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Does genotype affect the ability to form root suckers? A case study in black poplar

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Abstract: It is believed that poplar species from the *Aigeiros* section form root suckers less often than other poplars. Nevertheless, juvenile plants growing from root suckers can be found in natural populations of black poplar. As they are clones only of certain trees, it seems that the emergence of root suckers may be under genetic control. Unfortunately, there are no studies carried out in more controlled conditions to verify this observation. Here we genotyped root suckers in the clone archive of black poplar. Samples were collected from the vicinity of 21 individuals originating from two natural populations located in the Vistula and Warta river valleys in Poland. One individual was also used in a pot experiment under control and drought-stress conditions. Most clones from the clone archive formed root suckers. Their number was positively correlated with the above-ground size of the plants. However, it did not depend on the origin of the trees. In the pot experiment, we observed root suckers only in control plants after their root system had been damaged. The outcomes of our study suggest that black poplar's ability to form root suckers may be controlled by genotype-environment interactions. As shown by the pot experiment results, individuals may not have enough resources to invest in root suckers when environmental conditions are unfavorable. This is probably one of the reasons why only some genets in natural conditions form root suckers. Our findings are discussed in the context of their ecological significance.

Keywords: *Populus*, vegetative and asexual reproduction, clones, genetic variation, climate change

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Introduction

Black poplar (*Populus nigra* L.) is a dioecious, pioneer, and heliophilous tree. It constitutes one of the main components of riparian forests in Europe and Asia. Its short-lived seeds are transported by wind and water. They require specific water and soil conditions to successfully germinate and grow, i.e. bare,

moist, and sandy surfaces created by periodic flooding (Guilloy-Froget et al., 2002). Once widespread, black poplar is now considered threatened in many European countries. River regulation, deforestation, and intensive land use of riverside areas have caused a drastic decrease in the number of black poplar individuals over the last decades (Hughes et al., 2005). This species has now great difficulties with natural

regeneration, especially in the most transformed sections of the river valleys (Vanden Broeck et al., 2004; Wójkiewicz et al., 2019, 2021; Żukowska et al., 2021). Additional threats include seasonal, illegal burning of riverside meadows and a dynamically growing population of European beaver. Black poplar also hybridizes with fast-growing poplar cultivars of Euramerican hybrids (*P. × canadensis* Moench) and varieties of *P. nigra* such as Lombardy poplar (*P. nigra* cv. 'Italica' Duroi) (Vanden Broeck et al., 2004). This fact can lead to the progressive loss of the genetic integrity of this valuable tree.

Black poplar reproduces mainly generatively with impressive seed yields ($25\text{--}50 \times 10^6$ seeds annually per tree; Bessey, 1904). In favorable environmental conditions, a large number of seedlings can be found, reaching even 4,000 specimens per 1 m^2 (Braatne et al., 1996). Nevertheless, only a few of them survive the first year (Johnson, 2000). This very high mortality rate seems to be at least partially compensated by asexual reproduction. After damage or when environmental conditions drastically change, black poplar can start sprouting from its trunk, roots, or even from completely detached fragments of the parental stem carried with the river current (Barsoum, 2002; Barsoum et al., 2004). Shoots produced by the root system are known as root suckers. This phenomenon can be observed in many angiosperm trees, but not all species retain this ability throughout their lifetime (Del Tredici, 2001). Poplar species from the *Aigeiros* section are believed to form fewer root suckers than other poplar species (Bugala, 1973). Root suckers can lead to the formation of duplicate clones of the same individual (=ramets) in the close neighborhood, but suckering has its primary role as a reparative response that only secondarily results in clonal growth (Del Tredici, 2001). Individual ramets share water and nutrients which allow them to remain functional for many years (De Byle, 1964).

To date, it is unknown whether the ability to form root suckers is species-specific or varies among individuals of the same species. So far there are no studies that were carried out in the same environmental conditions that could help to resolve this issue. The available research comprised natural populations where only some trees were clones located in close proximity (Smulders et al., 2008; Wójkiewicz et al., 2021). The results suggest that not all genets are able to form root suckers. Sometimes clones were found at some distance from one another. They had been most likely formed from fragments of shoots carried along the river current (Barsoum et al., 2004; Smulders et al., 2008). The level of clonality differs in stands of different ages (Barsoum et al., 2004). Unfortunately, it is hard to draw any definite conclusions because previous studies were carried out in highly transformed river valleys where conditions

are often far from natural, and several factors may initiate or suppress the regrowth of specific individuals. To our best knowledge, our research is the first one to assess how genotype affects the ability to form root suckers by black poplar trees growing in the same environmental conditions.

In this paper, we aimed to verify if the formation of root suckers by black poplar is under genetic control. To do so, we analyzed the genotypes of root suckers growing in the black poplar clone archive located at the Institute of Dendrology, Polish Academy of Sciences in Kórnik, Poland (ID PAS). We also carried out a pot experiment. Specifically, we asked the following questions: (1) Do only some genotypes form root suckers as a result of direct damage? (2) Is this ability retained in unfavorable environmental conditions?

Materials and methods

Our research was carried out using root suckers growing in the black poplar clone archive at ID PAS. The archive was established in 2015 by rooting cuttings collected from natural populations of black poplar. Each clone in the archive is represented by three repetitions. The clone archive is regularly mowed and the shoots of the clones are cut annually. This acts as an impulse for the formation of root suckers which are visible in the close vicinity of some specimens. In this study, we chose two fragments of the archive with uniform environmental conditions where all individuals survived and were in good condition. The first part is occupied by 14 clones of black poplar that come from the natural population located in the Vistula River valley near Dęblin (pop. D) in Poland ($51^{\circ}34'00''\text{N}$, $21^{\circ}51'41''\text{E}$). This area used to be a military training zone and is strongly affected by human activities. The population comprises over 50 pure black poplar trees – remnants of a natural floodplain forest. The trees are close to terminal age – more than 150 years old. We have not observed any natural regeneration on this site. The population is characterized by a high level of genetic variation and high genotypic richness ($R = 0.91$) (Lewandowski & Litkowiec, 2017). The second area comprises seven clones originating from the natural black poplar population found in the Warta River valley near Czeszewo (pop. C) in Poland ($52^{\circ}08'22''\text{N}$, $17^{\circ}30'49''\text{E}$). This location has been heavily transformed by humans. Currently, there is a ferry crossing. The trees are close to terminal age and there is no natural regeneration. Pop. C is less numerous than pop. D and is characterized by lower genotypic richness.

We collected leaves from all parent individuals ($21 \times 3 = 63$ samples) and root suckers (61 samples) in the chosen fragment of the clone archive

in August 2022. Leaves were frozen at -20°C . DNA was extracted from leaf tissue ground in liquid nitrogen according to the CTAB protocol (Dumolin et al., 1995). Genotyping and subsequent clonality and kinship analyses were performed using 13 nuclear microsatellite loci (Smulders et al., 2001; Van der Schoot et al., 2000), as described by Żukowska et al. (2021). This set of microsatellite loci allows the identification of individuals with a probability exceeding 99.99%. All parent clones were also divided into four classes depending on the size of their above-ground part. The correlation between the size class and the number of root suckers was checked by calculating Spearman's rank correlation coefficient. We used the Mann-Whitney U test to check the differences between the mean number of root suckers in pop. D vs. pop. C. For both populations, we calculated basic genetic variation parameters, including the mean number of alleles (A), mean effective number of alleles (A_E), number of private alleles (A_p), as well as observed (H_o) and expected heterozygosity (H_e) using GenAlEx ver. 6.5 (Peakall & Smouse, 2006, Peakall & Smouse, 2012). The mean rarefied allelic richness (A_R) and inbreeding coefficients (F_{IS}) were computed in FSTAT ver. 2.9.4 (Goudet, 2003). Genetic differentiation was assessed based on the values of fixation index (F_{ST}) and Slatkin's analog of F_{ST} (R_{ST}). To visualize genetic relationships among the studied individuals we carried out the Principal Coordinates Analysis (PCoA), available in GenAlEx.

One male clone (D11) with a moderate number of root suckers was also used in the pot experiment established in 2020 (Fig. 1). This clone was vegetatively propagated and rooted in two replicates in 20 pots. Plants grew in the tent in optimal conditions throughout the first growing season. In 2021, the

pots were divided into control (optimal hydration, i.e. water on pot stands) (10 pots) and stressed (10 pots) variants. The stressed plants were subjected to 11 series of drought stress starting from the middle of April until the end of August 2021 in such a way that they were not watered until external signs of water deficiency. At the end of March 2022, specimens from each pot were separated by cutting the root systems and planted individually in bigger pots. Afterward, the experiment was continued in the same way as in 2021.

Results

All 21 black poplar clones from the clone archive had different genotypes, repeated three times, which confirms that they were planted according to the plan. The genotypes of all root suckers corresponded to the genotypes of parent individuals. The results showed that the majority of the studied clones (17 out of 21) formed root suckers but their number was variable (1–11 in pop. D; 1–6 in pop. C) and they reached different distances. In both populations two clones had no root suckers (Table 1, Fig. 2). Nonetheless, the difference between the mean values of root suckers between pop. D and C was statistically insignificant ($p = 0.453$). There was a strong positive linear correlation between the size class of the clones and the number of root suckers they produced ($\rho = 0.771$; $p = 0.001$ for pop. D; and $\rho = 0.748$; $p = 0.05$ for pop. C). The pot experiment revealed that root suckers were present only in all control pots as a consequence of root damage after the plants were separated (Fig. 1).

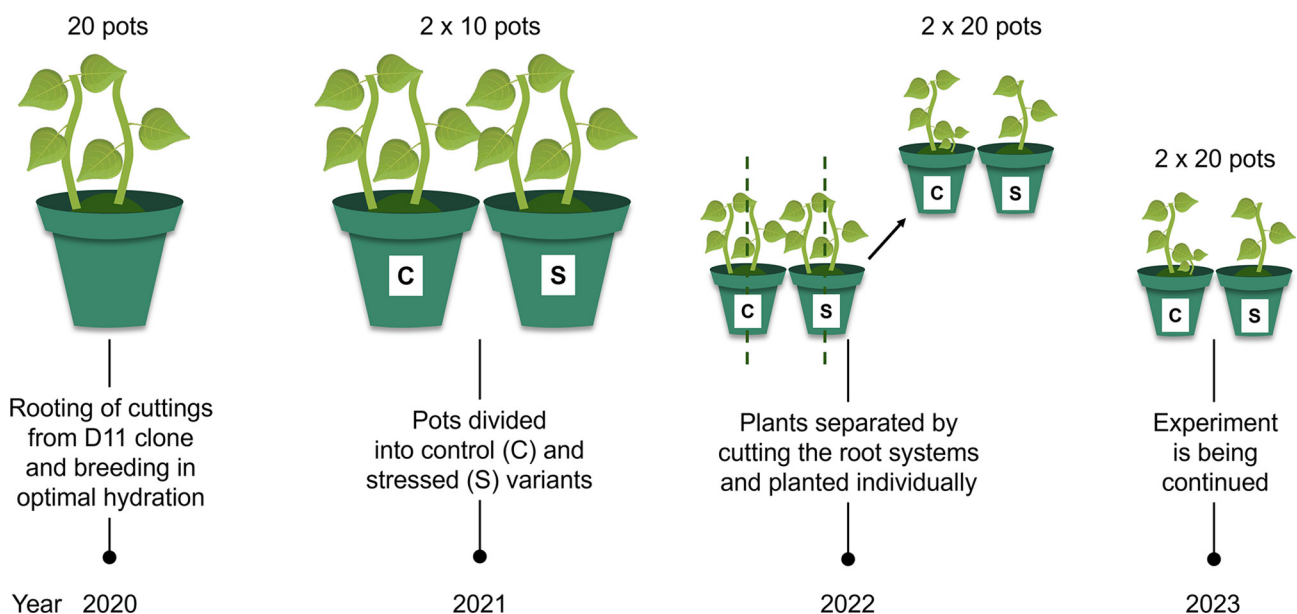


Fig. 1. The design and course of the pot experiment

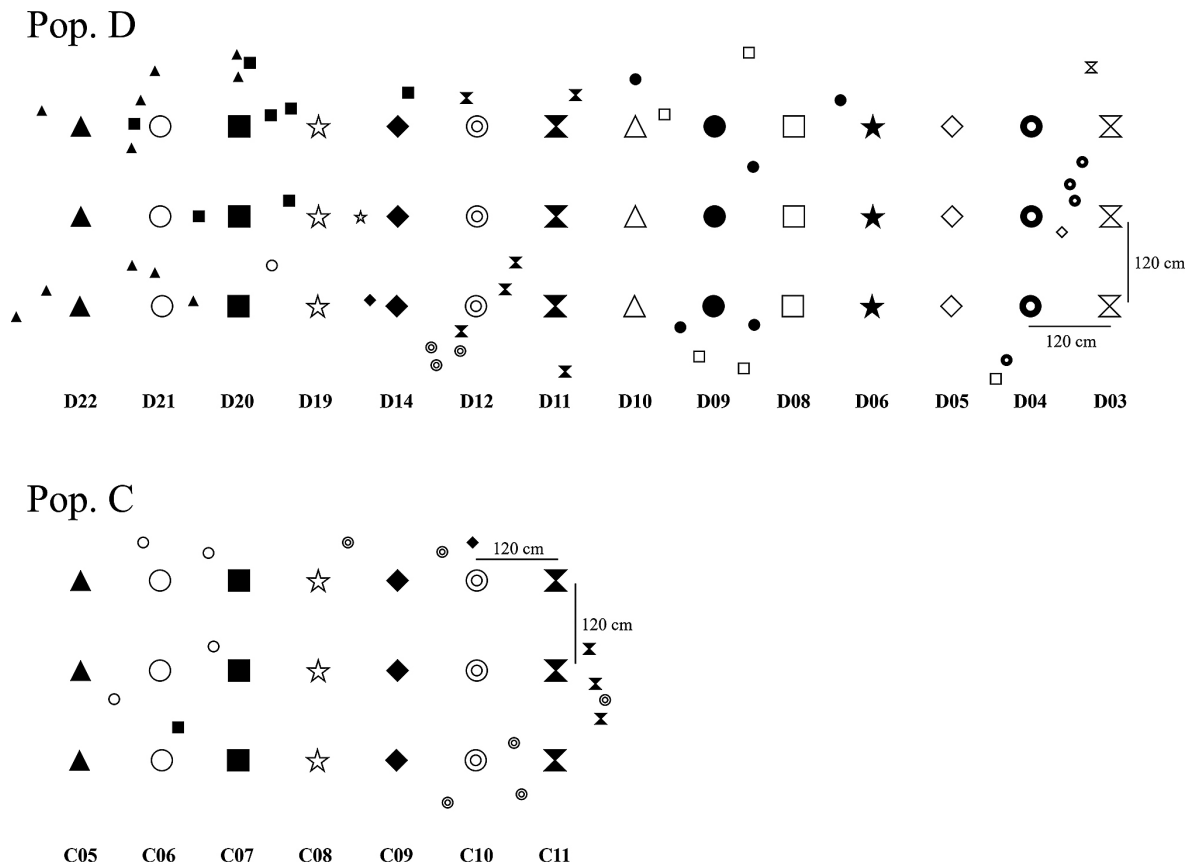


Fig. 2. Schematic representation of the two fragments of the black poplar clone archive used in this study. Each clone is planted in three repetitions. Smaller symbols correspond to the root suckers formed by the respective parent clones

Table 1. Sex, size class, and the number of root suckers formed by the black poplar clones analyzed in this study

Population	Clone	Sex	Size class	No. of root suckers
Dęblin	D22	-	4	11
	D21	M	3	1
	D20	F	3	7
	D19	F	2	1
	D14	F	1	1
	D12	M	3	3
	D11	M	3	6
	D10	F	2	0
	D09	M	3	5
	D08	-	3	5
	D06	M	2	0
	D05	M	3	1
	D04	M	3	4
D03	M	2	1	
Mean			2.64	3.29
Czeszewo	C05	-	2	0
	C06	-	3	4
	C07	-	3	1
	C08	-	3	0
	C09	-	3	1
	C10	-	4	6
C11	-	3	3	
Mean			3.00	2.14

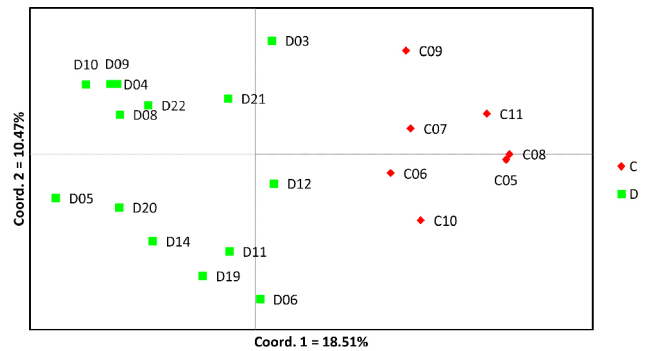


Fig. 3. Results of the Principal Coordinates Analysis (PCoA)

Table 2. Basic parameters of genetic variation. N – number of individuals; A – mean number of alleles; A_E – mean effective number of alleles; A_R – mean rarefied allelic richness; A_p – number of private alleles; H_O – observed heterozygosity; H_E – expected heterozygosity; F_{IS} – inbreeding coefficient

Population	N	A	A_E	A_R	A_p	H_O	H_E	F_{IS}
Dęblin	14	8.15	5.07	5.93	66	0.72	0.75	0.07
Czeszewo	7	4.08	2.49	4.08	13	0.70	0.57	-0.16
Mean	11	6.12	3.78	5.01	40	0.71	0.66	-0.05

According to the results of the kinship analysis, clones from Dęblin were not related. Conversely, we found that clones from Czeszewo were highly related. C05, C08, C09, and C11 were the first-degree siblings, while the other clones likely share one parent. The values of basic genetic variation parameters differed in most cases. Pop. C had lower values of allelic variation than pop. D. Nevertheless, the observed heterozygosity in pop. C significantly exceeded expected heterozygosity ($H_o = 0.70$ and $H_e = 0.57$, respectively), and the value of the inbreeding coefficient was below zero ($F_{IS} = -0.16$) (Table 2). Genetic differentiation between pop. D and C was high ($F_{ST} = 0.129$, $R_{ST} = 0.204$; $p = 0.001$). The studied populations represented two different gene pools as shown by the results of the PCoA (Fig. 3).

Discussion

In this study, we analyzed clones originating from two populations located in different river valleys. According to the results, they represent two different genetic pools. Lower values of allelic variation detected in pop. C were most likely the result of the two times lower number of analyzed clones, as well as from their high relatedness. Surprisingly, there was an excess of heterozygotes in pop. C, accompanied by a negative value of the inbreeding coefficient. This may reflect the heterozygote superiority phenomenon, where heterozygotes have higher relative fitness than homozygotes (Fisher, 1923). As shown by the results, the number of root suckers did not depend on the origin of the clones. More importantly, even closely related clones that shared the same parents produced different numbers of root suckers. However, this observation does not exclude the possibility that the occurrence and intensity of root suckering are influenced by genotype-environment interactions.

Although little is known about the physiology of sprouting in trees, the formation of root suckers certainly requires the allocation of resources to storage. This constitutes a trade-off between reproduction and growth (Iwasa & Kubo, 1997). In this way, vegetative recruits have the advantage of carbohydrate reserves but also possible pre-established links to water sources via the parent plant (Barsoum, 2002). In this study, we detected a strong positive linear correlation between the size class of the black poplar clones and the number of root suckers they produced. Therefore, it appears that the intensity of root suckering may depend on the size of an individual, probably mostly on the size of the root system and nutrient content. More importantly, our results suggest that the initiation of root suckering requires direct damage, either to the shoot (clone archive) or

root system (pot experiment). Consequently, flood training, beaver activity, and mowing are the most probable causes of sprouting observed in natural populations of black poplar. Barsoum et al. (2002) showed that the relative contribution of asexual regeneration increases during the first four years of the black poplar life cycle with each successive wave of flood disturbance.

Root suckering, as well as other types of sprouting, could be age-dependent. Many forest trees lose the ability to sprout as adults (Del Tredici, 2001), but it is the juvenile sprouting ability that can be considered part of the recruitment strategy of a species (Bond & Midgley, 2001). Therefore, the sprouting of seedlings and saplings has greater ecological significance than the sprouting of mature trees (Del Tredici, 2001). In the case of black poplar, regeneration from seeds is predominant in the first 2–3 years after seed release but poor survival rates lead to a shift in regeneration strategies over time (Barsoum, 2002). In the study of Barsoum et al. (2004), young stands of black poplar had significantly fewer replicated genotypes than middle-aged or old stands, while middle-aged stands had the greatest number of replicated genotypes. The most important result was the fact that there were variations in levels of clonality among stands from the same age class. These findings reflect the spatially heterogeneous physical impacts of floodwaters and differences in resource availability. The prevalence of vegetative propagation is thought to facilitate the persistence of local populations when the available sites for colonization are not suitable for seedling recruitment, both spatially and temporally (Chenault et al., 2011; Smulders et al., 2008).

In natural populations, the ramets of a specific mature tree are usually low in number and located in close vicinity. This may presumably be due to the increased competition with other species, i.e. more shade-tolerant and/or having fewer seedling recruitment requirements. Shady conditions in a closed forest generally suppress the formation of root suckers or inhibit their development beyond a meter in height (Del Tredici, 2001). For most woody species, light is not necessary to initiate root suckering but it is essential for their subsequent growth (Del Tredici, 1995). Still, seedlings are more demanding when it comes to sunlight. This is because the lack of endosperm makes seedlings highly dependent upon photosynthates from cotyledons and juvenile leaves, so full sunlight is crucial to their growth and development (Braatne et al., 1996). Water availability is also a limiting factor for seedling establishment. If the rate of water table decline exceeds the rate of root growth, water deficits lead to seedling mortality (Braatne et al., 1996). As shown by the results of our pot experiment, black poplar seems to need

optimal hydration also to preserve the ability to form root suckers. It is, therefore, likely that the survival of sister ramets may be greater when environmental resources are not limited.

Sprouting can have a significant impact on the genetic diversity of a particular population, especially in the case of root suckering which increases the number of ramets. This can lead to a reduction in effective population size, presumably negatively impacting long-term adaptability. Nevertheless, variation in genotypic diversity among clonal plant populations is considerable (Vallejo-Marín et al., 2010). High clonality indeed decreases genetic diversity but the effective number of alleles and heterozygosity should increase over time (Meloni et al., 2013). Many clonal species are successful invaders even when their populations have little if any genetic diversity (Barrett, 2015). Furthermore, vegetative dispersal through translocated fragments promotes outcrossing and thereby the maintenance of genetic diversity. Greater clonality also delays the time between generations, so the loss of alleles through genetic drift is decreased (Wei & Jiang, 2012). Asexual recruits can play a vital role in seedling regeneration by promoting sedimentation on the floodplain and thus stabilizing sand and gravel bars which may eventually lead to island formation (Lefèvre et al., 2001).

Our research provides preliminary results on how genotype and environmental conditions affect sprouting in black poplar. In natural populations, the probability of sprouting is the result of habitat conditions, neighboring vegetation, as well as the type and strength of disturbance. Climate change may cause a shift in the reproduction strategy of sprouters from mainly generative to vegetative one. Understanding the relationship between sexual vs. asexual recruitment is, therefore, crucial to develop successful conservation and management strategies for black poplar and other woody sprouters. It is equally important to monitor age-specific patterns of recruitment as a means of regulating, wherever possible, the age structure of stands (Lefèvre et al., 2001).

Study limitations

Our study was conducted with a limited number of clones originating from two populations. It therefore requires confirmation based on a larger sample size. Furthermore, we used nuclear microsatellite markers, which are considered selectively neutral. The varying number of root suckers cannot be directly linked to genetic variability found in our study, as this would require using a significantly higher number of markers of a different type. The number of markers we used may be also insufficient to determine whether the partial relatedness of some clones from pop. C is because they share one parent.

Black poplar is a dioecious species, so it has to be mentioned that the ability to form root suckers might also be sex-dependent. We knew the sex of 12 clones from pop. D (eight male vs. four female clones), but it was too little and biased to address this issue. Considering the age dependence, we estimated only the approximate age of the individuals from which cuttings were taken to establish the clone archive. Finally, in the case of the pot experiment, it would be necessary to examine physiological and biochemical parameters to properly assess whether and to what extent the availability of resources limits the production of root suckers.

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