2016). The genetic variability of *P. menziesii* in North American forest ecosystems is due to its extensive range and the spread of genetic material through pollen transfer. European populations of *P. menziesii*, derived from seeds collected in the USA, have revealed similarly high genetic variability as their North American counterparts (Klumpp, 1999; Krutovsky *et al*., 2009; Neophytou *et al*., 2020). The comparable level of genetic diversity between native and introduced populations is likely due to similar ecological conditions and selective pressures that favour well-adapted genotypes (Neophytou *et al*., 2020).

Niemczyk *et al*. (2021) identified several bioclimatic factors, including mean annual temperature, annual temperature range (continentality), mean annual precipitation sum, isothermality, and precipitation seasonality, as having an effect on quantitative traits of *P. menziesii*. In our study (Fig. 1), we selected two bioclimatic factors: number of frost days (nfd) and precipitation seasonality (bio15), intentionally excluding mean annual temperature and annual precipitation due to their generalized nature (Puchałka *et al*., 2021).

Douglas-fir populations growing in Poland occupy a climatic niche within the secondary range of the species in Europe, bordering on the climatic niche of its natural North American habitat, likely corresponding to the 'interior' populations (Fig. 1b; Boiffin *et al*., 2017). Thus, populations from northern Poland experience conditions resembling the interior of North

America, despite being situated on the Baltic coast (Fig. 1b). Although it remains uncertain whether the conditions of their secondary distribution were optimal for local adaptation within their natural range, we can speculate on the environmental pressures shaping their genetic structure. The mechanism driving genetic diversity of *P. menziesii* under environmental pressure is likely natural selection (Klumpp, 1999; Krutovsky *et al*., 2009), as observed in populations in America and Europe (Neophytou *et al*., 2020).

Old-growth *P. menziesii* populations in northern Poland exhibit considerable genetic variability, as indicated by observed heterozygosity (Table 4; H0=0.69-0.80), except for the younger ELK stand (H0=0.49). The notably lower genetic variability of the ELK population suggests that it originated from seeds of a local population, which is supported by Neophytou *et al*. (2020). Our results support the hypothesis of a bottleneck effect in younger *P. menziesii* populations in Poland and potentially in Europe (Table 4), likely driven by a limited number of reproducing trees.

Studying the genetic diversity of local populations is crucial for understanding the adaptation of European *P. menziesii* populations to projected climate change and alterations in the species' secondary range (Puchałka *et al*., 2023). Differences in genetic variation in the ELK population are reflected in genetic distance indices, with ELK exhibiting a 300% higher genetic distance than other populations (Fig. 3). The PCoA diagram of genetic distance (Fig. 4) clearly sets ELK apart from the other populations, suggesting a different origin. This disparity in genetic parameters between old-growth stands and the ELK population likely arises from the species' introduction history in Europe, characterized by seed collection from limited mother trees.

Following a period of increased interest in seed collection in the 19th century, large quantities of reproductive material were shipped from the natural range of *P. menziesii*, creating a broad but random genetic base (Lavender and Hermann, 2014). However, the import of seeds to Poland ceased after the Second World War (due to the Communist Bloc), so the use of local seed sources became necessary (Związek *et al*., 2023) Thus, the ELK population likely originates from seeds collected locally, resulting in reduced genetic diversity. The locally adverse climatic conditions during ELK's growth (predominantly continental; Fig. 1) contributed to nearly halving observed heterozygosity and allelic richness compared to other populations (Table 4).

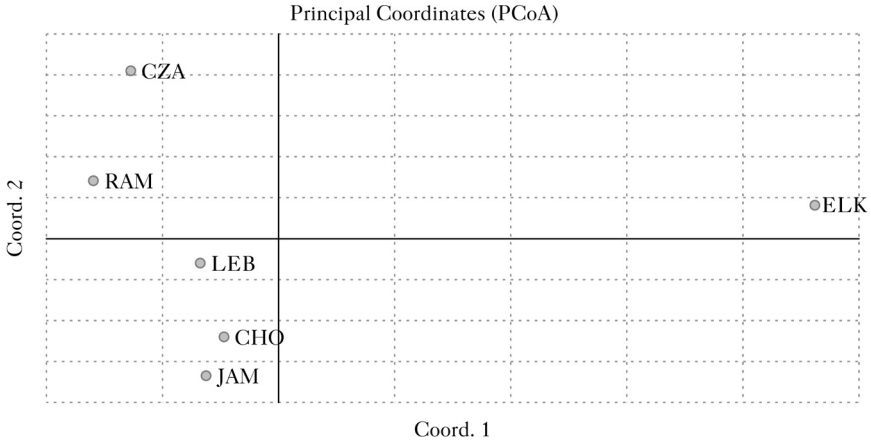

Fig. 4.

Grouping of studied Douglas-fir locations using principal coordinates analysis (PCoA) based on D_{ST} – Nei genetic distance (Nei, 1978)

Our analyses revealed a moderate correlation coefficient (r=0.45) between geographical and genetic distance. While the experimental design does not cover the entire species range in Poland, genetic differentiation was expected due to the species' secondary range in Europe (Puchałka *et al*., 2023), geographical isolation, and climatic disparities (Fig. 1). Our genetic differentiation results are consistent with the potential distribution of Douglas-fir in Europe (Fig. 1; Puchałka *et al*., 2023).

Second-generation reproduction of *P. menziesii* may lead to reduced genetic variability and increased inbreeding, which is particularly evident in the ELK population (Table 4). Therefore, the use of reproductive material from isolated populations from relatively young stands may jeopardize genetic diversity due to environmental selection pressure. Hence, it is essential to consider the minimum age of harvested populations to ensure their natural origin. Populations younger than 130 years may originate from local collections, especially in Eastern Europe, which requires further studies to corroborate this hypothesis.

In the absence of sufficient old reproductive material in Poland, importing *P. menziesii* seeds from acclimatised populations in other European countries or from the species' natural range in North America may be necessary. The choice of seed source is crucial, especially given changing climatic conditions and increasing interest in *P. menziesii* as an alternative species for mixed stands (Thurm *et al*., 2016). As the species' natural regeneration capacity in Europe is limited (Eberhard and Hasenauer, 2018), breeding efforts may be required if the decision to promote this alien species in Europe is pursued. With the predicted reduction in ecological niches for *P. menziesii* under new climatic conditions, understanding its acclimatization potential is crucial as climatic anomalies become more frequent.

Conclusions

- The average correlation between genetic and geographical distance may be a consequence of the dispersed distribution of the species in this part of Europe (isolated populations) and the resulting limited gene flow between them. However, this does not exclude that the old populations of *P. menziesii* were established from genetically similar seed material.
- Our results indicate that there is a high probability of the 'bottleneck effect' in the youngest populations (likely second-generation) established from seeds collected in first-generation stands from Poland. The reduction in genetic variability of the progeny that occurs in this case is a phenomenon widely observed among European populations of this species, but there is still a lack of research to determine the direction of such selection.
- Three out of the six populations (JAM, LEB, ELK) show a significant inbreeding index, which may indicate directional selection leading to the elimination of certain genotypes from the populations, or the use of a limited genetic pool (from a limited number of mother trees from the native range) to establish these populations.

Authors' contribution

A.G. was responsible for field data and the testing material collection, field measurements, participation in the conceptualization, funding acquisition, editing, P.P. was responsible for the conceptualization, methodology, writing-original draft preparation, writing-review and editing, review process; S.J. was responsible for the project conceptualization, writing, editing, methodology; V.M. was responsible for methodology, statistical analyses; M.K. was responsible for methodology, writing-original draft preparation, writing-review and editing.

Conflict of interest

The Authors declare no conflict of interest.

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References

Aas, G., 2008. Die Douglasie (*Pseudotsuga menziesii*) in Nordamerika: Verbreitung, Variabilität und Ökologie. *LWF – Wissen*, 59: 7-12.

Alizoti, P., Bastien, J.C., Chakraborty, D., Klisz, M.M., Kroon, J., Neophytou, C., Schueler, S., van Loo, M., Westergren, M., Konnert, M., 2022. Non-native forest tree species in Europe: The question of seed origin in afforestation. *Forests*, 13: 273. DOI: https://doi.org/10.3390/f13020273.

Amarasinghe, V., Carlson, J.E., 2002. The development of microsatellite DNA markers for genetic analysis in Douglas-fir'. *Canadian Journal of Forest Research*, 32 (11): 1904-1915. DOI: https://doi.org/10.1139/x02-110.

Berney, J.L.A., 1972. Studies on the probable origin of some European Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) plantations. British Columbia: University of British Columbia. DOI: https://doi.org/10.14288/1.0075390.

Boiffin, J., Badeau, V., Bréda, N., 2017. Species distribution models may misdirect assisted migration: Insights from the introduction of Douglas-fir to Europe. *Ecological Applications*, 27 (2): 446-457. DOI: https://doi.org/10.1002/eap.1448.

Brus, R., Pötzelsberger, E., Lapin, K., Brundu, G., Orazio, Ch., Straigyte, L., Hasenauer, H., 2019. Extent, distribution and origin of non-native forest tree species in Europe. *Scandinavian Journal of Forest Research*, 34 (7): 533-544. DOI: https://doi.org/10.1080/02827581.2019.1676464.

Burzyński, G., 1990. Wrażliwość jedlicy na mrozy zimowe w latach 1975-1980 na powierzchniach proweniencyjnych Instytutu Badawczego Leśnictwa. (Sensitivity of Douglas fir to winter frost in the years 1975-1980 on provenance areas of the Forest Research Institute). *Sylwan*, 134 (1): 37-49.

Caudullo, G., Welk, E., San-Miguel-Ayanz, J., 2017. Chorological maps for the main European woody species. *Data in Brief*, 12: 662-666. DOI: https://doi.org/10.1016/j.dib.2017.05.007.

Chakraborty, D., Wang, T., Andre, K., 2016. Adapting Douglas-fir forestry in Central Europe: Evaluation, application, and uncertainty analysis of a genetically based model. *European Journal Forest Research*, 135: 919-936. DOI: https://doi.org/10.1007/s10342-016-0984-5.

Chałupka, W., 2014. Zasoby genowe i potencjał hodowlany daglezji (*Pseudotsuga menziesii* (Mirb.) Franco) w lasach niektórych krajów europejskich. [Genetic resources and silvicultural potential of Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) in some European countries]. *Sylwan*, 158 (5): 331-339. DOI: https://doi.org/10.26202/sylwan.2013099.

Dimitrova, A., Csilléry, K., Klisz, M., Lévesque, M., Heinrichs, S., Cailleret, M., Andivia, E., Madsen, P., Böhenius, H., Cvjetkovic, B., De Cuyper, B., de Dato, G., Ferus, P., 2022. Risks , benefits , and knowledge gaps of non-native tree species in Europe. *Frontiers in Ecology and Evolution*, 10: 908464. DOI: https://doi.org/10.3389/fevo.2022.908464.

Dyderski, M.K., Paź, S., Frelich, L.E., Jagodziński, A.M., 2018. How much does climate change threaten European forest tree species distributions? *Global Change Biology*, 24: 1150-1163. DOI: https://doi.org/10.1111/gcb.13925.

Eberhard, B., Hasenauer, H., 2018. Modeling regeneration of Douglas fir forests in Central Europe. *Austrian Journal of Forest Science*, 135: 33-51.

Eckert, C.G., Samis, K.E., Lougheed, S.C., 2008. Genetic variation across species' geographical ranges: The central-marginal hypothesis and beyond. *Molecular Ecology*, 17 (5): 1170-1188. DOI: https://doi.org/10.1111/j.1365-294X.2007.03659.x.

Eckhart, T., Walcher, S., Hasenauer, H., van Loo, M., 2017. Genetic diversity and adaptive traits of European versus American Douglas-fir seedlings. *European Journal Forest Research*, 136: 811-825. DOI: https://doi.org/10.1007/s10342-017-1072-1.

Excoffier, L., Lischer, H.E.L., 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, 10: 564-567. DOI: https://doi.org/10.1111/j.1755-0998.2010.02847.x.

Fonder, W., Matras, J., Załęski, A., 2007. Leśna baza nasienna w Polsce. Warszawa: Centrum Informacyjne Lasów Państwowych, 300 pp.

Fontes, L., Savill, P., Luis, J.S., 2003. Identification of the origin of Portuguese Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] provenances. *Silvae Genetica*, 52 (5-6): 266-273.

GBIF, 2012. Global Biodiversity Information Facility (GBIF), Natural History. Available from: http://www.gbif.org/ [accessed: 21.11.2012].

Goudet, J., 2003. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.4). Available from: http://www2.unil.ch/popgen/softwares/fstat.htm [accessed: 21.11.2003].

Hartl, D.L., Clark, A.G., 2007. Principles of population genetics. *Écoscience*, 14 (4): 544-545.

Herman, R.K., 2014. Adverse abiotic factors. In: D.P. Lavender, R.K. Hermann, eds. Douglas-fir: The genus *Pseudotsuga*. Corvallis: Oregon State University, pp. 245-272.

Hintsteiner, W.J., van Loo, M., Neophytou, C., Schueler, S., Hasenauer, H., 2018. The geographic origin of old Douglas-fir stands growing in Central Europe. *European Journal Forest Research*, 137: 447-461. DOI: https://doi.org/10.1007/s10342-018-1115-2.

Isaac-Renton, M., Dawid, R., Hamann, A., Spiecker, H., 2014. Douglas-fir plantations in Europe: A retrospective test of assisted migration to address climate change. *Global Change Biology*, 20 (8): 2607-2617. DOI: https://doi.org/10.1111/gcb.12604.

Jastrzębowski, S., Ukalska, J., Guziejko, A., Puchałka, R., 2021. Current and predicted future winter warm spells would affect Douglas Fir (*Pseudotsuga menziesii* (Mirb.), Franco) seeds in the early stage of germination more than in the late stage of germination. *Forests*, 12: 796. DOI: https://doi.org/10.3390/f12060796.

Karger, D., Conrad, O., Böhner, J., 2017. Climatologies at high resolution for the earth's land surface areas. *Scientific Data*, 4: 170122. DOI: https://doi.org/10.1038/sdata.2017.122.

Klumpp, R.T., 1999. Untersuchungen zur Genökologie der Douglasie (*Pseudotsuga menziesii* [Mirb.] Franco). Doctoral dissertation. Niedersächsische Staats-und Universitätsbibliothek Göttingen. DOI: http://dx.doi.org/10.53846/goediss-2310.

Krutovsky, K.V., St. Clair, J.B., Saich, R., Hipkins, V.D., Neale, D.B., 2009. Estimation of population structure in coastal Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco var. menziesii] using allozyme and microsatellite markers. *Tree Genetics and Genomes*, 5: 641-658. DOI: https://doi.org/10.1007/s11295-009-0216-y.

Lange, F., Ammer, C., Leitinger, G., Seliger, A., Zerbe, S., 2022. Is Douglas fir [*Pseudotsuga menziesii* (Mirbel) Franco] invasive in Central Europe? A case study from south-west Germany. *Frontiers in Forests and Global Change*, 5: 844580. DOI: https://doi.org/10.3389/ffgc.2022.844580.

Lefèvre, F., Fady, B., Fallour-Rubio, D., Ghosn, D., Bariteau, M., 2004. Impact of founder population, drift and selection on the genetic diversity of a recently translocated tree population. *Heredity*, 93 (6): 542-550. DOI: https://doi.org/10.1038/sj.hdy.6800549.

Little, E.L., Viereck, L.A., 1971. Atlas of United States Trees: volume 1 Conifers and important hardwoods. United States: Agricultural Research Service. Forage and Range Research Branch.

Mejnartowicz, L., 1976. Genetic investigations on Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) populations. *Arboretum Kórnickie*, 21: 126-179.

Mejnartowicz, L., 2007. Productivity of Douglas fir populations in the IUFRO provenance experiment at age 39. *Raport dla ID PAN*.

Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, 89 (3): 583-590.

Neophytou, C., van Loo, M., Hasenauer, H., 2020. Genetic diversity in introduced Douglas-fir and its natural regeneration in Central Europe. *Forestry: An International Journal of Forest Research*, 93: 535-544. DOI: https://doi.org/10.1093/forestry/cpz055.

Nicolescu, V.N., Mason, W.L., Bastien, J.C., Vor, T., Petkova, K., Podrázský, V., ..., Mihăilescu, G., 2023. Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) in Europe: An overview of management practices. *Journal of Forestry Research*, 34 (4): 871-888. DOI: https://doi.org/10.1007/s11676-023-01607-4.

Niemczyk, M., Chmura, D.J., Socha, J., Wojda, T., Mroczek, P., Gil, W., Thomas, B.R., 2021. How geographic and climatic factors affect the adaptation of Douglas-fir provenances to the temperate continental climate zone in Europe. *European Journal of Forest Research*, 140: 1341-1361. DOI: https: //doi.org/10.1007/s10342-021-01398-5.

Peakall, R.O.D., Smouse, P.E., 2006. GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, 6 (1): 288-295. DOI: https://doi.org/10.1111/j.1471-8286.2005.01155.x.

Podhorsky, J., 1927. New information on Douglas-fir in Europe. *Allgemeine Forst und Jagdzeitung*, 103: 255-258.

Przybylski, P., Mohytych, V., Rutkowski, P., Tereba, A., Tyburski, Ł., Fyalkowska, K., 2021. Relationships between some biodiversity indicators and crown damage of *Pinus sylvestris* L. in natural old growth pine forests. *Sustainability*, 13 (3): 1239. DOI: https://doi.org/10.3390/su13031239.

Puchałka, R., Paź-Dyderska, S., Jagodziński, A.M., Sádlo, J., Vítková, M., Klisz, M., ..., Dyderski, M.K., 2023. Predicted range shifts of alien tree species in Europe. *Agricultural and Forest Meteorology*, 341: 109650. DOI: https://doi.org/10.1016/j.agrformet.2023.109650.

Puchałka, R., Dyderski, M., Vítková, M., Sádlo, J., Klisz, M., Netsvetov, M., Prokopuk, Y., Matisons, R., Mionskowski, M., Wojda, T., Koprowski, M., Jagodziński, A., 2021. Black locust (*Robinia pseudoacacia* L.) range contraction and expansion in Europe under changing climate. *Global Change Biology*, 27 (8): 1587-1600. DOI: https://doi.org/10.1111/gcb.15486.

Schmid, M., Pautasso, M., Holdenrieder, O., 2014. Ecological consequences of Douglas fir (*Pseudotsuga menziesii* [Mirb.] Franco) cultivation in Europe. *European Journal of Forest Research*, 133: 13-29. DOI: https://doi.org/10.1007/s10342-013-0745-7.

Slavov, G.T., Howe, G.T., Yakovlev, I., Edwards, K.J., Krutovskii, K.V., Tuskan, G.A., ..., Adams, W.T., 2004. Highly variable SSR markers in Douglas-fir: Mendelian inheritance and map locations. *Theoretical and Applied Genetics*, 108: 873-880. DOI: https://doi.org/10.1007/s00122-003-1490-y.

Thurm, E.A., Uhl, E., Pretzsch, H., 2016. Mixture reduces climate sensitivity of Douglas-fir stem growth. *Forest Ecology and Management*, 376: 205-220. DOI: https://doi.org/10.1016/j.foreco.2016.06.020.

van Loo, M., Dobrowolska, D., 2019. Douglas-fir's role in European forests in the 21st century. *Douglas-fir – an option for Europe. What Science Can Tell Us*, 9: 30-32.

van Loo, M., Hintsteiner, W., Pötzelsberger, E., Schüler, S., Hasenauer, H., 2015. Intervarietal and intravarietal genetic structure in Douglas-fir: Nuclear SSR s bring novel insights into past population demographic processes, phylogeography, and intervarietal hybridization. *Ecology and Evolution*, 5 (9): 1802-1817. DOI: https://doi.org/10.1002/ece3.1435.

van Loo, M., Lazic, D., Chakraborty, D., Hasenauer, H., Schüler, S., 2019. North American Douglas-fir (*P. menziesii*) in Europe: establishment and reproduction within new geographic space without consequences for its genetic diversity. *Biological Invasions*, 21 (11): 3249-3267. DOI: https://doi.org/10.1007/s10530-019-02045-2.

van Oosterhout, C., Hutchinson, W.F., Wills, D.P., Shipley, P., 2004. MICRO-CHECKER: Software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, 4 (3): 535-538. DOI: https://doi.org/10.1111/j.1471-8286.2004.00684.x.

Wohlgemuth, T., Gossner, M.M., Campagnaro, T., Marchante, H., van Loo, M., Vacchiano, G., ..., Silva, J.S., 2022. Impact of non-native tree species in Europe on soil properties and biodiversity: A review. *NeoBiota*, 78: 45-69. DOI: https://doi.org/10.3897/neobiota.78.87022.

Związek, T., Łuców, D., Popek, J., Klisz, M., Obremska, M., Sobechowicz, Ł., ..., Swoboda, P., 2023. Addressing multiple perspectives in studying environmental changes in forest landscapes during the modernization period (18-19th centuries). *The Anthropocene Review*. DOI: https://doi.org/10.1177/20530196231205485.

STRESZCZENIE

Zróżnicowanie genetyczne daglezji zielonej *Pseudotsuga menziesii* (Mirb.) Franco w północnej Polsce

Wśród introdukowanych drzew iglastych daglezja zielona *Pseudotsuga menziesii* (Mirb.) Franco wydaje się być jednym z najlepiej zaaklimatyzowanych gatunków w swoim wtórnym zasięgu (ryc. 1). Ten sukces introdukcyjny był najprawdopodobniej wynikiem dopasowania wtórnych warunków wzrostu do wymagań ekologicznych rodzimych populacji (tab. 1). Wiedza na temat struktury genetycznej pierwszego pokolenia jest jednak nadal niepełna, co generuje pytanie, czy zmienność genetyczna gatunku jest wystarczająca w celu adaptacji do zmieniającego się klimatu. Badania proweniencyjne przeprowadzone w Polsce wskazują też, że dla przetrwania daglezji istotne znaczenie ma region jej pochodzenia: czy jest to odmiana „przybrzeżna", czy „kontynentalna". Najnowsze badania Niemczyk i in. (2021) wskazują na przewagę adaptacyjną w Polsce odmiany „kontynentalnej". Niestety, stare drzewostany daglezji, mogące stanowić źródło nasion w kraju, w większości nie mają udokumentowanego pochodzenia, więc analizy wykorzystujące narzędzia biologii molekularnej wydają się konieczne.

Głównym celem badań było określenie zróżnicowania genetycznego pierwszego pokolenia populacji *Pseudotsuga menziesii* (Mirb.) Franco w północnej Polsce przy wykorzystaniu neutralnych *loci* mikrosatelitarnego DNA. Ponadto postawiono hipotezę badawczą, że populacje pierwszego pokolenia charakteryzują się wysoką różnorodnością genetyczną w porównaniu z wtórnymi, czyli młodszymi populacjami. W badaniach całkowite DNA wyizolowano z igieł zebranych w 5 starych populacjach i jednym młodszym drzewostanie (ELK). Wyizolowany materiał genetyczny charakteryzował się wysoką jakością i ilością DNA. W analizach molekularnych wykorzystano

4 jądrowe markery mikrosatelitarne (tab. 2), które zostały użyte do określenia podstawowych wskaźników genetycznych: liczby alleli na *locus* (*Na*), liczby efektywnych alleli na *locus* (*Ne*), obserwowanej heterozygotyczności (*Ho*) i oczekiwanej heterozygotyczności (*He*) oraz bogactwa allelicznego (*Rh*) (tab. 4; ryc. 2). Dodatkowo przeprowadzono analizę istotności wpływu alleli zerowych (tab. 3) – uzyskany wynik udowodnił ich obecność, lecz nie zostały one przypisane do konkretnego *locus*.

Wyniki potwierdziły polimorficzność wszystkich badanych *loci*, z których najwięcej alleli miał PmOSU_4A7. W badaniach uzyskano wysokie wskaźniki liczby alleli i bogactwa allelicznego, a rezultaty nie korespondowały z liczbą przebadanych próbek. Wysoka zmienność genetyczna 5 drzewostanów pierwszego pokolenia odpowiada różnorodności populacji rodzimych, podczas gdy młodszy drzewostan (ELK) charakteryzuje się znacznie niższą zmiennością genetyczną i wyższym wskaźnikiem wsobności (tab. 4). Analiza dystansu genetycznego wskazuje na genetyczne oddzielenie młodszej populacji od populacji starszych (ryc. 4).

W pracy po raz pierwszy zaprezentowano wyniki analizy różnorodności genetycznej *Pseudotsuga menziesii* w północnej Polsce. Biorąc pod uwagę dystans genetyczny między populacjami pierwszego pokolenia a młodszym, najprawdopodobniej wtórnym pokoleniem, a także jego wysoki współczynnik wsobności, zaleca się genetyczną weryfikację populacji wchodzących w skład bazy nasiennej tego gatunku w Polsce. Uzyskane w prezentowanej pracy wyniki są tożsame z publikacją Neophytou i in. (2020), według której odtworzenie drzewostanów daglezji z drugiego pokolenia może istotnie zmniejszyć zmienność genetyczną populacji, prowadząc do krzyżowania między osobnikami spokrewnionymi. Takie zjawisko wystąpiło najprawdopodobniej w najmłodszej populacji ELK (tab. 4). Dlatego też wykorzystanie materiału rozrodczego z izolowanych populacji lub ze względnie młodych drzewostanów (poniżej 130 lat) może być narażone na znaczne zmniejszenie, a w późniejszym stadium nawet erozję zmienności genetycznej ze względu na selekcję środowiskową i naturalną. Sugeruje się więc, że minimalny wiek populacji, z których planowany jest zbiór nasion, powinien wynosić 130 lat. Hipoteza ta wymaga jednak dalszych badań i uzupełnienia poprzez badania z zakresu historii leśnictwa. W przypadku braku zweryfikowanych źródeł nasion w Polsce należy także rozważyć wykorzystanie importu z USA lub Kanady.