

Dyscyplina: nauki biologiczne

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Rozprawa doktorska

**Uwarunkowania czynnej ochrony zagrożonych gatunków roślin z rodzaju *Salix*
metodami translokacji w perspektywie globalnych zmian klimatycznych**

***Determinants of active conservation of endangered plant species of the genus *Salix*
using translocation methods in the context of global climate change***

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Streszczenie

Postępująca degradacja siedlisk naturalnych spowodowana antropopresją oraz zmianami klimatu jest obecnie jednym z największych zagrożeń dla różnorodności biologicznej. Szczególnie podatne na ekstynkcję są wrażliwe na zmiany warunków siedliskowych roślinne relikty glacialne funkcjonujące w ekosystemach torfowiskowych. Celem niniejszej pracy była ocena efektywności zabiegów ochrony czynnej zagrożonych gatunków flory mokradłowej (z rodzaju *Salix*) metodą translokacji gatunkowych, w perspektywie prognozowanych zmian klimatycznych. Dokonano empirycznej weryfikacji zdolności aklimatyzacyjnych materiału roślinnego pochodzącego z uprawy ex situ, przejawiającej się w potencjale do przeżycia krótko- i długoterminowej ekspozycji na czynniki stresu abiotycznego. Przyjęta strategia badawcza opierała się na podejściu komplementarnym: obejmowała zarówno eksperymenty laboratoryjne, w których w warunkach kontrolowanych symulowano określone gradienty stresogenne, jak i badania prowadzone bezpośrednio w naturalnych siedliskach docelowych. Na każdym z etapów badań kondycja roślin była oceniana za pomocą wskaźników morfologicznych i fizjologicznych.

Uzyskane wyniki pozwoliły na stwierdzenie, że sukces translokacji osobników *Salix myrtilloides* pochodzących z upraw ex situ jest istotnie uzależniony od sposobu aklimatyzacji i ram czasowych jej przebiegu. Reakcje fizjologiczne osobników *S. lapponum* na stres abiotyczny stanowią wiarygodne i czułe wskaźniki efektywności ich aklimatyzacji do warunków siedliskowych stanowisk zastępczych, a uzupełnione o subiektywną ocenę cech morfologicznych mogą stanowić zoptymalizowany system kontrolno-pomiarowy introdukowanych osobników. Wyniki eksperymentu terenowego wykazały, iż osobniki *S. lapponum* mogą nie tylko przetrwać, ale również rozwijać się i zapoczątkować proces rozmnażania w stanowiskach zastępczych, których warunki siedliskowe różnią się znacząco od preferowanych przez badany gatunek. Siedliska torfowiskowe, które ulegają transformacjom na skutek zmian klimatu mogą pełnić funkcję stanowisk zastępczych dla *S. lapponum*. Kluczowym czynnikiem determinującym sukces translokacji badanego gatunku jest utrzymanie stabilnych warunków hydrologicznych w siedlisku.

Słowa kluczowe: *Salix lapponum*, *Salix myrtilloides*, torfowiska, translokacje gatunkowe, zmiany klimatu

Abstract

The progressive degradation of natural habitats caused by anthropogenic pressure and climate change is currently one of the greatest threats to biodiversity. Sensitive to changes in habitat conditions, plant glacial relicts inhabiting peatland ecosystems are particularly vulnerable to extinction. The aim of this study was to assess the effectiveness of translocating threatened wetland plants of the genus *Salix* as an active conservation method in the context of projected climate change. The acclimatization capacity of plant material cultivated ex situ was evaluated empirically in terms of its ability to survive short- and long-term exposure to abiotic stressors. The research strategy adopted a complementary approach, combining laboratory experiments, in which defined levels of abiotic stress were simulated under controlled conditions, with field studies conducted directly at natural recipient habitats. At each stage of the study, plant condition was assessed using morphological and physiological indicators.

The obtained results indicated that the successful translocation of ex situ-grown *Salix myrtilloides* plants depended substantially on both the acclimatization procedure and the duration of acclimatization. Physiological responses of *S. lapponum* to abiotic stress provide sensitive and reliable indicators of how successfully translocated plants acclimatize to conditions at recipient sites. Combined with visual assessment of morphological traits, these indicators may form an integrated framework for monitoring the condition of translocated plants. The field experiment revealed that *S. lapponum* plants could survive, grow, and initiate reproduction at recipient sites where habitat conditions differed substantially from those typically preferred by the species. Peatland habitats undergoing changes driven by climate change may therefore serve as recipient sites for *S. lapponum*. Maintaining stable hydrological conditions within these habitats is a key factor determining the success of translocation of the species.

Key words: climate changes, peatlands, species translocations, *Salix lapponum*, *Salix myrtilloides*

1. Wprowadzenie teoretyczne

Współcześnie utrata różnorodności biologicznej charakteryzuje się tempem i skalą, które znacznie przekraczają naturalną dynamikę wymierania gatunków (Palombo, 2021). Procesy zanikania lokalnych populacji oraz ekstynkcji kolejnych taksonów są w większości wynikiem antropopresji, która bezpośrednio i pośrednio powoduje przekształcania i fragmentację siedlisk przez nadmierną eksploatację zasobów, zanieczyszczenie środowiska naturalnego oraz nasilające się zmiany klimatu (Johnson i in., 2017). W latach 2000–2020 utratę siedlisk stwierdzono w około 19% obszarów chronionych na świecie, a wzrost ich fragmentacji w 34% (Yuan i in., 2024). Degradacja i fragmentacja siedlisk mają szczególnie poważne konsekwencje w przypadku małych i izolowanych populacji, gdyż redukcja możliwości dyspersyjnych prowadzi bezpośrednio do zahamowania przepływu genów, co uniemożliwia naturalną regenerację struktur populacyjnych. W ostatnich dekadach, pomimo intensyfikacji działań na rzecz ochrony przyrody, m.in. rozwoju sieci obszarów chronionych i wdrażania programów restytucyjnych, wciąż odnotowuje się utratę różnorodności florystycznej, co wskazuje na ograniczoną efektywność dotychczasowych strategii konserwatorskich (Heywood, 2017). Skuteczna ochrona gatunków wymaga zatem nie tylko wyznaczania obszarów chronionych, lecz również aktywnego zarządzania siedliskami i bezpośredniego wspierania populacji gatunków wrażliwych na zmiany zachodzące w naturalnym środowisku (Heywood, 2019).

W celu ograniczenia ryzyka utraty cennych taksonów roślinnych coraz częściej wdrażane są działania z zakresu translokacji gatunkowych, stanowiące istotny element współczesnej ochrony czynnej (Bellis i in., 2024). Obejmują one: reintrodukcję, polegającą na przywróceniu gatunków na stanowiska, w których obrębie wyginęły, zasilanie istniejących populacji, a także introdukcję do stanowisk zastępczych, podejmowaną w sytuacjach, gdy dotychczasowe warunki siedliskowe uległy silnym przekształceniom uniemożliwiającym ich renaturyzację (Plein i in., 2016). Skuteczność translokacji gatunkowych w przypadku roślin jest wypadkową wielu sprzężonych ze sobą czynników o charakterze biotycznym, abiotycznym i metodologicznym. Wśród nich kluczową rolę odgrywają m.in. biologia i autekologia gatunku, pochodzenie materiału roślinnego, warunki siedliska docelowego oraz reżim techniczny realizacji i monitorowania prowadzonych zabiegów (Batson i in., 2015; Abeli i Dixon, 2016; Bellis i in., 2024). Ewaluacja efektów translokacji gatunkowych zrealizowanych na terenie Europy wykazała, że blisko 50% nowopowstałych populacji uległo ekstynkcji w przeciągu pięciu lat od momentu

przeprowadzenia zabiegu. Zaledwie 28% z nich osiągnęło status populacji samopodtrzymujących się. Główną barierą sukcesu była wysoka śmiertelność osobników w fazie inicjalnej, w 40% poddanych analizie przypadków przeżywalność roślin po pierwszym roku wegetacji nie przekraczała bowiem 20% (Godefroid i in., 2025). Niezadowalająca skuteczność działań z zakresu ochrony czynnej zagrożonych gatunków roślin sprawia, że konieczna jest weryfikacja, modyfikacja oraz optymalizacja dotychczasowych procedur prowadzenia translokacji. Działania te są niezbędne, aby uzyskiwane efekty ochrony gatunków były proporcjonalne do nakładów pracy oraz ponoszonych kosztów finansowych. Należy również pamiętać, że każdy gatunek wymaga zastosowania procedur dostosowanych do jego specyfiki wzrostu i rozwoju oraz wymagań ekologicznych na każdym etapie procesu jego translokacji.

W pierwszym etapie działań czynnej ochrony gatunków roślin wyzwaniem jest pozyskanie odpowiedniej ilości materiału roślinnego, który w dalszym etapie służy zasilaniu lub tworzeniu nowych populacji w siedliskach naturalnych. Jedną ze szczególnie efektywnych metod jest mikrorozmnażanie. Technika ta umożliwia uzyskanie w stosunkowo krótkim czasie dużej liczby osobników potomnych z niewielkiej ilości materiału wyjściowego, co pozwala ograniczyć ingerencję w naturalnie funkcjonujące populacje (González-Benito i Martín, 2011; Ayuso i in., 2019). Rośliny w kulturach *in vitro* rozwijają się w specyficznym mikroklimacie, cechującym się wysoką wilgotnością powietrza, stabilną temperaturą oraz niskim natężeniem światła. Ponadto wszystkie niezbędne składniki mineralne, także węglowodany, jako źródło węgla, pobierają bezpośrednio z pożywki hodowlanej (Hazarika, 2006). Z tego względu kluczowym etapem przed ich przeniesieniem do gruntu jest stopniowa aklimatyzacja. Ma ona na celu przystosowanie sadzonek do zmiennych warunków środowiska naturalnego, co znacznie zwiększa ich szanse na prawidłowy wzrost i rozwój w docelowych stanowiskach (Sharma i in., 2023). Aklimatyzacja jest etapem newralgicznym, ponieważ to właśnie w tym okresie odnotowywana jest największa śmiertelność roślin otrzymanych w kulturach tkankowych. Ze względu na to, że proces ten zależy od wielu zmiennych, a reakcja organizmu jest specyficzna gatunkowo, opracowanie skutecznych procedur wymaga indywidualnego podejścia oraz dostosowania parametrów do wymagań konkretnego taksonu. Kluczowe staje się zatem optymalne zdefiniowanie warunków środowiskowych, a także identyfikacja wskaźników morfologicznych i fizjologicznych umożliwiających obiektywną ocenę kondycji roślin na poszczególnych etapach aklimatyzacji (Brito i in., 2009; Osório i in.,

2013). Monitorowanie tych cech pozwala na bieżąco śledzić dynamikę zmian adaptacyjnych, wskazać momenty krytyczne oraz zweryfikować efektywność zastosowanego protokołu (Pogorzelec i in., 2022). Szczególne znaczenie przypisuje się parametrom fizjologicznym, ponieważ ich wartości mogą wskazywać na reakcję stresową rośliny znacznie wcześniej, niż pojawią się widoczne symptomy morfologiczne, co umożliwia wczesną diagnostykę pogorszenia stanu zdrowotnego sadzonek (Schönbeck i in., 2023).

Kolejne istotne wyzwanie stanowi właściwy dobór stanowisk docelowych. Globalne analizy dowodzą, że nieodpowiednio dobrane warunki siedliskowe są jedną z głównych przyczyn niepowodzenia projektów introdukcji i reintrodukcji (Silcock i in., 2019; Bellis i in., 2024). W praktyce ochrony przyrody wytypowanie optymalnych lokalizacji dla nowych populacji opiera się na zróżnicowanych kryteriach. Do najważniejszych z nich zalicza się dane historyczne o rozmieszczeniu gatunku, stopień spójności parametrów klimatyczno-siedliskowych z jego wymaganiami ekologicznymi, odległość od populacji źródłowej, a także dostępność odpowiednich mikrosiedlisk (Bellis i in., 2025). Ocena ograniczona wyłącznie do aktualnego stanu siedliska może okazać się niewystarczająca w obliczu postępujących zmian klimatycznych, które stanowią źródło dodatkowej niepewności podczas prowadzenia działań ochrony czynnej gatunków. Przydatność potencjalnej lokalizacji stanowisk zastępczych należy zatem rozpatrywać w sposób perspektywiczny, uwzględniając zarówno bieżące uwarunkowania abiotyczne, jak i prognozowane trendy długoterminowe (Ferrarini i in., 2016). Równolegle, kluczowym elementem procedury powinno być określenie walencji ekologicznej gatunku wobec czynników stresowych, których oddziaływanie może nasilać się wraz ze zmianami klimatu i przekształcaniem siedlisk (Tarszisz i in., 2014). Prognozowany wzrost częstotliwości i intensywności ekstremalnych zjawisk klimatycznych, takich jak przedłużające się susze i fale upałów, może prowadzić do zaburzeń w funkcjonowaniu ekosystemów oraz bezpośrednio ograniczać potencjał rozwojowy i stabilność fizjologiczną flory, determinując podwyższone ryzyko ekstynkcji taksonów wrażliwych (Dreesen i in., 2014; Zahi i Lee, 2024; Rupngam i Messiga, 2024). Odtwarzanie populacji zagrożonych gatunków powinno być zatem ściśle powiązane z równoległą ochroną siedlisk, w przeciwnym razie znacząco wzrasta ryzyko niepowodzenia prowadzonych zabiegów (Godefroid i in., 2011).

Proces translokacji nie ogranicza się do fazy wprowadzenia materiału roślinnego do siedliska docelowego. Jego integralnym elementem jest odpowiednio zaplanowany

i długofalowy monitoring efektów podjętych zabiegów. Kluczowym wyznacznikiem powodzenia translokacji jest zdolność roślin do przejścia pełnego cyklu rozwojowego, obejmującego wzrost wegetatywny, kwitnienie oraz rekrutację następnego pokolenia (Godefroid i in., 2011). Ewaluacja tych parametrów ma jednak charakter długoterminowy i wymaga wieloletnich, kosztochłonnych obserwacji terenowych. W związku z tym istnieje potrzeba identyfikacji wczesnych markerów diagnostycznych, w szczególności wskaźników fizjologicznych i morfologicznych, które umożliwiłyby szybką, nieinwazyjną ocenę kondycji roślin oraz predykcję powodzenia translokacji (Schönbeck i in., 2023). Podejście to ma istotne znaczenie aplikacyjne, ponieważ wiele czynników ograniczających sukces translokacji jest związanych ze zmianami jakości siedliska lub zagrożeniami, których wpływ można ograniczyć dzięki podjęciu wczesnej interwencji. Dlatego też połączenie długoterminowego monitoringu z zarządzaniem adaptacyjnym optymalizuje skuteczność prowadzonych działań ochronnych i zmniejsza ryzyko ekstynkcji nowo utworzonych populacji (Bellis i in., 2024)

Znaczenie uwarunkowań środowiskowych prowadzenia zabiegów ochrony czynnej flory wzrasta szczególnie w przypadku stenotopowych gatunków o wąskiej walencji ekologicznej, których przetrwanie w siedlisku docelowym jest ściśle determinowane przez zachowanie specyficznych, a współcześnie wysoce niestabilnych parametrów mikroklimatycznych i edaficznych. Dotyczy to między innymi roślinnych reliktyw glacialnych związanych z terenami podmokłymi (Dítě i in., 2018; Słowińska i in., 2026). Ekosystemy hydrogeniczne należą obecnie do najbardziej zagrożonych siedlisk na świecie. W ciągu ostatnich stuleci uległy one znacznym przekształceniom i degradacji. Główną przyczyną tych zmian były intensywne melioracje odwadniające na potrzeby rolnictwa, leśnictwa oraz eksploatacji torfu. W Europie proces ten trwał od XVII do połowy XX wieku, prowadząc do bezpowrotnego zniszczenia wielu cennych przyrodniczo obszarów (Joosten i in., 2017). W rezultacie szacuje się, że około 25% europejskich torfowisk jest obecnie w stanie zdegradowanym, natomiast w wielu krajach, w tym w Polsce, odsetek ten wynosi ponad 80% (Tanneberger i in., 2021). Negatywne skutki wcześniej wspomnianych przekształceń antropogenicznych są dodatkowo potęgowane przez postępujące zmiany klimatyczne. Skutki globalnego ocieplenia, takie jak wzrost temperatury, wydłużenie okresów suszy i zmiany sezonowości opadów, stanowią istotne zagrożenie dla torfowisk, prowadząc do obniżenia poziomu wód gruntowych, odsłaniania powierzchni torfu oraz przyspieszonej mineralizacji materii organicznej i uwalniania dwutlenku węgla (Dise, 2009;

Bu i in., 2011; Macrae i in., 2013; Salimi i in., 2021). Zjawiska te sprzyjają także przyspieszeniu procesu sukcesji wtórnej na torfowiskach prowadząc do wielokierunkowych zmian w składzie gatunkowym fitocenozy. Szczególnie istotne jest zmniejszanie się udziału torfowców, odgrywających kluczową rolę w procesach torfotwórczych i utrzymywaniu wody w strefie akrotelmu, a także wzrost udziału gatunków trawiastych oraz drzew i krzewów odpornych na przesuszanie siedliska, które jednocześnie poprzez intensywną transpirację pozbawiają siedliska retencjonowanej tam wody (Weltzin i in., 2000; Gunnarsson, 2005; Hájková i in., 2011; Dieleman i in., 2015). Innym zagrożeniem związanym z osuszaniem torfowisk jest nasilenie procesu eutrofizacji (Venterink, 2002). Torfowiska pełnią funkcję naturalnych filtrów, zatrzymując i akumulując znaczne ilości związków azotu i fosforu, pochodzących m.in. z działalności rolniczej i ścieków komunalnych (Beusen i in., 2016; Zarkami i in., 2022; Borgström i in., 2024). Mineralizacja torfu prowadzi jednak do uwolnienia zgromadzonych w nim biogenów, zwiększając ich dostępność dla roślin. Powoduje to zmianę warunków troficznych, która sprzyja rozwojowi wysokich, konkurencyjnych roślin zielnych oraz drzew i krzewów, prowadząc do ograniczenia występowania wyspecjalizowanych gatunków torfowiskowych (Khan i Ansari, 2005; Khan i Mohammad, 2014). Odwadnianie torfowisk może pociągać za sobą również inne negatywne konsekwencje. Chroniczne przesuszanie torfowisk obniża ciśnienie hydrostatyczne, umożliwiając przenikanie zasolonej wody gruntowej, pochodzącej m.in. z nawadniania upraw, przemysłu, górnictwa oraz soli do odladzania, do wysychającego podłoża. W warunkach obniżonego poziomu wód gruntowych i zwiększonego parowania może także dochodzić do koncentracji rozpuszczonych soli w przypowierzchniowych warstwach torfu. Negatywnie wpływa to na zdolność torfowiska do retencji wody i akumulacji materii organicznej (Herbert i in., 2015). Sole zawarte w podłożu mogą spowodować suszę fizjologiczną u roślin, co bezpośrednio przekłada się na upośledzenie tempa ich wzrostu, może także doprowadzić do uszkodzenia komórek aparatu fotosyntetycznego (Parihar i in., 2015; Safdar i in., 2019).

W świetle wieloaspektowych presji środowiskowych, fundamentalnego znaczenia nabiera weryfikacja naukowa, czy relikty glacialne, które funkcjonują na torfowiskach, w często izolowanych lub zlokalizowanych poza granicami zwartego zasięgu geograficznego populacji, wykazują dostateczną plastyczność adaptacyjną, by przetrwać postępującą degradację siedlisk. Równolegle, kluczowym celem badawczym staje się ocena,

czy programy restytucji mogą stanowić efektywną i długoterminową metodę ochrony czynnej zabezpieczającą te krytycznie zagrożone taksony przed lokalną ekstynkcją.

Przedstawicielami grupy roślinnych reliktyw glacialnych związanych z ekosystemami torfowiskowymi są wierzba lapońska (*Salix lapponum*) i wierzba borówkolistna (*Salix myrtilloides*). Są to niewielkie, dwupienne krzewy dorastające zazwyczaj do ok 0,5 – 1,5 metra wysokości. Charakterystyczną cechą *S. lapponum* są eliptyczne szarozielone liście, które pokryte są gęstym kutnerem. *S. myrtilloides* wyróżnia się natomiast drobnymi niebieskozielonymi liśćmi pokrytymi warstwą wosku oraz cienkimi, wyprostowanymi pędami (Kłosowski i Kłosowski, 2006).

Zwarty zasięg *S. lapponum* obejmuje północno-wschodnią Europę oraz Syberię. W Polsce, podobnie jak na Wyspach Brytyjskich, w Sudetach, Pirenejach czy górach Bułgarii funkcjonują izolowane stanowiska tego gatunku, stanowiące relikty z okresów glacialnych (Kruszelnicki, 2014). Wierzba lapońska preferuje słoneczne bądź częściowo zacienione stanowiska na torfowiskach przejściowych o kwaśnym, mezo- lub oligotroficznym podłożu, natomiast w górach może zasiedlać torfowiska źródliskowe, m.in. w zbiorowiskach z kosodrzewiną. Występuje w zespołach roślinnych z klasy *Scheuchzerio-Caricetea fuscae*, natomiast w siedliskach górskich może tworzyć własny zespół *Salicetum lapponum* (Hroneš i in., 2018).

S. myrtilloides jest gatunkiem o borealno-kontynentalnym typie rozmieszczenia. Występuje przede wszystkim w borealnej i subarktycznej części Eurazji, natomiast populacje w Europie Środkowej są niewielkie i silnie izolowane. Wierzba borówkolistna zasiedla nizinne i podgórskie torfowiska przejściowe. Najlepiej rozwija się w miejscach niezakrzewionych. Preferuje stanowiska torfowe o niskim odczynie (pH 3,5-5,5). Optimum warunków biocenotycznych stanowią dla niej zbiorowiska turzycowe z klasy *Scheuchzerio-Caricetea fuscae* (Kruszelnicki i Gostyńska-Jakuszevska, 2014, Kołos i in., 2025).

Oba gatunki są w Polsce objęte ścisłą ochroną gatunkową i zostały wpisane do Polskiej Czerwonej Księgi Roślin. *S. myrtilloides* posiada status gatunku zagrożonego (EN), natomiast *S. lapponum* krytycznie zagrożonego wyginięciem (CR; Kruszelnicki, 2014; Kruszelnicki i Gostyńska-Jakuszevska, 2014). Na większości polskich stanowisk populacje *S. lapponum* i *S. myrtilloides* wyginęły, czego przyczyną były zmiany stosunków wodnych w siedliskach. Na terenie Polesia Lubelskiego, niegdyś bardzo liczne i rozmieszczone na dużych powierzchniach siedlisk populacje, obecnie są odizolowane od siebie i w większości

składają się z kilku do kilkunastu osobników (Pogorzelec, 2008a; Pogorzelec i in., 2014; Kruszelnicki i Gostyńska-Jakuszevska, 2014).

Dotychczasowe badania nad gatunkami *S. lapponum* i *S. myrtilloides* koncentrowały się wokół identyfikacji ich preferencji siedliskowych, analizy struktury i różnorodności genetycznej populacji, a także oceny potencjału reprodukcyjnego w warunkach ex situ (Stamati i in., 2007; Pogorzelec, 2008a, 2008b, 2010; Hroneš i in., 2011, 2018, 2019; Pogorzelec i in., 2014, 2015, 2016, 2020a; Serafin i in., 2015, 2020, 2022; Parzymies i in., 2020, 2023; Finger i in., 2023). Ponadto podjęto kilka prób wzmocnienia lub odtworzenia populacji obu gatunków we wschodniej Polsce (Parzymies i in., 2020; Pogorzelec i in., 2022; Arciszewski i in., 2023). Niemniej jednak zasób danych empirycznych dotyczących zdolności do efektywnej aklimatyzacji, plastyczności fenotypowej w reakcji na stres środowiskowy oraz przeżywalności osobników wierzb borealnych introdukowanych do ekosystemów torfowiskowych wciąż pozostaje niewystarczający. Kwestia ta nabiera szczególnego znaczenia w kontekście prognozowanych przekształceń strukturalno-funkcjonalnych tych siedlisk w dobie postępujących zmian klimatycznych. W związku z tym podjęto próbę kompleksowego uzupełnienia stanu wiedzy w zakresie mechanizmów aklimatyzacyjnych oraz plastyczności reakcji obu gatunków na dynamicznie zachodzące zmiany czynników środowiskowych.

2. Cel badań i hipotezy badawcze

Głównym celem podjętych badań była ocena efektywności zabiegów ochrony czynnej zagrożonych gatunków flory mokradłowej (reliktów glacialnych z rodzaju *Salix*) metodą translokacji gatunkowych, w perspektywie prognozowanych zmian klimatycznych.

Dokonano empirycznej weryfikacji zdolności aklimatyzacyjnych materiału roślinnego pochodzącego z uprawy ex situ, przejawiającej się w potencjale do przeżycia krótko- i długoterminowej ekspozycji na czynniki stresu abiotycznego. Przyjęta strategia badawcza opierała się na podejściu komplementarnym: obejmowała zarówno eksperymenty laboratoryjne, w których w warunkach kontrolowanych symulowano określone gradienty stresogenne, jak i badania prowadzone bezpośrednio w naturalnych siedliskach docelowych.

Określono następujące cele szczegółowe:

1. Ocena zdolności osobników *Salix myrtilloides* otrzymanych metodą mikrorozmnażania do efektywnej aklimatyzacji ex vitro oraz identyfikacja morfologicznych i fizjologicznych wskaźników umożliwiających monitorowanie przebiegu tego procesu (P1).
2. Określenie wpływu krótkotrwałych ekstremów temperaturowych na kondycję aklimatyzowanych osobników *Salix lapponum* w celu oceny zdolności ich odpowiedzi na stres środowiskowy związany z prognozowanymi w scenariuszach klimatycznych anomaliami pogodowymi (P2).
3. Ilościowa i jakościowa ocena wpływu podwyższonej dostępności azotu azotanowego oraz zasolenia podłoża na parametry morfologiczne i fizjologiczne *Salix lapponum* w kontekście determinacji sukcesu translokacji tego taksonu (P3).
4. Weryfikacja efektywności translokacji jako metody czynnej ochrony *Salix lapponum* w siedliskach torfowiskowych podlegających transformacji hydrologicznej na skutek antropopresji i zmian klimatycznych (P4).
5. Ocena przydatności wybranych parametrów fizjologicznych jako markerów kondycji osobników *Salix lapponum* introdukowanych do siedlisk docelowych, ze szczególnym uwzględnieniem ich efektywności we wczesnej detekcji reakcji roślin na stres środowiskowy (P5).

Postawiono następujące hipotezy badawcze:

- 1) Efektywność translokacji gatunkowych zagrożonych taksonów z rodzaju *Salix* pozyskanych z uprawy ex situ jest ściśle zdeterminowana przez ramy czasowe oraz procedury ich aklimatyzacji do specyficznych warunków stanowisk zastępczych.
- 2) Warunkowane zespołem mechanizmów fizjologicznych, ujawniające się fenotypowo reakcje roślin na stres środowiskowy są wiarygodnym wskaźnikiem efektywności działań ochrony czynnej *Salix lapponum*, na poszczególnych etapach procesu translokacji.
- 3) Translokacje z wykorzystaniem osobników otrzymanych w warunkach ex situ mogą stanowić skuteczne narzędzie czynnej ochrony *Salix lapponum* tylko w stanowiskach spełniających specyficzne wymagania siedliskowe gatunku, a ich skuteczność maleje w siedliskach szczególnie podatnych na przekształcenia związane z działalnością człowieka i zmianami klimatycznymi.

3. Metody badań

Material roślinny

Przedmiotem badań były dwa gatunki wierzb: wierzba borówkolistna (*Salix myrtilloides*; publikacja P1) oraz wierzba lapońska (*Salix lapponum*; publikacje P2–P5), które były objęte działaniami ochrony czynnej metodą translokacji gatunkowej na terenie wschodniej Polski (Pogorzelec i in., 2020b).

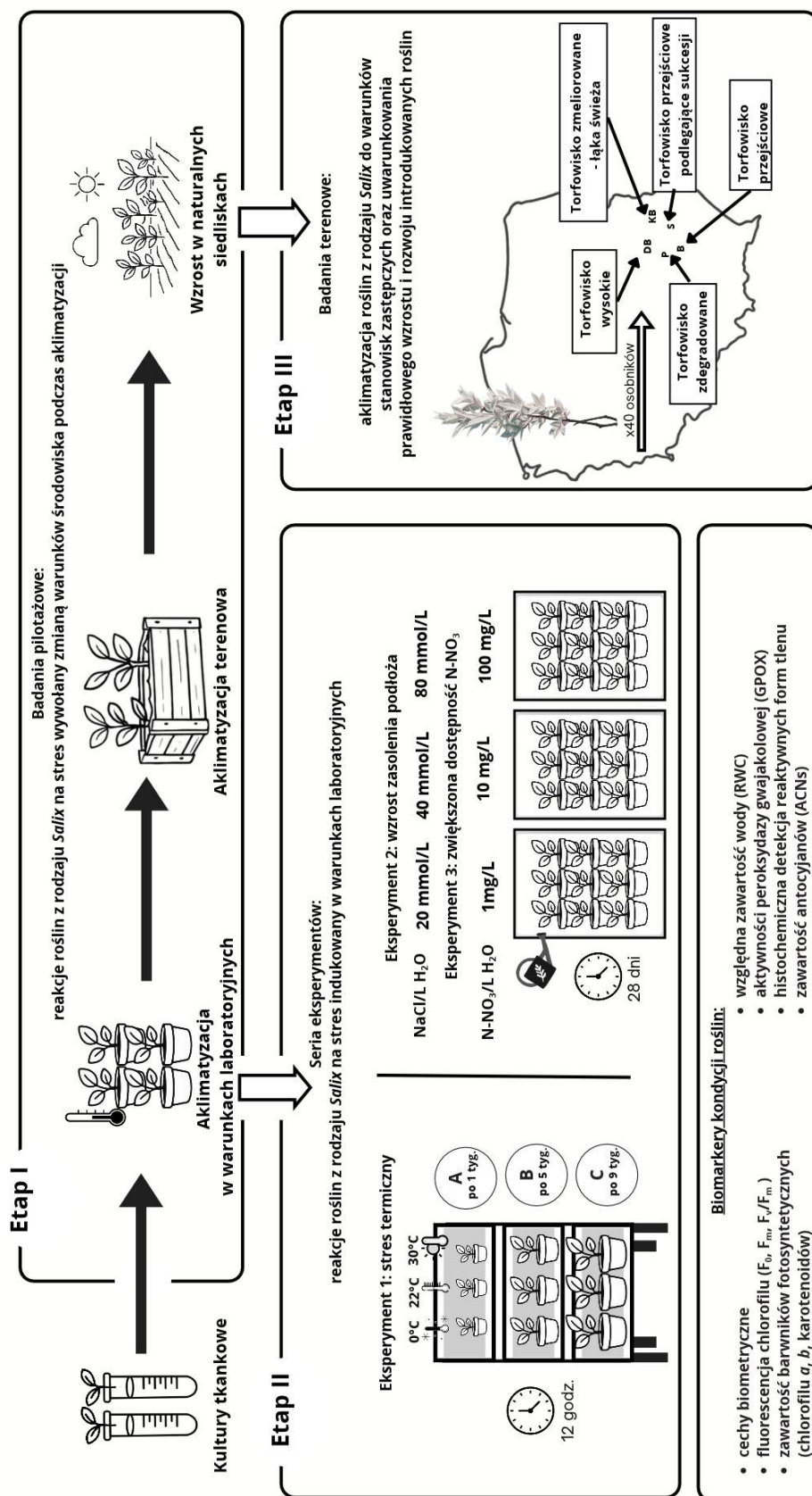
Do przeprowadzonych eksperymentów laboratoryjnych i terenowych wykorzystano sadzonki roślin, które były uprawiane w warunkach *ex vitro* w podłożu stałym. Materiał roślinny uzyskano metodą mikrorozmnażania (dzięki wcześniej opracowanym i zoptymalizowanym protokołom; Parzymies i in., 2020; Parzymies i in., 2023) w Laboratorium Kultur Tkankowych, Instytutu Produkcji Ogrodniczej Uniwersytetu Przyrodniczego w Lublinie, z fragmentów pędów pobranych z naturalnych populacji funkcjonujących na torfowisku nad Jeziolem Bikcze (Pojezierze Łęczyńsko-Włodawskie, wschodnia Polska; Głębocka i Pogorzelec 2017).

Organizacja i przebieg badań

Badania przeprowadzono w latach 2022-2025 w trzech niezależnych etapach (ryc. 1):

Etap 1 - reakcje *Salix myrtilloides* na stres wywołany zmianą warunków środowiska podczas aklimatyzacji (**P1**)

Otrzymane metodami mikrorozmnażania sadzonki *Salix myrtilloides* poddawano kolejnym etapom aklimatyzacji. Pierwszy etap stanowiła *aklimatyzacja laboratoryjna* w fitotronie w stałych warunkach oświetlenia, wilgotności i temperatury. W celu przygotowania roślin do wzrostu w warunkach naturalnych, po około 3 miesiącach sadzonki przeniesiono do inspektów z piaskowo-torfowym podłożem, zabezpieczonych siatką przeciw owadom, gdzie poza laboratorium przebywały przez kolejne 2 miesiące (*aklimatyzacja terenowa*). Ostatnim etapem procesu aklimatyzacji było przetransportowanie roślin do wcześniej wyznaczonej lokalizacji i posadzenie ich w stanowisku naturalnym (*aklimatyzacja w siedlisku*).



Ryc. 1. Schemat badań.

Wybrane cechy morfo-fizjologiczne roślin oraz ich zmienność w różnych warunkach środowiskowych posłużyły jako wskaźniki diagnostyczne, pozwalające śledzić procesy zachodzące podczas przenoszenia *S. myrtilloides* z kontrolowanych warunków laboratoryjnych do docelowego siedliska w ramach reintrodukcji.

Na każdym etapie aklimatyzacji kondycja osobników była oceniana w wyznaczonych terminach na podstawie pomiarów wybranych cech morfo-fizjologicznych roślin tj. tempo przyrostu pędu głównego, zmiana liczby liści, zawartość barwników asymilacyjnych w tkankach roślin, wybrane parametry fluorescencji chlorofilu oraz obecność reaktywnych form tlenu (analiza histochemiczna).

Etap 2 - seria eksperymentów: reakcje *Salix lapponum* na stres indukowany w warunkach laboratoryjnych (**P2, P3**)

- Eksperyment 1

Eksperyment miał na celu ocenę kondycji osobników *Salix lapponum* oraz ich fizjologicznej reakcji na krótkotrwały stres termiczny oddziałujący na rośliny podczas aklimatyzacji w warunkach laboratoryjnych. W kontrolowanych warunkach symulowano zjawiska nasilające się w środowisku naturalnym na skutek zmian klimatu – tj. nagłe, krótkotrwałe i ekstremalne spadki lub wzrosty temperatury otoczenia, które mogą wpływać na rośliny introdukowane do stanowisk naturalnych.

W badaniach wykorzystano sadzonki *S. lapponum* otrzymane metodą mikrorozmnażania. Rośliny aklimatyzowano w warunkach *ex vitro* w podłożu stałym przez: 1 tydzień, 5 tygodni lub 9 tygodni, a następnie umieszczano na 12 godzin w komorze klimatycznej (Climatic chamber - MLR-352H-PE, PHCbi) w warunkach stałej wilgotności (75%), oświetlenia ($53 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) oraz w temperaturze 0°C (wariant 1) i 30°C (wariant 2). Próbę kontrolną stanowiły sadzonki eksponowane na temperaturę 22°C (wariant 3). W każdym wariantcie wykorzystano po 90 sadzonek.

Bezpośrednio po upływie 12 godzin materiał roślinny był poddawany analizom laboratoryjnym celem określenia wartości wybranych wskaźników morfo-fizjologicznych, pomocnych w określeniu kondycji oraz ewentualnej reakcji roślin na oddziaływanie stresu termicznego. Określono zawartość barwników asymilacyjnych, wybrane parametry fluorescencji chlorofilu, zawartość antocyjanów, aktywność peroksydazy gwajakolowej oraz katalazy, względną zawartość wody w tkankach roślin oraz przeprowadzono histochemiczne wykrywanie reaktywnych form tlenu.

- Eksperyment 2 i 3

Celem badań było określenie w jakim stopniu zmiany warunków siedliskowych obserwowane w środowisku naturalnym, a mogące wynikać z presji antropogenicznej i zmian klimatu, takie jak postępująca eutrofizacja czy zasolenie gleby, mogą ograniczać wzrost i kondycję osobników *Salix lapponum*.

Przeprowadzono dwa niezależne eksperymenty w celu określenia wpływu wodnego roztworu chlorku sodu o różnych stężeniach (eksperyment 1) oraz zróżnicowanych dawek azotu azotanowego (eksperyment 2) na wybrane parametry morfo-fizjologiczne osobników *Salix lapponum*. W każdym doświadczeniu wykorzystano po 60 sadzonek (po 15 roślin na wariant w obu eksperymentach), które przeszły wcześniej trzymiesięczną aklimatyzację laboratoryjną w podłożu stałym. W obu doświadczeniach pojemniki z roślinami umieszczono w kuwetach wypełnionych do poziomu 2 cm roztworami NaCl lub N-NO₃ w różnych stężeniach. Przez cały okres doświadczenia uzupełniano poziom roztworów. W pierwszym doświadczeniu analizowano wpływ stresu solnego, stosując wodne roztwory NaCl o stężeniach 20 mM, 40 mM oraz 80 mM. W drugim doświadczeniu oceniano reakcję roślin na zróżnicowane stężenia azotu azotanowego (N-NO₃), stosując roztwory o stężeniach 1 mg/l, 10 mg/l oraz 100 mg/l. Próby kontrolne stanowiły rośliny podlewane wodą bez dodatku NaCl lub N-NO₃.

Po 2 oraz po 4 tygodniach trwania eksperymentów przeprowadzono badania laboratoryjne w celu oznaczenia wybranych parametrów morfologicznych i fizjologicznych badanych osobników takich jak: tempo przyrostu pędu głównego oraz zmiana liczby liści, względna zawartość wody, zawartość barwników fotosyntetycznych, wybrane parametry fluorescencji chlorofilu, gromadzenie się reaktywnych form tlenu, aktywność peroksydazy gwajakolowej oraz zawartość antocyjanów.

Etap 3 - Badania terenowe: aklimatyzacja *Salix lapponum* do warunków stanowisk zastępczych oraz uwarunkowania prawidłowego wzrostu i rozwoju introdukowanych roślin (P4, P5)

Eksperyment terenowy był prowadzony w okresie od września 2023 do czerwca 2025. Jego celem było zbadanie przebiegu aklimatyzacji introdukowanych roślin do zróżnicowanych pod względem warunków abiotycznych i biocenotycznych stanowisk zastępczych (określanych dalej również jako stanowiska eksperymentalne). Zaplanowane

działania obejmowały translokację roślin do wyznaczonych stanowisk, regularny monitoring warunków siedliskowych oraz obserwacje wybranych parametrów wzrostu i rozwoju roślin.

Lokalizację stanowisk eksperymentalnych wyznaczono na podstawie analizy dostępnej literatury oraz wstępnych obserwacji terenowych na Pojezierzu Łęczyńsko-Włodawskim (województwo lubelskie, wschodnia Polska). Stanowiska do przeprowadzenia eksperymentu translokacji gatunkowej zostały wybrane w taki sposób, aby reprezentowały (i) siedliska charakterystyczne dla torfowisk regionu, zróżnicowane pod względem warunków abiotycznych i biocenotycznych, (ii) siedliska niezaburzone, jak i podlegające przyspieszonej sukcesji ekologicznej oraz zmianom spowodowanym celową działalnością człowieka, (np. osuszanie), (iii) stanowiska zasiedlane przez populacje *S. lapponum* oraz takie, dla których nie notowano występowania gatunku w przeszłości (Pogorzelec i in. 2014).

Do eksperymentu translokacji wytypowano pięć stanowisk zlokalizowanych na: 1) torfowisku przejściowym (Bikcze, B) - stanowisko referencyjne, na którym funkcjonuje jedna z największych populacji *Salix lapponum* w badanym regionie (Pogorzelec, 2008b; Głębocka i Pogorzelec, 2017); 2) torfowisku wysokim (Durne Bagno, DB), 3) torfowisku przejściowym podlegającym przyspieszonej sukcesji ekologicznej (Spławy, S), 4) zmeliorowanej łące świeżej na glebie murszowej (Krowie Bagno, KB) oraz 5) zdegradowanym torfowisku (Piaseczno, P). Do każdego ze stanowisk eksperymentalnych we wrześniu 2023 roku wprowadzono po 40 osobników *S. lapponum*. Rośliny sadzono ręcznie z całą bryłą korzeniową w pło torfowiskowe lub do gleby w zależności od charakteru siedliska. Sadzonki rozmieszczono losowo na powierzchni około 25 m² w każdym stanowisku.

Obserwacje introdukowanych do środowiska osobników *S. lapponum* prowadzono regularnie, w miesięcznych odstępach w każdym stanowisku. Oceniano ogólną kondycję roślin na podstawie wybranych cech morfologicznych o charakterze jakościowym oraz ich zmian zachodzących podczas trwania eksperymentu terenowego. Określono także wartości parametrów fizjologicznych: wybrane parametry fluorescencji chlorofilu, zawartość barwników asymilacyjnych, zawartość antocyjanów, względną zawartość wody, aktywność peroksydazy gwajakolowej oraz wykonano histochemiczną detekcję reaktywnych form tlenu. Udział osobników *S. lapponum*, które przetrwały okres zimowy w stanowiskach eksperymentalnych i podjęły wzrost (SR, *ang. survival rate*), oraz osobników kwitnących (FR, *ang. flowering rate*) w nowopowstałych populacjach oceniono w dwóch kolejnych

sezonach wegetacyjnych: 2024 i 2025. Oceny przeżywalności dokonywano każdorazowo wczesną wiosną, po rozpoczęciu wegetacji.

Od początku okresu wegetacyjnego (kwiecień 2024) przez pięć kolejnych miesięcy prowadzono pomiary wybranych parametrów fizyczno-chemicznych płytkiej wody gruntowej dostępnej dla roślin. In situ badano odczyn i przewodność elektrolityczną, w warunkach laboratoryjnych oznaczono natomiast zawartość azotu ogólnego N_{tot} , azotanów $N-NO_3$, stężenie fosforu ogółem P_{tot} , fosforanów $P-PO_4$, udział ogólnego węgla organicznego (TOC), a także chemiczne (COD) i biochemiczne zapotrzebowanie na tlen (BOD).

Skład florystyczny oraz jego strukturę ilościową i jakościową na stanowiskach eksperymentalnych określono na podstawie zdjęć fitosocjologicznych wykonanych klasyczną metodą Braun-Blanqueta (1932). W obrębie każdego stanowiska wykonano po trzy zdjęcia fitosocjologiczne w ciągu sezonu wegetacyjnego 2024 roku. W celu uwzględnienia i udokumentowania sezonowej dynamiki oraz zmienności gatunkowej flory, badania terenowe przeprowadzono w trzech terminach: wiosennym (kwiecień), letnim (czerwiec) oraz późnoletnim (sierpień). Identyfikacji oraz przynależności syntaksonomicznej wyróżnionych zbiorowisk roślinnych do odpowiednich jednostek dokonano na podstawie kryteriów podanych przez Matuszkiewicza (2012).

Analizy statystyczne

Metody analizy statystycznej dobrano odpowiednio do charakteru, struktury oraz rozkładu danych empirycznych uzyskanych w poszczególnych etapach badań. Do oceny różnic między grupami stosowano metody parametryczne, w tym jedno- i dwuczynnikową analizę wariancji (ANOVA) z testem post hoc Tukeya (P1, P2, P3), oraz metody nieparametryczne, obejmujące test Kruskala–Wallisa z testem post hoc Dunna i korektą Bonferroniego (P3, P4, P5). W badaniach obejmujących pomiary prowadzone w czasie wykorzystano modele dla danych z powtarzanymi pomiarami (P3). W celu identyfikacji głównych czynników różnicujących analizowane parametry fizjologiczne i środowiskowe zastosowano analizę głównych składowych (PCA; P2, P4), natomiast do oceny zależności pomiędzy składem gatunkowym roślinności, a zmiennymi środowiskowymi wykorzystano analizę redundancji (RDA; P4). Wszystkie analizy przeprowadzono przy poziomie istotności $\alpha = 0,05$ z wykorzystaniem programu STATISTICA 13.3 software (TIBCO Software Inc. 2017, Palo Alto, CA, USA; P1) oraz środowiska R (R Core Team (2023; P2–P5).

4. Wyniki badań

Etap I: reakcje *Salix myrtilloides* na stres wywołany zmianą warunków środowiska podczas aklimatyzacji (P1).

Proces aklimatyzacji osobników *Salix myrtilloides* obejmował trzy kolejne etapy: aklimatyzację w kontrolowanych warunkach laboratoryjnych, aklimatyzację w warunkach terenowych w stacji aklimatyzacji roślin (w inspektach) oraz wzrost w stanowisku naturalnym. Na każdym z etapów analizowano zmiany wybranych parametrów morfologicznych i fizjologicznych, a końcowym kryterium oceny skuteczności aklimatyzacji była przeżywalność roślin po 12 miesiącach od introdukcji do naturalnego siedliska.

W kolejnych etapach procesu aklimatyzacji *S. myrtilloides* obserwowano wyraźne zmiany parametrów związanych z funkcjonowaniem aparatu fotosyntetycznego oraz zróżnicowane nasilenie stresu oksydacyjnego. W okresie aklimatyzacji laboratoryjnej obserwowano stopniowy wzrost zawartości chlorofilu *a* oraz *b* w tkankach liści, przy jednoczesnym braku istotnych zmian zawartości karotenoidów. Przeniesienie roślin z fitotronu do terenowej stacji aklimatyzacji spowodowało początkowo obniżenie zawartości chlorofilu *a* i *b* w tkankach roślin, jednakże po 2 tygodniach aklimatyzacji w inspektach odnotowano wzrost zawartości wszystkich badanych pigmentów. Ponowny spadek stężenia barwników w tkankach roślinnych odnotowano po przeniesieniu roślin na torfowisko. Z kolei w trakcie rozwoju roślin w siedlisku naturalnym odnotowano wzrost wartości stosunku chlorofilu *a/b* który osiągnął maksimum po 12 miesiącach od momentu introdukcji.

Zmiana warunków aklimatyzacji wpłynęły także na parametry fluorescencji chlorofilu *a*. Po pięciu tygodniach od przeniesienia regenerantów z kultur *in vitro* do podłoża, wartość maksymalnej wydajności kwantowej fotosystemu II (F_v/F_m) zbliżyła się do poziomu optymalnego dla prawidłowo funkcjonującego aparatu fotosyntetycznego (0,8), natomiast po trzech miesiącach aklimatyzacji laboratoryjnej odnotowano przekroczenie tej wartości. Po rozpoczęciu etapu aklimatyzacji terenowej stwierdzono przejściowy, niewielki spadek wartości analizowanych parametrów fluorescencji chlorofilu *a*. Niemniej jednak, już po miesiącu rośliny osiągnęły najwyższą w tej fazie doświadczenia wartość wskaźnika F_v/F_m . Największą zmianę wartości parametrów fluorescencji odnotowano po introdukcji roślin do stanowiska naturalnego. Po dwóch tygodniach od posadzenia odnotowano wyraźne obniżenie wartości F_m oraz F_v/F_m , przy jednoczesnym wzroście F_0 . Po 12 miesiącach

wzrostu w siedlisku naturalnym wartości parametrów fluorescencji uległy stabilizacji, chociaż wydajność PSII utrzymywała się na niższym poziomie niż podczas wcześniejszych etapów aklimatyzacji.

W celu uzupełnienia oceny odpowiedzi fizjologicznej *S. myrtilloides* przeprowadzono jakościowe oznaczanie akumulacji reaktywnych form tlenu (ROS, ang. *Reactive Oxygen Species*) w tkankach liści. Obecność ROS stwierdzono u roślin na wszystkich analizowanych etapach aklimatyzacji, przy czym produkty barwnych reakcji były szczególnie skoncentrowane w obrębie ogonków liściowych oraz głównych wiązek przewodzących. Najintensywniejsze wybarwienie wskazujące na największą akumulację nadtlenu wodoru oraz anionorodnika ponadtlenkowego odnotowano bezpośrednio po przeniesieniu roślin z warunków laboratoryjnych do terenowej stacji aklimatyzacji.

W okresie aklimatyzacji terenowej obserwowano systematyczny przyrost elongacyjny pędów *S. myrtilloides*. Najwyższe tempo wzrostu odnotowano w drugim miesiącu tego etapu kiedy przyrost dobowy wynosił średnio 1,74 mm. W kolejnych tygodniach tempo przyrostu nieznacznie zmalało, zaś u osobników introdukowanych do stanowiska naturalnego było stosunkowo niskie i wynosiło średnio 0,57 mm na dobę.

Wskaźnik przeżywalności roślin w trakcie całego procesu aklimatyzacji utrzymywał się na wysokim poziomie. Podczas trzymiesięcznej aklimatyzacji w warunkach laboratoryjnych śmiertelność sadzonek wyniosła zaledwie 5%, natomiast w fazie aklimatyzacji terenowej nie odnotowano żadnych strat materiału roślinnego. W ostatnim etapie procesu aklimatyzacji wprowadzono do naturalnego siedliska 48 osobników *S. myrtilloides*, z czego po 12 miesiącach przetrwało 79%.

Etap II: reakcje *Salix lapponum* na wybrane czynniki stresu abiotycznego (P2, P3)

W warunkach laboratoryjnych przeprowadzono trzy eksperymenty, w których badano odpowiedź roślin *S. lapponum* na działanie krótkotrwałego stresu termicznego, zwiększone zasolenie i dostępność azotu azotanowego. Oceniano reakcje fizjologiczne roślin oraz zmiany wybranych parametrów morfologicznych w celu określenia, które z badanych czynników mogą negatywnie wpłynąć na kondycję roślin w trakcie procesu translokacji.

Reakcja *S. lapponum* na stres termiczny (P2)

Odpowiedź fizjologiczna roślin na krótkotrwały stres termiczny zależała od ich fazy rozwojowej. Dwunastogodzinna ekspozycja na temperatury 0°C oraz 30°C zainicjowała zmiany w zawartości antocyjanów oraz aktywności peroksydazy gwajakolowej (GPOX) w tkankach badanych roślin. Z kolei zawartość barwników fotosyntetycznych nie różniła się statystycznie pomiędzy analizowanymi wariantami temperatury. Wartości względnej zawartości wody (RWC, *ang. Relative Water Content*) utrzymywały się na wysokim poziomie we wszystkich wariantach doświadczenia.

Rośliny rosnące w podłożu stałym przez tydzień, poddane działaniu stresu termicznego, wykazywały najsilniejszą reakcję na niską temperaturę. W efekcie ekspozycji na temp. 0°C stwierdzono u nich zwiększoną zawartość antocyjanów oraz podwyższoną aktywność peroksydazy gwajakolowej w porównaniu z roślinami z pozostałych wariantów eksperymentalnych. W przypadku roślin rosnących w podłożu przez pięć tygodni zaobserwowano zwiększoną zawartość antocyjanów oraz istotny wzrost aktywności peroksydazy gwajakolowej w ich tkankach po ekspozycji na temperaturę 30°C. U roślin rosnących w laboratorium przez dziewięć tygodni, po ekspozycji na niską jak i wysoką temperaturę odnotowano zwiększoną aktywność GPOX. W tej grupie roślin stwierdzono także większe stężenie antocyjanów w odpowiedzi na działanie wysokiej temperatury, jednakże ich zawartość była niższa niż u roślin rosnących w laboratorium przez pięć tygodni. Nasiloną reakcję roślin na stres cieplny potwierdziły wyniki analizy histochemicznej. Najintensywniejsze wybarwienie produktów reakcji dla H_2O_2 i $O_2^{\cdot -}$ obserwowano po ekspozycji na 30°C, podczas gdy w wariantcie niskiej temperatury akumulacja H_2O_2 była niewielka.

Reakcja osobników *S. lapponum* na zasolenie podłoża (P3)

Zwiększone zasolenie podłoża wywołało u badanych osobników *S. lapponum* wyraźne zmiany cech morfologicznych oraz wartości parametrów fizjologicznych, których nasilenie pogłębiało się wraz z czasem trwania eksperymentu. Wyniki pomiarów morfometrycznych wykazały, że już w pierwszych dwóch tygodniach ekspozycji na roztwory NaCl rośliny charakteryzowały się obniżoną dynamiką wzrostu oraz wolniejszym tempem przyrostu liczby liści w porównaniu z obiektami kontrolnymi. Po czterech tygodniach trwania eksperymentu negatywny wpływ zasolenia podłoża utrzymywał się, a najniższe tempo wzrostu oraz nasiloną defoliację zaobserwowano u roślin podlewanych roztworem NaCl o najwyższym stężeniu (80 mM).

Ekspozycja roślin na działanie roztworów chlorku sodu istotnie zmodyfikowała również stan uwodnienia tkanek. Przed rozpoczęciem eksperymentu, wskaźnik RWC u osobników *S. lapponum* wynosił około 97%, natomiast po dwóch tygodniach trwania doświadczenia obniżył się do około 88%. Dalsza ekspozycja na stres solny doprowadziła do progresywnego odwodnienia liści, w efekcie czego końcowe wartości RWC spadły do 66–69%.

Negatywny wpływ zasolenia podłoża na funkcjonowanie aparatu fotosyntetycznego osobników *S. lapponum* odnotowano dopiero przy długotrwałej ekspozycji na działanie tego czynnika stresowego. Po dwóch tygodniach trwania eksperymentu wartości maksymalnej wydajności kwantowej fotosystemu II (F_v/F_m) nie różniły się istotnie od tych zanotowanych na początku eksperymentu. Nie stwierdzono także istotnych różnic w zawartości barwników asymilacyjnych w tkankach roślin. Jednakże po czterech tygodniach wzrostu w zasolonych podłożach, u roślin stwierdzono pogorszenie wartości parametrów fluorescencji chlorofilu *a*, wartość F_v/F_m spadła do około 0,67. Odnotowano także spadek wartości fluorescencji maksymalnej F_m oraz wzrost wartości fluorescencji minimalnej F_0 . W tym okresie odnotowano także spadek zawartości barwników asymilacyjnych w tkankach roślin.

Zasolenie podłoża indukowało także odpowiedź związaną ze stresem oksydacyjnym. Już po 2 tygodniach ekspozycji na zasolenie zaobserwowano istotny wzrost zawartości antocyjanów w tkankach badanych roślin. Wraz z czasem trwania eksperymentu obserwowano sukcesywne zwiększanie aktywności peroksydazy gwajakolowej. Ocena histochemiczna wykazała obecność nadtlenu wodoru i anionorodnika ponadtlenkowego w tkankach osobników *S. lapponum*, z czego najbardziej intensywne wybarwienie produktów reakcji stwierdzono po czterech tygodniach traktowania roślin roztworem NaCl o stężeniu 80 mM.

Reakcja osobników *S. lapponum* na dostępność azotu azotanowego (P3)

Zróżnicowana zawartość azotu azotanowego w roztworze glebowym wpływała na wybrane parametry morfologiczne i fizjologiczne osobników *S. lapponum*, jednakże reakcja roślin była wyraźnie słabsza niż w przypadku ekspozycji na stres solny.

Analiza zmian parametrów morfologicznych roślin wykazała iż po dwóch tygodniach ekspozycji na działanie azotu azotanowego, ograniczenie tempa wzrostu występowało wyłącznie u roślin podlewanych roztworem N-NO₃ o stężeniu 100 mg/L. W dalszej części eksperymentu rośliny traktowane roztworami o niższych stężeniach N-NO₃ (1 i 10 mg/L) wykazywały natomiast szybsze tempo wzrostu zarówno w odniesieniu do

obiektów kontrolnych, jak i osobników traktowanych roztworem o najwyższym stężeniu tego związku.

Zwiększona zawartość azotu w roztworze glebowym nie powodowała wyraźnego pogorszenia parametrów związanych z funkcjonowaniem aparatu fotosyntetycznego. Wartości minimalnej i jak i maksymalnej fluorescencji pozostawały na podobnym poziomie przez cały czas trwania eksperymentu, natomiast wartości maksymalnej wydajności fotosystemu II (F_v/F_m) uległy obniżeniu dopiero po czterech tygodniach ekspozycji na działanie roztworów N-NO₃. Jednocześnie w końcowym stadium doświadczenia zaobserwowano spadek zawartości chlorofilu *a* i karotenoidów w tkankach roślin w porównaniu do wartości odnotowanych na początku doświadczenia.

Odpowiedź antyoksydacyjna roślin na obecność azotu azotanowego, wyrażona zarówno poprzez syntezę antocyjanów, jak i aktywność peroksydazy gwajakolowej (GPOX), miała charakter ograniczony. Nie odnotowano istotnych statystycznie różnic pomiędzy wartościami analizowanych parametrów w kolejnych terminach pomiarowych. Ponadto histochemiczna ocena akumulacji reaktywnych form tlenu wykazała jedynie niewielkie gromadzenie się ROS w tkankach liści.

Etap III: aklimatyzacja *Salix lapponum* do zróżnicowanych warunków stanowisk zastępczych oraz uwarunkowania prawidłowego wzrostu i rozwoju introdukowanych roślin (P4, P5).

Zróżnicowanie warunków siedliskowych stanowisk eksperymentalnych (P4)

Stanowiska wybrane do przeprowadzenia eksperymentu translokacji różniły się zarówno stopniem przekształcenia siedliska, jak również warunkami abiotycznymi i strukturą roślinności. Wyniki analiz statystycznych potwierdziły występowanie istotnych różnic w wartościach odczynu (pH) wody dostępnej dla roślin w stanowisku na torfowisku wysokim DB (najniższe pH) i na łące świeżej KB (najwyższe pH). Istotne statystycznie różnice w wartościach parametrów związanych z zawartością materii organicznej (BOD, COD i TOC) odnotowano między stanowiskiem DB, a stanowiskami KB i P (siedliska ubogie w materię organiczną). Jednocześnie w stanowisku KB odnotowano najwyższe wartości przewodności elektrolitycznej (EC).

Biorąc pod uwagę charakterystykę stanowisk eksperymentalnych, opartą na wynikach analiz czynników fizyczno-chemicznych wody, przeprowadzono analizę głównych składowych (PCA), która wskazała podział na naturalne siedliska torfowiskowe

i siedliska przekształcone. Stanowisko DB, zlokalizowane na torfowisku wysokim, wyróżniało się na tle pozostałych stanowisk torfowiskowych (S i B), i było najsilniej powiązane z wyższą zawartością fosforanów oraz materii organicznej. Stanowisko KB było silnie skorelowane z EC, wskazującym na wyższy stopień mineralizacji. Z kolei stanowisko na zdegradowanym torfowisku (P) było najbardziej oddalone w przestrzeni ordynacyjnej od pozostałych stanowisk i skorelowane z najniższymi wartościami EC i względnie wysokim pH, co podkreślało jego całkowicie odmienny charakter związany z degradacją torfowiska.

Zróznicowanie stanowisk wybranych do translokacji *S. lapponum* dotyczyło także składu florystycznego i struktury fitocenoz. Stanowiska eksperymentalne zlokalizowane na torfowiskach reprezentowały odmienne typy roślinności. W stanowisku referencyjnym (B) dominowała roślinność ze związku *Caricion nigrae*, fitocenozę stanowiska DB na torfowisku wysokim sklasyfikowano do związku *Sphagnion magellanici*, natomiast fitocenoza w stanowisku S wykazywała cechy zbiorowiska przejściowego między torfowiskiem a zbiorowiskiem ze związku *Alnion glutinosae*. Odmienne typy roślinności odnotowano w stanowiskach KB i P. W fitocenozie stanowiska KB dominowały gatunki łąk ostrożeńiowych ze związku *Calthion palustris*, zaś w stanowisku P dominowała roślinność szuwarowa ze związku *Phragmition*.

Wpływ głównych gradientów parametrów abiotycznych wyodrębnionych w analizie PCA na strukturę składu gatunkowego oceniono za pomocą analizy redundancji (RDA), z wykorzystaniem osi PC1 i PC2 jako zmiennych objaśniających. Uzyskany model RDA był granicznie istotny statystycznie (test permutacyjny, $p \approx 0,05$) i wyjaśniał około 61% całkowitej wariancji składu gatunkowego fitocenoz stanowisk eksperymentalnych. Zbliżony układ stanowisk na biplocie PCA i RDA wykazał, że struktura składu gatunkowego badanych fitocenoz stanowiła odzwierciedlenie warunków fizykochemicznych wody, a stanowiska KB i P zarówno w analizie środowiskowej, jak i gatunkowej pozostawały wyraźnie oddalone od siedlisk torfowiskowych.

Przeżywalność i kwitnienie introdukowanych osobników *S. lapponum* (P4)

Monitoring przeprowadzony wiosną w pierwszym roku po introdukcji roślin do stanowisk eksperymentalnych wykazał, że najwyższą wartością wskaźnika przetrwania (SR) charakteryzowała się nowo utworzona populacja *S. lapponum* zlokalizowana w siedlisku łąki świeżej (KB). W 2025 roku w tym stanowisku liczebność osobników spadła jednak drastycznie (wegetację wznowiło jedynie 13 osobników). W pozostałych populacjach wskaźnik SR w 2024 roku mieścił się w przedziale 75–77,5%, natomiast w roku 2025 uległ

on nieznacznemu obniżeniu na dwóch stanowiskach (DB i S). Na torfowisku przejściowym (B) i zdegradowanym torfowisku (P) liczba osobników w populacji pozostała stabilna, przy wskaźniku SR wynoszącym odpowiednio 77,5% i 75%.

W roku 2024, we wszystkich utworzonych populacjach odnotowano osobniki *S. lapponum*, które wytworzyły kwiatostany. Najwyższą wartość wskaźnika kwitnienia w pierwszym roku badań (FR = 30%) stwierdzono w populacji zasiedlającej torfowisko przejściowe podlegające przyspieszonej sukcesji ekologicznej (S). W kolejnym sezonie wegetacyjnym odnotowano tam jednak regres tego parametru do poziomu 12%. Ogółem we wszystkich analizowanych populacjach odsetek osobników kwitnących w obu latach po introdukcji wahał się od 8% do 30%.

Zmienność cech morfologicznych osobników *S. lapponum* w stanowiskach zastępczych (P4)

Obserwacje roślin prowadzone w roku 2024 w ramach monitoringu po translokacji umożliwiły ocenę zmian cech morfologicznych osobników *S. lapponum* w poszczególnych stanowiskach eksperymentalnych. Część z nich wynikała z naturalnych procesów fenologicznych (np. defoliacji czy zmiany barwy blaszek liściowych), ale niektóre były bezpośrednią odpowiedzią na specyficzne warunki mikrosiedlisk. Wiosną 2024 roku osobniki *S. lapponum* w stanowiskach zlokalizowanych na torfowiskach (B, S, DB) oraz na łące świeżej (KB) charakteryzowały się pokrojem i morfologią charakterystyczną dla gatunku, szczególnie dla ekotypu reprezentatywnego w ekosystemach torfowiskowych we wschodniej Polsce. Odmianą architekturę pędów obserwowano w stanowisku na torfowisku zdegradowanym (P), gdzie przy utrzymującym się wiosną wysokim poziomie wody, pędy roślin były silnie wydłużone, a liście wyrastały głównie na szczycie pędów w dużym zagęszczeniu.

W okresie letnim w 2024 roku w stanowisku na torfowisku przejściowym Spławy (S) obserwowano zamieranie starszych pędów introdukowanych osobników przy jednoczesnej intensywnej inicjacji młodych pędów u podstawy roślin. Wyraźne zmiany morfologiczne zaobserwowano u roślin wzrastających w siedlisku łąki świeżej (KB), gdzie latem 2024 r. odnotowano redukcję gęstości kutneru, przebarwienia i deformacje blaszek liściowych oraz obumieranie części pędów. Wiosną kolejnego roku procesowi degradacji starszych pędów towarzyszyło masowe tworzenie pędów odroślowych. Na zdegradowanym torfowisku nad jeziorem Piaseczno (P) po obniżeniu się poziomu wody w siedlisku (latem 2024 r.) część silnie wydłużonych pędów uległa pokładaniu i ukorzenieniu w podłożu dzięki

wykształceniu korzeni przybyszowych. Jednocześnie obficie ulistnione pędy boczne podjęły wzrost pionowy. Wiosną 2025 roku z zakorzenionych w poprzednim sezonie pędów wyrosły liczne, młode i nierozgałęzione pędy, charakteryzujące się obfitym ulistnieniem o gęstym owłosieniu. Na stanowiskach zlokalizowanych na torfowisku przejściowym (B) oraz na torfowisku wysokim (DB) nie stwierdzono istotnych odchyleń od typowego rozwoju morfologicznego *S. lapponum*, a obserwowane zmiany miały charakter zgodny z naturalnym przebiegiem wzrostu i fenologii gatunku.

Wskaźniki fizjologiczne w monitoringu osobników *S. lapponum* w stanowiskach zastępczych (P5)

Po wprowadzeniu roślin do stanowisk eksperymentalnych, w dwóch kolejnych sezonach wegetacyjnych prowadzono monitoring ich stanu fizjologicznego z wykorzystaniem wybranych biomarkerów. Analizy obejmowały parametry związane z funkcjonowaniem aparatu fotosyntetycznego, odpowiedzią antyoksydacyjną i uwodnieniem tkanek roślin.

Wartości wybranych parametrów fluorescencji chlorofilu *a* osobników *S. lapponum* zmieniały się w trakcie sezonu wegetacyjnego i były zróżnicowane w zależności od miejsca translokacji. W pierwszym roku prowadzenia monitoringu kondycji roślin, wartości maksymalnej wydajności kwantowej fotosystemu II (F_v/F_m) wzrastały od maja do września, osiągając pod koniec sezonu najwyższe wartości (0,81-0,84). W sezonie wegetacyjnym w 2024 r. różnice w wartościach tego parametru pomiędzy stanowiskami były niewielkie, przy czym relatywnie najniższy poziom F_v/F_m odnotowano w stanowisku na zdegradowanym torfowisku (P). Wyraźne zróżnicowanie pomiędzy stanowiskami ujawniło się na początku kolejnego sezonu wegetacyjnego. Spadek wartości F_v/F_m do około 0,48 stwierdzono u roślin rosnących w stanowisku na torfowisku wysokim (DB) oraz na łące świeżej (KB). Różnice w wartościach pozostałych analizowanych parametrów fluorescencji chlorofilu (F_0 i F_m) u roślin w badanych stanowiskach były niewielkie, a największą zmienność sezonową odnotowano u roślin na torfowisku zdegradowanym (P).

Poziom akumulacji barwników asymilacyjnych w tkankach badanych roślin odzwierciedlał zróżnicowane warunki ekologiczne panujące w stanowiskach eksperymentalnych. Zawartość chlorofilu *a* oraz chlorofilu *b* w tkankach osobników *S. lapponum* rosnących na stanowisku referencyjnym (B) pozostawała względnie stabilna w obu sezonach wegetacyjnych. W pozostałych siedliskach parametry te charakteryzowały się większą zmiennością, w szczególności na torfowisku zdegradowanym (P), gdzie

odnotowano wyraźne fluktuacje stężenia tych barwników. Zawartość karotenoidów w tkankach roślin wprowadzonych do siedlisk eksperymentalnych zmieniała się dynamicznie w trakcie sezonu wegetacyjnego, jednakże w obrębie poszczególnych terminów badań nie odnotowano statystycznie istotnych różnic.

Wartości względnej zawartości wody (RWC) w liściach *S. lapponum* wykazywały wyraźne zróżnicowanie zarówno w ujęciu sezonowym jak i pomiędzy stanowiskami. W sezonie wegetacyjnym w 2024 roku w miesiącach maj-lipiec względna zawartość wody w tkankach roślin ulegała fluktuacjom we wszystkich stanowiskach eksperymentalnych i osiągnęła najniższe wartości w czerwcu (RWC=52% u roślin w stanowisku DB). W kolejnych miesiącach wartość tego wskaźnika istotnie wzrosła u roślin i w większości przypadków oscylowała na poziomie 90%. W 2025 roku RWC w tkankach *S. lapponum* utrzymywało się na stosunkowo wyrównanym, wysokim poziomie, przy czym szczególnie wysokie wartości (ok. 99%) odnotowano w maju 2025 roku u roślin w stanowisku referencyjnym (B).

Ocena odpowiedzi antyoksydacyjnej badanych roślin obejmowała oznaczenie aktywności peroksydazy gwajakolowej, zawartości antocyjanów w tkankach, a także histochemiczne barwienie reaktywnych form tlenu. Aktywność GPOX w tkankach roślin była zróżnicowana w zależności od stanowiska, co ujawniło się szczególnie w pierwszych miesiącach prowadzenia obserwacji. W stanowisku referencyjnym (B) na torfowisku przejściowym, aktywność peroksydazy gwajakolowej pozostawała na podobnym poziomie w trakcie całego eksperymentu ($0,008-0,010 \text{ U} \cdot \text{mg}^{-1} \text{ FW}$). W stanowisku na torfowisku wysokim (DB) odnotowano istotny wzrost aktywności enzymatycznej w tkankach roślin w maju 2025 w porównaniu z analogicznym okresem w 2024 roku. W pozostałych stanowiskach aktywność peroksydazy gwajakolowej ulegała dużym wahaniom w trakcie sezonu wegetacyjnego w roku 2024. Istotne różnice w aktywności peroksydazy gwajakolowej stwierdzono u roślin w stanowisku na osuszonym torfowisku (KB) i na torfowisku zdegradowanym (P), gdzie w maju 2024 roku aktywność była najniższa, natomiast w miesiącach letnich aktywność enzymu znacznie wzrosła.

W sezonie wegetacyjnym 2024 r. zawartość antocyjanów w badanym materiale roślinnym wzrastała we wszystkich stanowiskach translokacji, osiągając maksimum w sierpniu (około $0,15-0,17 \text{ mg} \cdot \text{g}^{-1} \text{ FW}$). W większości terminów badawczych nie stwierdzono istotnych statystycznie różnic w poziomie akumulacji tych barwników pomiędzy poszczególnymi powierzchniami eksperymentalnymi. W aspekcie wiosennym

kolejnego roku (2025) zawartość antocyjanów w tkankach badanych roślin uległa wyraźnej redukcji względem wartości odnotowanych w analogicznej fazie pierwszego roku badań.

Histochemiczne barwienia z wykorzystaniem NBT oraz DAB wykazały obecność nadtlenu wodoru oraz anionorodnika ponadtlenkowego u roślin we wszystkich analizowanych stanowiskach zarówno wiosną jak i latem 2024 roku. Intensywność wybarwienia była wyraźnie większa wiosną, przy czym najbardziej nasiloną akumulację ROS obserwowano u osobników zasiedlających siedliska antropogenicznie przekształcone.

5. Dyskusja

Translokacje zagrożonych gatunków roślin stanowią cenne narzędzie w ochronie przyrody. Są one jednakże przedsięwzięciem wymagającym starannego planowania, przygotowania materiału roślinnego, selekcji stanowisk zastępczych i utrzymania odpowiedniego stanu ich siedlisk oraz długotrwałego monitoringu, co może wiązać się ze znacznymi nakładami finansowymi (Godefroid i in., 2011; Silcock i in., 2019; D'Agostino i in., 2024). Efektywność tych działań zależy od wielu czynników związanych zarówno z kondycją materiału roślinnego jak i specyfiką warunków ekologicznych panujących w siedliskach, do których rośliny są wprowadzane (Bellis i in., 2024; D'Agostino i in., 2024). Badania przeprowadzone w ramach niniejszej rozprawy umożliwiły prześledzenie kolejnych etapów procesu translokacji roślinnych reliktyw glacialnych z rodzaju *Salix* uzyskanych w warunkach ex situ. Obejmowały one w szczególności ocenę przebiegu aklimatyzacji (P1), ale także reakcję osobników badanego gatunku na różne czynniki stresowe (P2, P3). Przeprowadzono również terenowy eksperyment translokacji osobników *S. lapponum* do stanowisk we wschodniej Polsce, zróżnicowanych pod względem warunków biocenotycznych i abiotycznych (P4). Poza klasycznymi kryteriami oceny efektywności zabiegów translokacji analizie poddano także stan fizjologiczny roślin, co umożliwiło określenie wpływu skumulowanej presji środowiskowej na kondycję introdukowanych osobników (P5).

Wykorzystanie roślin pochodzących z kultur tkankowych w działaniach z zakresu ochrony czynnej wymaga ich odpowiedniego przygotowania i zaaklimatyzowania do warunków siedlisk naturalnych. Wyniki uzyskane dla *S. myrtilloides* wskazują, że krytycznym etapem tego procesu były pierwsze doby po zmianie warunków w jakich funkcjonowały sadzonki. Zarówno po przeniesieniu roślin z fitotronu do terenowej stacji aklimatyzacji, jak i po wprowadzeniu ich do stanowisk naturalnych stwierdzono istotne pogorszenie parametrów związanych z funkcjonowaniem aparatu fotosyntetycznego oraz wzmożoną akumulację reaktywnych form tlenu. Obserwowane zmiany zawartości barwników fotosyntetycznych mogły wynikać z przejścia od kontrolowanych warunków oświetlenia w warunkach laboratoryjnych do zmiennego natężenia światła i naturalnego fotoperiodu. Dostosowanie profilu pigmentowego do natężenia promieniowania stanowi kluczowy element aklimatyzacji, aczkolwiek fluktuacje temperatury powietrza oraz uwodnienia podłoża mogą dodatkowo modyfikować tempo syntezy i degradacji tych związków (Mane i in., 2010; Osório i in., 2013; Grzeszczuk i in., 2018). Innym

z badanych parametrów kondycji roślin, który umożliwił określenie optymalnego czasu trwania kolejnych etapów aklimatyzacji był wskaźnik maksymalnej wydajności fotosystemu II (F_v/F_m). Po pięciu tygodniach wzrostu w fitotronie, wartości F_v/F_m zbliżały się do poziomu charakterystycznego dla poprawnie funkcjonującego aparatu fotosyntetycznego (Murchie i Lawson, 2013), jednak dopiero po trzech miesiącach aklimatyzacji przekroczyły próg wartości 0,8. Sugeruje to, że trzymiesięczny okres uprawy w warunkach laboratoryjnych sprzyjał optymalnemu przygotowaniu regenerantów do kolejnego etapu, jakim był transfer do terenowej stacji aklimatyzacji. Dalsza analiza parametrów fluorescencji chlorofilu i akumulacji reaktywnych form tlenu wykazała iż optymalny czas aklimatyzacji roślin w inspektach wynosi około miesiąca i po tym czasie rośliny powinny być wprowadzone do naturalnych siedlisk. Finalnie monitoring przeprowadzony po roku od reintrodukcji osobników *S. myrtilloides* wykazał, że 79% roślin przetrwało, co wspiera ocenę zastosowanej wieloetapowej procedury jako skutecznej w badanych warunkach.

Kolejny etap badań dotyczył oceny reakcji osobników *S. lapponum* na wybrane czynniki stresowe w kontrolowanych warunkach laboratoryjnych. Chociaż w warunkach naturalnych czynniki te rzadko występują pojedynczo i działają najczęściej synergistycznie lub antagonistycznie z różnym nasileniem w czasie i przestrzeni, eksperymenty z zastosowaniem pojedynczych stresorów pozwalają rozpoznać reakcję na każdy z nich i stanowią punkt wyjścia do interpretacji wyników uzyskanych w warunkach terenowych (Mittler, 2006; Suzuki i in., 2014; Zandalinas i in., 2021). Zmiany tempa przyrostu pędów i rozwoju liści odzwierciedlają skumulowany efekt działania czynników środowiskowych na metabolizm roślin, są także pierwszymi, uwidaczniającymi się fenotypowo objawami stresu abiotycznego (Munns i Tester, 2008). Ponieważ zmiany w parametrach biometrycznych obserwowane na poziomie morfologicznym są efektem zaburzeń fizjologicznych, analiza zawartość barwników fotosyntetycznych i antocyjanów, wydajności aparatu fotosyntetycznego, względnej zawartości wody czy aktywności enzymów antyoksydacyjnych umożliwia lepsze zrozumienie wewnątrzkomórkowych mechanizmów adaptacji do warunków stresowych (Zhu, 2001; Hernández i in., 2001).

Wyniki badań obrazujące reakcję osobników *S. lapponum* na krótkotrwałe działanie skrajnych temperatur wykazały, że charakter odpowiedzi fizjologicznej roślin był uwarunkowany aktualną fazą ich wzrostu. We wczesnych fazach aklimatyzacji rośliny reagowały na stres wywołany niską temperaturą (0°C), podczas gdy osobniki starsze wykazywały większą wrażliwość na działanie wysokiej temperatury (30°C). Zróżnicowanie

to uwidaczniało się przede wszystkim w zmianach zawartości antocyjanów i wzmożonej aktywności peroksydazy gwajakolowej, wskazujących na uruchomienie zarówno nieenzymatycznych jak i enzymatycznych mechanizmów ochrony antyoksydacyjnej. Wzrost syntezy antocyjanów w tkankach *S. lapponum*, w odpowiedzi na działania niskiej temperatury jak i przy stresie cieplnym, oraz wzrost aktywności enzymów antyoksydacyjnych związane mogły być z ograniczaniem skutków nadmiernego powstawania reaktywnych form tlenu (Suzuki i Mittler, 2006; Shao i in., 2007; Li i in., 2015). Zmiany wartości pozostałych badanych parametrów nie były znaczne, co wskazuje, iż 12 godzinna ekspozycja na działanie różnych wartości temperatur nie doprowadziła do istotnych zaburzeń funkcjonowania aparatu fotosyntetycznego oraz nie wpłynęła na uwodnienie tkanek roślin. W badaniach dotyczących innych gatunków spadek zawartości barwników asymilacyjnych obserwowano po dłuższej ekspozycji na działanie wysokiej temperatury, co potwierdza znaczenie czasu ekspozycji dla obserwacji uszkodzeń aparatu fotosyntetycznego (Awasthi i in., 2014; Wassie i in., 2019). Zaprezentowany w publikacji P2 przykład pozwala wnioskować, iż rośliny *S. lapponum* pochodzące z upraw ex situ są w stanie tolerować okresy krótkotrwałego stresu termicznego dzięki aktywacji szeregu mechanizmów obronnych. Niemniej jednak nie można jednoznacznie prognozować, w jaki sposób gatunek reagował będzie na długotrwały stres termiczny.

Wyniki eksperymentów, które uwzględniały czynniki związane z antropogenicznym przekształceniem warunków siedliskowych, wykazały, że niezależnie od zastosowanego stężenia roztworu oraz czasu ekspozycji zasolenie podłoża w każdym przypadku prowadziło do ograniczenia tempa wzrostu i wpływało na pogorszenie stanu aparatu asymilacyjnego, objawiające się rozległą nekrozą blaszek liściowych oraz ogólnym pogorszeniem kondycji zdrowotnej ulistnienia. Obserwowanym modyfikacjom morfologicznym towarzyszyły zmiany zawartości barwników fotosyntetycznych i parametrów fluorescencji chlorofilu, wskazujące na zaburzenia funkcjonowania aparatu fotosyntetycznego. Negatywny wpływ stresu solnego wynikać może zarówno z zakłóceń równowagi jonowej oraz toksycznego oddziaływania jonów Na^+ i Cl^- , co bezpośrednio zaburza syntezę chlorofilu (Ashraf i Harris, 2013). Uzyskane wyniki dla *S. lapponum* są zgodne z przedstawionymi przez Ran i in. (2021) dla *Salix alba*, gdzie stres solny znacząco pogorszył wydajność fotosyntezy zarówno poprzez mechanizmy szparkowe, jak i pozaszparkowe, a także wywołał stres oksydacyjny na poziomie PSII prowadząc do częściowej inaktywacji centrum reakcji PSII. Odnotowane wahania parametrów fluorescencji chlorofilu, a w szczególności parametru maksymalnej

wydajności fotosystemu II (F_v/F_m) potwierdzają przydatność tego wskaźnika do wczesnego wykrywania zaburzeń fizjologicznych, przed ujawnieniem się widocznych symptomów makroskopowych oraz zaawansowanych uszkodzeń morfologicznych (Bertamini i in., 2019). Zmniejszenie zawartości chlorofilu w tkankach roślin oraz pogorszenie parametrów fluorescencji chlorofilu pod wpływem zasolenia obserwowano również u innych gatunków roślin. Są to zjawiska będące powszechnie dokumentowaną odpowiedzią fizjologiczną roślin na ten typ stresu środowiskowego (Bayat i in., 2022; Yousefi i Karamian, 2025).

Reakcja *S. lapponum* na zwiększoną dostępność N-NO₃ była słabsza niż na zasolenie i zależała od zastosowanej dawki oraz czasu ekspozycji. Niższe stężenia azotu działały stymulująco na wzrost roślin, natomiast jego nadmiar przy dłuższej ekspozycji prowadził do zahamowania tego procesu. Wyniki te wpisują się w przedstawianą w literaturze zależność reakcji roślin od dostępności i formy azotu (Andrews i in., 2013; Ahmed i in., 2020). Badania prowadzone w naturalnych siedliskach *S. lapponum* we wschodniej Polsce wykazały bardzo niskie stężenia związków azotu w wodzie gruntowej dostępnej dla roślin (Serafin i in., 2022). Wyniki tych badań sugerują, iż wierzba lapońska jest w stanie prawidłowo funkcjonować w siedliskach ubogich w azot, jednakże nie poznano dotąd górnej granicy tolerancji ekologicznej gatunku na ten czynnik. Przedstawione w publikacji P3 wyniki eksperymentu wykazały, że badane rośliny tolerowały zastosowane dawki azotu azotanowego lepiej niż testowane warianty zasolenia. Mając jednak na względzie, iż azot jest jednym z kilku biogenów powodujących eutrofizację, a także fakt iż w niniejszych badaniach pod uwagę wzięto jedynie formę azotanową tego pierwiastka, istnieje potrzeba kontynuacji badań, szczególnie z uwzględnieniem innych form azotu.

Ostatni etap badań obejmował translokację osobników *S. lapponum* do stanowisk zlokalizowanych na Pojezierzu Łęczyńsko-Włodawskim. Ocena przydatności siedlisk do realizacji działań ochronnych takich jak translokacje wymaga kompleksowego uwzględnienia zarówno struktury gatunkowej i składu fitocenozy jak i kluczowych warunków abiotycznych w siedliskach. Jednocześnie w przypadku postępujących i wielokierunkowych zmian środowiskowych, przewidywanie długoterminowego kierunku przekształceń ekosystemów torfowiskowych obarczone jest dużą niepewnością. Podkreśla to konieczność ostrożnego doboru stanowisk do prowadzenia translokacji, opartego na szczegółowej analizie parametrów siedliskowych, a także potrzebę prowadzenia działań o charakterze eksperymentalnym (Albrecht i Long 2019; Pogorzelec i in., 2020a; Stadtmann i Seddon, 2020). Badania prowadzone w polskich i czeskich populacjach *S. lapponum* wykazały, że

gatunek ten może występować w fitocenozach o zróżnicowanym składzie gatunkowym (Pogorzelec 2008a; Hroneš i in., 2018), jednak jak wskazuje Hroneš i in. (2018), tylko część fitocenz zapewnia warunki zbliżone do optimum ekologicznego gatunku, sprzyjające jego prawidłowemu funkcjonowaniu. Stanowiska wybrane do przeprowadzenia eksperymentu translokacji w latach 2023-2025 (P4), w których dominowały gatunki charakterystyczne dla torfowisk przejściowych (B i S) i szuwarów przybrzeżnych (P) okazały się być najbardziej odpowiednie dla wierzby lapońskiej. Tego typu fitocenozy Hroneš i in. (2018) określają jako sprzyjające pełnej funkcjonalności populacji, co przejawia się m.in. wyższym udziałem osobników kwitnących. Badania prowadzone przez Kołosa i in. (2015) oraz Serafina i in. (2015), wskazują że nawet przy stabilnych warunkach hydrologicznych siedliska skład gatunkowy fitocenz, a w szczególności stan zaawansowania sukcesji ekologicznej i towarzysząca jej presja konkurencyjna drzew i krzewów, może stanowić główny czynnik ograniczający funkcjonowanie populacji *S. lapponum*.

Wyniki eksperymentu translokacji osobników *S. lapponum* do stanowisk zastępczych wykazały, że w pierwszym roku po translokacji, wartość wskaźnika przeżycia (SR) we wszystkich stanowiskach była wyższa od średniego wyniku notowanego w światowych zestawieniach translokacji roślin (Godefroid i in., 2011). Wysoka przeżywalność roślin w początkowym okresie nie przesądziła jednak o dalszej stabilności utworzonych populacji, co było szczególnie widoczne w stanowisku świeżej łąki (KB), gdzie w drugim roku po wprowadzeniu roślin wartość SR spadła do 32%. Uzasadnia to zatem potrzebę prowadzenia wieloletniego monitoringu roślin po przeprowadzeniu translokacji, obejmującego nie tylko początkową aklimatyzację osobników, lecz także dalsze utrzymywanie się ich liczebności oraz zdolność do reprodukcji i rekrutacji nowego pokolenia (Godefroid i in., 2011; Whitehead i in., 2023). Potwierdza się jednak tezę, że wraz z upływem czasu wartość wskaźnika przeżywalności się zmniejsza. Wskazują na to również inni autorzy przedstawiający wyniki działań ochrony czynnej prowadzonej na różnych gatunkach roślin na świecie (Drayton i Primack, 2012; Haskins i Keel, 2012; Segovia-Rivas i in., 2018).

Kolejnym ze zbadanych wskaźników powodzenia procesu translokacji było określenie liczby osobników kwitnących w nowopowstałych populacjach (FR). W pierwszym roku po wprowadzeniu osobników *S. lapponum* do stanowisk eksperymentalnych stwierdzono kwitnienie roślin we wszystkich stanowiskach. Udział osobników, które wytworzyły kwiaty mieścił się w zakresie porównywalnym ze średnią

wartością 19% uzyskaną dla innych zabiegów translokacji roślin prowadzonych na świecie (Godefroid i in., 2011), co potwierdza, że rośliny były zdolne do rozpoczęcia reprodukcji także w warunkach innych niż w stanowisku referencyjnym. Pełna ocena sukcesu translokacji wymaga jednak potwierdzenia przebiegu kolejnych etapów rozmnażania roślin oraz pojawienia się nowego pokolenia zdolnego do wzrostu w siedlisku docelowym (Menges 2008; Albrecht i in., 2019).

Integralnym elementem monitoringu osobników *S. lapponum* w nowopowstałych populacjach była ocena ich odpowiedzi fizjologicznej na stres środowiskowy (P5). Wyniki badań fluorescencji chlorofilu prowadzonych podczas eksperymentu terenowego wskazują, że parametr F_v/F_m był najbardziej czułym narzędziem diagnostycznym oceny kondycji *S. lapponum*. Analiza wyników pomiarów F_v/F_m u roślin w stanowiskach zastępczych pokazała zróżnicowane reakcje osobników badanego gatunku w zależności od warunków siedliskowych w jakich wzrastały. Ujawniła także wyraźną reakcję wszystkich badanych introdukowanych roślin na stres w okresie wiosennym (w maju 2025 roku). Na potencjalną użyteczność tego wskaźnika w monitoringu kondycji roślin introdukowanych do nowych stanowisk wskazują badania prowadzone na innych gatunkach wymagających ochrony czynnej na świecie (Soares i in., 2020; Liu i in., 2025; Li i in., 2025). Potwierdzają to także wyniki badań prowadzonych przez Pogorzelec i in. (2022) i Arciszewskiego i in. 2024 (P1) na gatunkach z rodzaju *Salix*, na początkowym etapie funkcjonowania roślin w środowisku naturalnym. Natomiast w wyniku eksperymentów prowadzonych w warunkach laboratoryjnych, polegających na indukowaniu stresu za pomocą jednego czynnika środowiskowego (temperatury, zasolenia lub przeżyźnienia podłoża) stwierdzono zdecydowanie mniejsze wahania wartości F_v/F_m u *S. lapponum* (P2, P3) niż w przypadku oddziaływania zespołu czynników stresowych w siedliskach zastępczych podczas badań prowadzonych w latach 2024-2025.

Dopełnienie oceny stanu fizjologicznego roślin stanowiły analizy względnej zawartości wody w tkankach (RWC) oraz aktywność peroksydazy gwajakolowej (GPOX). Wskaźnik RWC jest uznawany za jeden z kluczowych wyznaczników stresu wodnego roślin, odzwierciedlający bilans między tempem pobierania a transpiracją wody oraz stanem uwodnienia tkanek (Lawlor i Cornic, 2002). Uzyskane podczas eksperymentu terenowego wyniki wskazują na wyraźną zmienność sezonową RWC u osobników *S. lapponum* w stanowiskach zastępczych, co potwierdza wysoką czułość tego wskaźnika na zmiany warunków hydrologicznych siedlisk. Aktywność peroksydazy gwajakolowej była

szczególnie wysoka u osobników *S. lapponum* rosnących w siedliskach przekształconych: torfowisku osuszonym (KB) i zdegradowanym (P). Może to wskazywać na nasilenie enzymatycznej odpowiedzi antyoksydacyjnej roślin na stres środowiskowy, związanej z usuwaniem nadmiaru reaktywnych form tlenu (Jebara i in., 2005; Lofti i in., 2010).

Ewaluacja przydatności poszczególnych biomarkerów, przeprowadzona na podstawie wyników badań, umożliwiła wyselekcjonowanie grupy wskaźników fizjologicznych, stanowiących narzędzie do obiektywnej oceny efektywności zabiegów ochrony czynnej badanych gatunków wierzb. Należy jednak podkreślić, że kluczowym, wstępnym etapem weryfikacji przebiegu aklimatyzacji oraz identyfikacji osobników o obniżonej kondycji powinna być ocena cech morfologicznych roślin przez eksperta. Dopiero w dalszej kolejności makroskopowa ocena wizualna powinna być uzupełniana o analizę wysoce czułych markerów fizjologiczno-biochemicznych (Bussotti i Pollastrini, 2017).

6. Wnioski

Na podstawie wyników badań przeprowadzonych w ramach niniejszej rozprawy doktorskiej, sformułowano następujące wnioski w odniesieniu do kolejnych hipotez:

Aklimatyzacja roślin do warunków stanowisk zastępczych **(H1)**:

- Sukces translokacji osobników *Salix myrtilloides* pochodzących z upraw ex situ jest istotnie uzależniony od sposobu aklimatyzacji i ram czasowych jej przebiegu. Wydłużenie etapu prowadzonego w warunkach laboratoryjnych oraz skrócenie aklimatyzacji terenowej może przyczynić się do zwiększenia wydajności fotosyntetycznej, przywrócenia homeostazy ROS, a tym samym może wpływać pozytywnie na ogólną kondycję roślin na dalszych etapach translokacji.

Reakcje osobników *Salix lapponum* na stres środowiskowy związany bezpośrednio i pośrednio ze zmianami klimatu **(H2)**:

- Osobniki *S. lapponum* pochodzące z upraw ex situ są w stanie przetrwać okresy krótkotrwałego stresu termicznego (zarówno niskiej jak i wysokiej wartości temperatury otoczenia), ale zdolność aktywacji antystresowych mechanizmów fizjologicznych wykształca się u roślin sukcesywnie w kolejnych fazach wzrostu.
- Osobniki *S. lapponum* uruchamiają mechanizmy obronne w postaci aktywacji enzymów antyoksydacyjnych, syntezy antocyjanów i utrzymywania wysokich wartości RWC, gdy w środowisku oddziałuje na nie czynnik stresowy jakim jest zasolenie podłoża, bez względu na jego natężenie (stężenie soli w roztworze glebowym).
- Dostępność przyswajalnych form azotu w niskich stężeniach w roztworze glebowym działa stymulująco na wzrost roślin, ale znaczny wzrost stężenia azotu azotanowego powoduje pogorszenie parametrów morfo-fizjologicznych *S. lapponum*, co może być przyczyną spadku kondycji introdukowanych osobników w razie postępowania procesu eutrofizacji w siedlisku.
- Reakcje fizjologiczne na stres środowiskowy osobników *S. lapponum* introdukowanych do stanowisk naturalnych, stanowią wiarygodne i czułe wskaźniki efektywności ich aklimatyzacji do warunków siedliskowych stanowisk zastępczych oraz pozwalają na ocenę kondycji roślin na każdym etapie procesu translokacji.

- Wykorzystanie wskaźników funkcjonowania aparatu fotosyntetycznego (F_v/F_m), statusu wodnego (RWC) oraz odpowiedzi antyoksydacyjnej (aktywność peroksydazy gwajakolowej), uzupełnione o subiektywną ocenę cech morfologicznych może stanowić zoptymalizowany system kontrolno-pomiarowy introdukowanych osobników *S. lapponum*.

Potencjał wykorzystania siedlisk suboptymalnych dla translokacji zagrożonego gatunku *S. lapponum* **(H3):**

- Translokacja z wykorzystaniem osobników otrzymanych w warunkach ex situ może stanowić skuteczne narzędzie czynnej ochrony *Salix lapponum* nie tylko w przypadku stanowisk zastępczych spełniających w pełni specyficzne wymagania gatunku w zakresie parametrów fizykochemicznych środowiska oraz warunków biocenotycznych.
- Wyniki badań potwierdzają znaczną plastyczność ekologiczną gatunku. Badane rośliny mogą nie tylko przetrwać, ale również rozwijać się (wzrastać wegetatywnie) i zapoczątkować proces rozmnażania (wytwarzać kwiaty) w stanowiskach zastępczych, których warunki siedliskowe różnią się znacząco od preferowanych przez badany gatunek.
- Siedliska torfowiskowe, które ulegają transformacjom na skutek zmian klimatu mogą pełnić funkcję stanowisk zastępczych dla *S. lapponum*. Kluczowym czynnikiem determinującym sukces translokacji badanego gatunku jest utrzymanie stabilnych warunków hydrologicznych w siedlisku. Niedostateczne zasoby wody glebowej, w połączeniu z zaawansowaną sukcesją drzew i krzewów w istotny sposób wpływają na krótko i długoterminowe przetrwanie osobników *S. lapponum*.

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8. Kopie opublikowanych prac wchodzących w skład cyklu publikacji

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Towards successful reintroduction of *Salix myrtilloides*: the importance of monitoring plant physiological indicators during acclimatization

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Abstract: *Salix myrtilloides* L. is a relict species in Poland, considered to be particularly endangered, for which it is necessary to carry out active protection through reintroduction and the replenishment of existing populations. The aim of the study was to test the capacity of *S. myrtilloides* plants obtained in *in vitro* cultures for effective acclimatization (under both laboratory and field conditions) during the translocation process. In order to trace the response of individuals to changes in environmental conditions on their way from the laboratory to the intended natural habitat, research was carried out on the selected parameters of the functioning of the photosynthetic apparatus (chlorophyll *a* fluorescence, concentration of photosynthetic pigments in plant tissues), the response of plants to environmental stress (accumulation of reactive oxygen species; ROS) and the variability of biometric traits during their growth (rate of daily stem growth). At each stage of the study, verification was carried out on the number of *S. myrtilloides* plantlets that survived the successive stages of acclimatization and the 12-month growth period in the natural stand. The results of the study reveal that the physiological condition of *S. myrtilloides* regenerants developed in tissue culture is dependent on changes in environmental conditions during acclimatization, and that the first few days of each stage of this process are crucial and most difficult for the plants. An elevated accumulation of ROS in leaf tissue and lower photosystem II (PS II) efficiency may indicate increased level of stress. However, these changes may also serve as signals to initiate metabolic, physiological and developmental processes crucial for proper plant acclimatization. Analysis of the results provides important clues for optimising this process by varying the duration of the various stages.

Keywords: swamp willow, ex situ conservation, photosynthetic pigments, chlorophyll fluorescence, ROS

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Introduction

Among the most endangered species of flora, climate relics are very frequently mentioned, especially those whose populations function far from their range, in isolated habitats undergoing systematic and irreversible changes. Species inhabiting natural peatland ecosystems make up a large proportion of this group, and among these, boreal relics (Kruszelnicki & Gostyńska-Jakuszevska, 2014). In the course of their evolution many plant species associated with wetlands have developed unique adaptations to be able to function in these specific environmental conditions. However, a high degree of specialization also increases the potential risk of extinction, especially in the case of sudden or progressive changes taking place in the habitat (Minayeva & Sirin, 2012). The impoverishment of biodiversity which is occurring on a global scale and the climate crisis have created an urgent need for action to protect as many species and entire ecosystems as possible (Bartholomeus et al., 2011; Reiter et al., 2016).

Besides standard *in situ* and *ex situ* species conservation methods widely practiced by organizations and services associated with nature conservation, an alternative is to preserve the gene resources of endangered species by keeping a gene bank, renewing resources through the creation of tissue cultures, and introducing species to horticultural cultivation (Dias et al., 2011; Osório et al., 2013; Gonçalves et al., 2017; Volis, 2017). Native species, owing to the specific structural features, forms of growth, habitat preferences, and adaptability, are predisposed to function as elements of nature-friendly gardens which fit well into regional landscapes. The use of native flora to create naturalistic plantings is a very important chapter in the history of horticulture (Zachariasz, 2007). The introduction of native plant collections to professional as well as amateur cultivation is currently becoming popular (Heywood, 2003).

Wetlands and water bodies are habitats abundant in plant species with specific requirements, but also with ornamental potential and often medicinal properties as well. These include grasses and sedges, as perennials, but also trees and shrubs. Many of these species are currently under legal protection, because their population sizes have decreased significantly in recent years. Bringing them into cultivation provides the opportunity to preserve the gene pool but also to learn about and make use of their pharmacological properties as well as their ornamental value (Serafin et al., 2017; 2018; 2023).

The swamp willow (*Salix myrtilloides* L.) is a short shrub with small, blue-green leaves coated with a layer of wax. The main stem and side shoots are thin and straight, initially bare and yellowish brown or red, changing over time to grey or reddish-brown.

The species is dioecious, both insect- and wind-pollinated. The swamp willow is a sun-loving species which occurs naturally in flooded and unforested transitional peat bogs in lowlands and foothills; it prefers acidic peat with pH 3.5–5.5 as a substrate (Churski & Danielewicz, 2008; Serafin et al., 2015). *S. myrtilloides* is considered an endangered relic species in many countries of Central Europe and is found mainly in isolated stands (Kozel et al., 2022). Its populations have been systematically declining in recent decades (Pogorzelec et al., 2015). For this reason, attempts at active conservation of the species using translocations methods have recently been made (Arciszewski et al., 2023). The most important step was successful attempts at micropropagation of specimens from material taken from natural stands (Parzymies et al., 2023).

The acclimatization process of plants derived from tissue cultures is aimed at adapting them to the prevailing atmospheric and soil conditions, which significantly improves the chances of normal growth and development later on. The condition of plants determined on the basis of variation in certain morpho-physiological traits may indicate its critical points at each stage of acclimatization (Pogorzelec et al., 2022). Their precise determination can help to make the introduction in *ex situ* cultivation or reintroduction more successful and to ensure the long-term survival of the new populations. In addition, determination of the physiological condition of the species during translocation can help to estimate the ongoing risk associated with the effectiveness of the procedures. Certain physiological parameters of plants, such as changes in the content of photosynthetic pigments, the functioning and efficiency of the photosynthetic apparatus, or the accumulation of reactive oxygen species (ROS), can provide important information indicating early biochemical and physiological modifications even before significant changes in phenological and morphological parameters appear (Schönbeck et al., 2023; Carevic, 2016). Well-designed scientific experiments which include testing of research hypotheses improve the chances of perfecting protocols for the development of guidelines for carrying out specific procedures during the plant translocation process (Guerrant & Kaye, 2007; Kaye, 2008).

Two species of relic willows (*Salix myrtilloides* and *Salix lapponum*) occurring in Poland, despite their common origin, differ significantly not only in terms of morphology, but also in terms of tolerance ranges to various environmental factors (Kruszelnicki & Gostyńska-Jakuszevska, 2014). Specific requirements and related adaptive abilities certainly affect the efficiency of acclimatization and survival of individuals of both species in alternative locations to which they are transferred. Previous studies

conducted by Pogorzalet et al. (2022) allowed for the specification of the conditions of acclimatization of *Salix lapponum* plantlets produced in the process of micropropagation, both in laboratory and field conditions. In the case of *Salix myrtilloides*, it is equally important to develop effective methods and determine the optimal conditions for acclimatization of seedlings. In the case of plants threatened with extinction, it is necessary to develop precise procedures used during translocation to minimize the risk of losses of valuable plants.

The aim of the study was to test the capacity of *S. myrtilloides* plants obtained in *in vitro* cultures for effective acclimatization. Selected morpho-physiological traits of regenerants and their variability in different environmental conditions were used as diagnostic indicators to follow the phenomena taking place along the path travelled by *S. myrtilloides* from the laboratory to the target habitat during the reintroduction process. The following research hypotheses were put forth: (1) a change in the acclimatization conditions of *S. myrtilloides* affects the content of photosynthetic pigments and the functioning of the photosynthetic apparatus, particularly the activity of photosystem II (PSII); (2) accumulation of ROS in the leaves can be a useful indicator of the intensity of stress symptoms influencing the physiological condition of *S. myrtilloides* at various stages of acclimatization; (3) assessment of selected parameters of photosynthetic activity of PSII and the level of oxidative stress makes it possible to identify the period when homeostasis is established in plants and they are ready for the next stages of acclimatization.

Materials and Methods

The research object was *S. myrtilloides* regenerants obtained by micropropagation in the Tissue Culture Laboratory of the Institute of Horticultural Production, University of Life Sciences in Lublin and the studies were conducted in 2022–2023. The 100 plantlets used in the research were clones of plants from a natural population of the species, functioning in a stand at the Lake Bikcze (51°22'46.3"N; 23°2'35.17"E; Łęczna-Włodawa Plain, eastern Poland). The plants were obtained in the laboratory using micropropagation methods which had been developed and optimized for the species (Parzymies et al., 2023). After the plants had developed a root system in the growth substrate, they were transplanted to pots with a specially composed substrate and placed in a phytotron in controlled temperature (day/night 22 °C/20 °C), humidity (60%) and light conditions ($35 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ with the 16/8 hour light/dark cycle) (acclimatization in the laboratory, according to the procedure described for *S. lapponum*

by Pogorzalet et al. (2020). There they were pruned several times to stimulate the production of lateral shoots. To prepare the plants for growth in natural conditions, after about three months, in mid-May, the plantlets were transferred to cold frames with a substrate of sand and peat, with a net for protection against insects, where they spent another two months outside the laboratory (average temperature 20 °C, average precipitation 50 mm, average day length 15 h during field acclimatization, at the plant acclimatization station). The final stage of the acclimatization process was to transport the plants to the previously determined location and plant them in the natural stand, suitable for the studied species, which was a raised bog located in a protected area – Poleski National Park (51°27'8.96"N; 23°13'43.71"E). Conditions at the reintroduction site during first 14 days after planting the plantlets: average temperature 20 °C/12 °C – day/night, average precipitation 80 mm, average day length 16 h (the data comes from the meteorological station located at the plant acclimatization station; Fig. 1).

Content of photosynthetic pigments

At each stage of the acclimatization process the content of chlorophyll *a* and *b* and carotenoids was determined in the leaves of 10 randomly selected specimens. Plant material was analysed: (i) four times in conditions of laboratory acclimatization, at weekly intervals from the start of acclimatization; (ii) twice during field acclimatization, 1 day and 14 days after the plants were transferred from the laboratory; and (iii) twice in natural stands, after 14 days and 12 months.

Leaf from the middle part of the shoots were homogenized in 96% ethanol for pigment extraction. The homogenates were then placed in a water bath for 5 min, after which the liquid phase was separated from the solid phase using a centrifuge ($9\,279 \times g$). A spectrophotometer (SPECORD 40, Analytik Jena GmbH, Germany) was used to measure the absorbance of the solutions at three wavelengths (470, 649 and 665 nm). The content of pigments was calculated using formulas given by Lichtenthaler & Wellburn (1983).

Selected parameters of chlorophyll *a* fluorescence

To assess the effect of variable habitat conditions on the physiological response of *S. myrtilloides*, chlorophyll *a* fluorescence was measured at each stage of acclimatization to monitor changes in the efficiency of photosystem II (on leaves located in the middle of the shoot in 10 randomly selected specimens).

Measurements were made on plants (i) transferred from *in vitro* cultures to the soil substrate (5 weeks and 3 months after transplantation; due to the small size of young plantlets there was no physical possibility to take measurements before the 5th week of their growth); (ii) in the group of plantlets undergoing field acclimatization (after 1 and 14 days and after 1, 1.5 and 2 months of growth in cold frames); and (iii) on plants transferred to the natural habitat (14 days and 12 months after planting at the sites). In the case of plants growing in the laboratory, at the acclimatization station and those introduced to

natural sites, measurements were carried out in the morning, from 9 to 11 a.m.

Chlorophyll fluorescence was measured using a fluorimeter (Handy PEA fluorimeter, Hanstech Instruments, UK). The following parameters of chlorophyll *a* fluorescence were analysed: the minimum (F_0) and maximum (F_m) possible level of chlorophyll fluorescence and the maximum quantum yield of PS II (F_v / F_m , where $F_v = F_m - F_0$). Prior to the measurements, special clips eliminating access to light for 15 minutes were placed on the leaves.

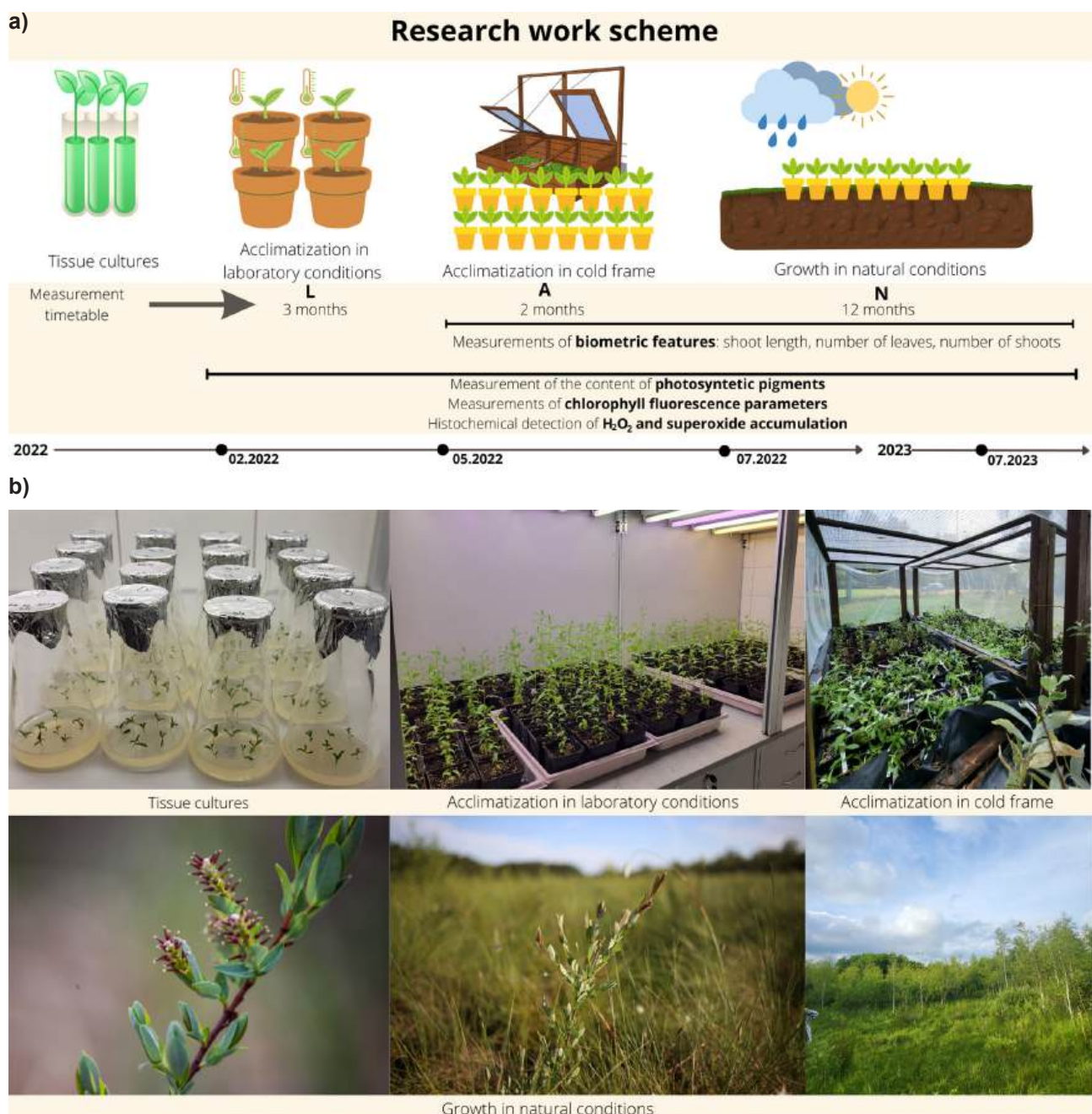


Fig. 1. Research design, taking into account the stages of *Salix myrtilloides* acclimatization and plant analyses (a). Photographic documentation of the reintroduction process (b)

Histochemical detection of H_2O_2 and superoxide anion

Histochemical analyses of the accumulation of H_2O_2 and $O_2^{\bullet-}$ in the tissues of *S. myrtilloides* leaves were carried out according to the method described by Kumar et al. (2014). Leaves were taken from the middle part of the shoot from plants at specific stages of acclimatization: (i) after 3 months of acclimatization in the laboratory, (ii) after the beginning of acclimatization using cold frame and after 1 month of growth in the cold frame, and (iii) 2 weeks and 12 months after transfer to the natural stand (the same times as for chlorophyll fluorescence measurements, environmental sampling took place in the morning, between 9 and 11 a.m.). Then the plant material was placed in test tubes filled with distilled water and immediately taken to the laboratory. H_2O_2 detection was carried out using 3,3'-diaminobenzidine (DAB). The leaves were immersed in a DAB solution (1 mg/mL) for 10–12 h and placed in the dark at room temperature. After that they were immersed in boiling water and then in hot 80% ethanol to remove chlorophyll from the tissues and visualize the brown product of DAB polymerization taking place in the presence of H_2O_2 .

Nitrotetrazolium blue chloride (NBT) was used to visualize $O_2^{\bullet-}$ in the leaf tissues. The oxidized form of NBT (yellow) is reduced by endogenous $O_2^{\bullet-}$ to the diformazan form (bluish purple). The leaves were immersed in 0.2% NBT solution in phosphate buffer (50 mM, pH = 7.5) and placed in the dark for 10–12 h. Then they were decolorized in hot 80% ethanol until the reduced, bluish-purple form of the pigment became visible.

About 10 leaves were stained for each of the experimental combinations, and 3–4 of the most representative leaves were chosen for photographic documentation.

Selected morphological traits of plants

To determine the daily growth rate of the plant shoots, their height was measured with a ruler (from the base of the plantlet to the top of the shoot). The total number of leaves and the number of side shoots was also determined on individual specimens. The measurements were initially made on the group of plantlets in the field acclimatization stage for a period of seven weeks, at seven-day intervals. In the next stage, the plants were divided into two groups: the first was transferred to a natural stand, while the other was left in the cold frames so that the growth rate of the two groups could be compared. Measurements of the previously determined morphological traits were made four times at 14-day intervals for plants from both groups.

Verification of the success of the translocation procedures

The number of *S. myrtilloides* plantlets which survived each stage of acclimatization and the 12-month period of growth at the natural stand was determined. Their percentage share was determined according to the following formula:

$$\text{Survival rate (\%)} = \frac{\text{number of surviving plantlets}}{\text{number of plantlets reintroduced}} \times 100$$

Statistical analyses

The Shapiro-Wilk test was used to check the normality of the numerical data distribution. Then one-way analysis of variance (ANOVA) was carried out to identify statistically significant differences between groups. In case the differences were significant, the post-hoc Tukey test was performed. All analyses were carried out for a significance level of $\alpha = 0.05$ using STATISTICA 13.3 software (TIBCO Software Inc. 2017, Palo Alto, CA, USA).

Results and Discussion

In the face of increasingly unpredictable changes in habitat conditions induced by climate change (both natural and anthropogenic) and deliberate human interference in natural ecosystems, the capacity of organisms for acclimatization and adaptation are crucial for their survival in the natural environment. In the case of endangered species, analysis of processes related to acclimatization on morphophysiological traits can provide valuable information on adaptation strategies and potential (Anderson & Song, 2020). A variety of physicochemical factors (light, soil moisture, etc.), and biocenotic factors (intra- and interspecific interactions) together create a complex mosaic of conditions which individual specimens encounter in their natural environment and which are confronted with the body's specific reaction to stress. The success of conservation procedures is to a great extent determined by basic knowledge as well as in-depth knowledge of the factors limiting plant growth and development, both in the natural environment and in *ex situ* conditions (Asgher et al., 2020; Liu et al., 2006; Gailite et al., 2023; Bose et al., 2022).

The results of ecophysiological tests conducted for the first time on *S. myrtilloides* regenerates obtained in *in vitro* conditions and later grown and acclimatized *ex vitro*, provided a broader picture of the response of this species to the variable conditions of the surrounding environment during the long process from the laboratory to the natural habitats.

The measurements of photosynthetic pigment content in the *S. myrtilloides* leaves, performed over four weeks in laboratory acclimatization conditions, revealed a systematic increase in the concentrations of chlorophyll *a* and *b* in the plant tissues. However, no significant changes were noted in the content of carotenoids (Fig. 2). This may have been due to inhibition of dynamic growth stimulated in *in vitro* conditions by the components of the growth medium. The plants most likely begin intensive photosynthesis at that time, which may be linked to enhanced production of photosynthetic pigments (Guan et al., 2008).

The change in conditions from laboratory acclimatization to field acclimatization also influenced the content of pigments in the species. Following transfer from the phytotron to the field acclimatization station, a statistically significant decrease in Chl *b* level was observed between the plantlets in the fourth week of laboratory acclimatization and those at the beginning of acclimatization in cold frame. In consequence, the Chl *a/b* ratio increased as well. A similar response of plants at this stage of acclimatization was observed in grapevine (*Vitis vinifera*) and wild olive (*Olea madarensis*). It has been suggested that the change in light conditions and exposure of plants to high intensity of this factor can lead to the phenomenon of photoinhibition or even photooxidation of chlorophyll (Amâncio et al., 1999; Brito et al., 2009). After two weeks of field acclimatization, a significant increase in the concentration of all photosynthetic pigments was noted in the *S. myrtilloides* leaves in comparison to the initial period. This was followed by a marked decrease in the concentrations of pigments in the plants transferred to the natural habitat. Under natural light conditions, despite a decrease in individual pigments, the Chl *a/b* ratio gradually

increases, reaching its highest value after 12 months. This may indicate that the plant is fully adapted to intense natural light, where the dominance of chlorophyll *a* allows maximum photosynthetic efficiency in the full spectrum of sunlight (Nyongesah et al., 2015; Fig. 2).

Depending on the conditions in which they function, plants efficiently adjust the content of photosynthetic pigments in the leaf tissues in order to optimize the course of photosynthesis (Grzeszczuk et al., 2018). A change in environmental conditions often induces stress responses in plants, which can disturb chlorophyll synthesis or the quantitative relationships between groups of pigments (Mane et al., 2010). The change in light conditions (from constant light in laboratory conditions to the natural photoperiod in field acclimatization) and the acceleration of the growth rate (increase in shoot length) observed at this stage of acclimatization may have been factors influencing the content of photosynthetic pigments in *S. myrtilloides*. During field acclimatization plants are exposed to frequent and unpredictable changes in temperature, light intensity, and substrate hydration. These factors can significantly modify both the biosynthesis/degradation of pigments and the intensity of photosynthesis (Osório et al., 2013).

In a normally functioning photosynthetic system, the F_v/F_m index indicating the maximum quantum yield of the PSII photosystem should range from 0.800 to 0.830 in most plant species (Murchie & Lawson, 2013). Analysis of parameters of chlorophyll fluorescence in the *S. myrtilloides* specimens in laboratory acclimatization conditions revealed that five weeks after the plants had been transferred from *in vitro* cultures to a soil substrate, the F_v / F_m value approach the optimal level. However, it was only after three months that the value exceed the threshold of 0.800. After this time, there was a statistically significant decrease in the minimum fluorescence value (F_0), and slight reduction in the maximum fluorescence (F_m ; Fig. 3). It can be concluded that extending the acclimatization period for *S. myrtilloides* in the phytotron to three months may increase the photosynthetic efficiency of the plantlets and thereby positively influence their overall condition at later stages of translocation.

Fluctuations in access to light, ambient temperature, humidity, and other environmental factors may affect the ability of plants to carry out photosynthesis and its efficiency (Brito et al., 2009). Measurements of chlorophyll fluorescence in the leaves of *S. myrtilloides* plants transferred from laboratory conditions to field acclimatization showed that the most favourable values for this parameter were noted after the plants had been in the cold frames for one month. In the case of the species *S. lapponum*, studied previously, the maximum quantum yield of PSII (F_v / F_m)

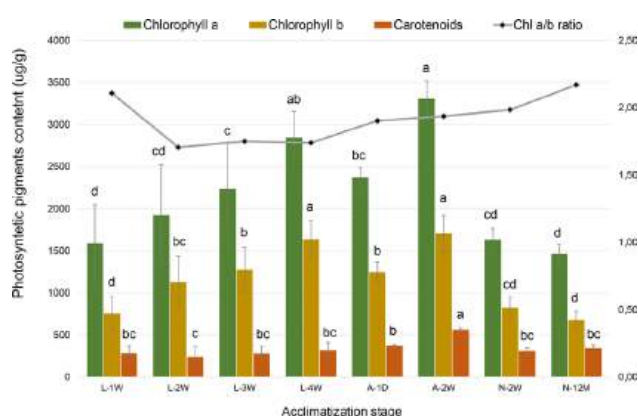


Fig. 2. Concentrations of photosynthetic pigments in *Salix myrtilloides* leaves at specific stages of plant acclimatization (L – laboratory acclimatization, A – acclimatization in cold frame, N – acclimatization in natural stand; D – day, W – week, M – month). Mean values ($\pm$ standard deviation; $n = 10$) for each class of pigments marked with different letters are significantly different ($p < 0.05$)

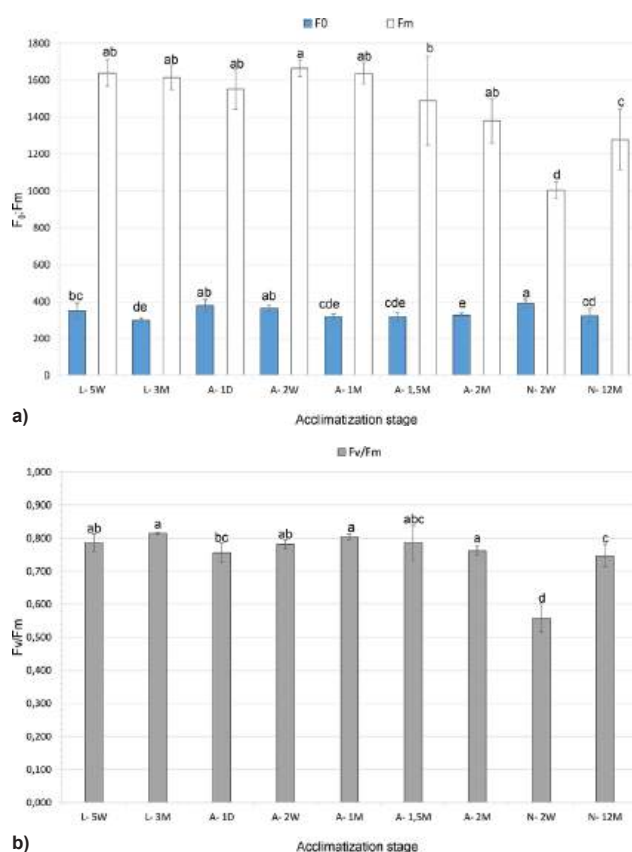


Fig. 3. Selected parameters of chlorophyll *a* fluorescence: (a) minimum fluorescence (F_0) and maximum fluorescence (F_m) value (b) maximum quantum yield of PSII photochemistry (F_v / F_m) in *Salix myrtilloides* leaves at each stage of acclimatization (L – laboratory acclimatization, A – acclimatization in cold frame, N – acclimatization in natural stand; D – day, W – week, M – month). Mean values ($\pm$ standard deviation; $n = 10$) marked with different letters are significantly different ($p < 0.05$)

was noted after about 1.5 months of growth in cold frames (Pogorzelec et al. 2022). Extending the acclimatization time for *S. myrtilloides* resulted in an F_v/F_m value below the expected minimum, and after 1.5–2 months at the acclimatization station their photosynthetic efficiency began to decrease slightly, showing a downward trend (Fig. 3).

In the case of *S. myrtilloides*, as noted previously in *S. lapponum*, 14 days after the plants had been transferred to natural stands, the parameters of maximum fluorescence (F_m) and F_v/F_m values chlorophyll fluorescence had deteriorated (Fig. 3). Since chlorophyll fluorescence is related to the state of photosynthesis and thus to the chlorophyll *a* content (Gan et al., 2023), the observed decrease in PS II efficiency may be caused by significant decrease in chlorophyll *a* concentration during this period. This changes can unquestionably be linked to the dramatic change in environmental conditions, where an increase in F_0 , accompanied by a decrease in F_m , is usually associated

with stress responses (Mathur et al., 2011). Similarly, Percival (2005) observed that the oak species' response to heat stress resulted in significant damage to PSII, as evidenced by a marked decrease in F_v/F_m values along with an increase in F_0 , indicating that such physiological responses might be a common reaction of plants to sudden environmental changes. The results of monitoring of the condition of *S. myrtilloides* specimens that had been in the natural stand for 12 months showed significant improvement in chlorophyll fluorescence parameters. However, as previously noted for *S. lapponum* (Pogorzelec et al., 2022), their efficiency of PSII was lower than in the specimens growing in conditions of laboratory and field acclimatization. This confirms the tendency towards substantial intensification of physiological processes associated with plant growth during their acclimatization.

The results of the statistical analysis indicate differences in F_v/F_m value when the stage of acclimatization changed from laboratory to field and after the plants were transferred from cold frames to natural habitats. This may indicate that the initial period (a few days), just after the change in the conditions of the growth environment, is a critical point of acclimatization, when significant changes are noted not only in the chlorophyll fluorescence, but also in the level of oxidative stress, which is confirmed by the histochemical analyses (visualization of ROS – H_2O_2 and $O_2^{\cdot-}$). In the leaves of plants acclimatized in the laboratory for about three months, increased accumulation of $O_2^{\cdot-}$ was noted over the entire surface of the leaf. On the other hand, H_2O_2 accumulated in small amounts only within the main vascular bundles (Fig. 4), which may have been due to mechanical damage to the leaf petioles when they were cut from the parent plant and the associated secondary oxidative stress. In the second stage, ROS accumulation in plants transferred from the phytotron to field acclimatization was visualized. The results obtained at this stage of the study indicate that after one day in the cold frames severe oxidative stress takes place in the plants, associated in particular with excessive accumulation of $O_2^{\cdot-}$ within the entire leaf blade. Local, point accumulation of H_2O_2 was observed as well. After a month of acclimatization in cold frames, oxidative stress subsided, and the accumulation of both ROS forms tested was much lower than after one day (Fig. 4), which was also confirmed by the improved parameters of chlorophyll fluorescence (Fig. 3).

ROS in the leaves of plants growing in natural conditions was visualized among specimens two weeks after reintroduction and also after 12 months in those that had survived the winter. Two weeks after transfer from the cold frames to the natural stand, there was significant ROS accumulation indicating increased oxidative stress. In the specimens that

survived the winter, accumulation of $O_2^{\cdot-}$ and H_2O_2 decreased, as indicated by the less intensive blue (in the case of $O_2^{\cdot-}$) or brown (H_2O_2) colour of the leaf blades. Visualization of ROS in *S. lapponum* specimens examined at the same stages of the acclimatization process as *S. myrtilloides* shows substantial similarity in accumulation of $O_2^{\cdot-}$ and H_2O_2 in the leaf tissues. Marked differences were noted only at the first stage of the process – in laboratory conditions increased accumulation of $O_2^{\cdot-}$ was observed over the entire surface of the leaf blade in *S. myrtilloides* (Fig. 4), while in *S. lapponum* it accumulated only in the main vascular bundles (Pogorzelec et al., 2022).

Here it is important to highlight the special function of ROS in plants as ubiquitous signalling and regulatory molecules with a significant metabolic function, which are also involved in acclimatization processes; their role in the early stages of signal transduction initiated by environmental stimuli and stress factors is well documented (Waszczak et al., 2018). For this reason, the substantially increased accumulation of these compounds in the cells in response to the initially drastic change in living conditions during individual stages of acclimatization, despite the induction of oxidative stress, may allow the plants to activate coordinated signalling networks regulated by ROS, so that the homeostasis can be restored

Plant growth conditions	$O_2^{\cdot-}$	H_2O_2
L-3M		
A-1D		
A-1M		
N-2W		
N-12M		

Fig. 4. Visualization of reactive oxygen species (ROS) in *Salix myrtilloides* leaf tissues at each stage of acclimatization (L – laboratory acclimatization, A – acclimatization in cold frame, N – acclimatization in natural stand; D – day, W – week, M – month)

as quickly as possible and the plants can adapt and become acclimatized to the surrounding conditions (Dietz et al., 2016; Devireddy et al., 2021).

During the observations made for this publication, the length of the main shoots of *S. myrtilloides* plantlets growing in cold frames, increased on average by 5 cm. The greatest daily increase was noted in the second month of field acclimatization and amounted to 1.74 mm/day. This confirms the intensification of the processes determining growth which take place during field acclimatization. In the weeks after the plantlets had been separated into two groups, the growth rate of the plants was lower, in the case of both plants left in the cold frame (1.67 mm/day) and those growing in the natural stands (on average 0.57 mm/day; Fig. 5). No significant changes were observed in the number of side shoots in the two groups. The average number of leaves underwent changes during the study, but these were associated mainly with the death of old organs and the appearance of young ones (data not shown).

Biometric traits and their modifications over time may be indicative of the condition of plants, which changes under the influence of environmental conditions. In the case of *S. lapponum*, as in the case of *S. myrtilloides*, the growth rate was also highest in the second month of field acclimatization, but was as high as 6 mm/day. Differences in leaf morphology were also clearly evident in *S. lapponum*, while the characteristic traits of the species (shape, size, and tomentum) did not become visible until after several months of growth in natural conditions (Pogorzelec et al., 2022). This phenomenon was not observed in the *S. myrtilloides* specimens.

In the study carried out in 2022–2023 on *Salix myrtilloides*, the percentage share of plants that survived each stage of acclimatization was determined. During three months of growth in the laboratory, 5% of all plantlets died. No losses were recorded during

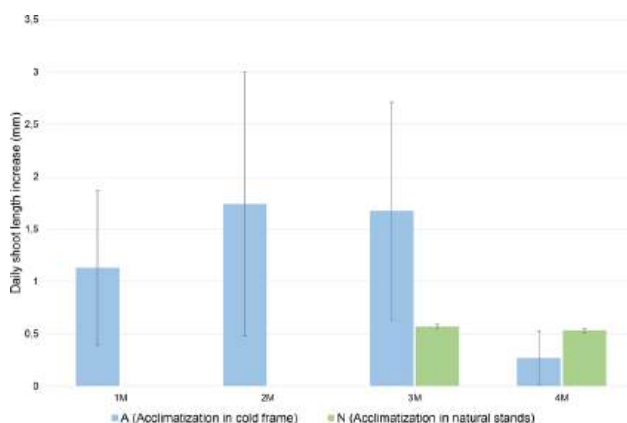


Fig. 5. Daily shoot length increase of *Salix myrtilloides* individuals during successive stages of acclimatization. (M – month). Mean values ($\pm$ standard deviation; $n = 9$)

the two-month field acclimatization or in the group of plants that remained in the cold frames for another eight weeks. After 12 months of growth in the natural stand, the survival of 79% of specimens from the group of 48 plants was noted. Literature data indicate that if even 50% of specimens survive the first growing season after translocation the result is considered satisfactory, or even very good in the case of woody plants (Fenu et al., 2019).

It should be stressed that ecophysiological study of the species *S. myrtilloides* has not previously been conducted, although its biology and elements of its population ecology are fairly well known, especially at the edge of the species' range. The first attempts at conservation of the species by translocation (reintroduction) in combination with *ex situ* cultivation have been made as well (Arciszewski et al., 2023; Zalewska et al., 2019; Pogorzelec et al., 2015; 2016; Parzymies et al., 2023; Serafin et al., 2015). The results of the current study, carried out specifically for this publication in 2022–2023 significantly expand knowledge of the responses of plants to stress associated with a change in living environment, but will also influence the planning of individual stages of conservation procedures for the species in the future.

Conclusion

The results of the study showed that the physiological condition of acclimatized *S. myrtilloides* specimens depended on changes in the conditions of the plant environment. Indicators of oxidative stress and photosynthesis efficiency as well as the biometric traits of plants changed during each stage of their transition from laboratory conditions to the natural habitat. The values for the parameters noted during the study provide important pointers for optimizing the acclimatization process of *S. myrtilloides* regenerants obtained in tissue cultures. For example, they suggest that extending the acclimatization period in the phytotron to three months and shortening field acclimatization to one month may increase the photosystem II efficiency of plants and restore ROS homeostasis, thereby positively influencing their overall condition at further stages of translocation. It was also noted that the first few days after the change in environmental conditions at each stage of growth are crucial and the most difficult for plants. This is manifested in a decline in efficiency of PSII, but also accumulation of ROS in the leaf tissues, which on the one hand indicates increased oxidative stress, but on the other hand may be essential for efficient signalling and activation of numerous metabolic, physiological and developmental processes crucial to acclimatization. The intensive response of plants to stress factors during this period may allow the plants

to activate mechanisms aimed at restoring homeostasis and to undertake growth and development processes.

An optimized, multi-stage acclimatization process guarantees the normal growth and development of plants in the conditions of natural habitats. This is evidenced by the final outcome of the study carried out in 2022–2023 study, i.e. the survival of as many as 79% reintroduced *S. myrtilloides* individuals from *ex situ* cultivation for a period of 12 months.

Author Contributions

Conceptualization, M.A. and M.P. (Magdalena Pogorzelec); methodology, M.A. M.P. (Magdalena Pogorzelec) B.H.-N.; validation, B.H.-N. and M.P. (Marzena Parzymies); formal analysis, M.A., M.P. (Mateusz Piejak); investigation, M.A., M.P. (Magdalena Pogorzelec), B.H.-N. M.P. (Marzena Parzymies), M.P. (Mateusz Piejak); data curation, M.A.; writing—original draft preparation, M.A.; writing—review and editing, M.P. (Magdalena Pogorzelec), B.H.-N., M.P. (Marzena Parzymies), M.P. (Mateusz Piejak); visualization, M.A. and B.H.-N.; supervision, M.A. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

Founding

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Article

Do Endangered Glacial Relicts Have a Chance for Effective Conservation in the Age of Global Warming? A Case Study: *Salix lapponum* in Eastern Poland

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Simple Summary: One of the challenges currently facing the conservation of endangered plant species is adapting the methods to the changing climate and increasing global warming. This problem is primarily linked to insufficient knowledge of whether and how quickly individuals of these species can adapt to changes in their natural habitats. Thus, it is difficult to predict whether their reintroduction into a natural environment will be successful. To come a little closer to answering this question, experiments were carried out on rare *Salix lapponum* plants grown in the growing room to determine how the plants respond to the stress of short-term exposure to extreme temperatures in the environment. It was found that the youngest plants were the most sensitive to low temperatures, while older ones reacted strongly to heat stress. This was indicated by an increased anthocyanin content in plant tissues and guaiacol peroxidase enzyme activity, which proved to be the most informative markers among the several used ones. The knowledge of the response of *Salix lapponum* to heat stress will help optimize plant acclimatization methods and perhaps contribute to the effective conservation of this valuable species of wetland flora in eastern Europe.



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Abstract: The abiotic stresses to which plants are exposed, especially in times of climate change, can result in the disruption of natural plant physiological processes. Sudden atmospheric phenomena may increase the risk of failure in protecting rare and extinction-threatened plant species by translocation. This study aimed to determine the effect of extreme ambient temperatures on the condition and physiological response of *Salix lapponum* plantlets used for their reintroduction into the natural habitat. *Salix lapponum* plants obtained by micropropagation methods at different stages of growth under laboratory conditions were subjected to a biological experiment. Plants were exposed for 12 h to temperature extremes (0 °C and 30 °C), after which the values of selected markers of the biochemical response were determined, such as photosynthetic pigments and anthocyanin content, guaiacol peroxidase and catalase activity, the presence of ROS and the RWC value. The study showed that plants at early growth stages were sensitive to low-temperature stress. In contrast, older ones showed a stronger response to high temperature, marked by an increased anthocyanin content and guaiacol peroxidase activity. It was also found that a short exposure to temperature extremes did not change the photosynthetic pigment content or catalase activity. The results of the study may be an important indication for the optimization of plant acclimatization methods in the process of their active protection by species translocation.

Keywords: climate change; downy willow; oxidative stress; temperature stress; translocation

1. Introduction

Sudden atmospheric events, which are a direct consequence of global warming, pose an increasing threat to both humans and natural ecosystems. Extreme weather phenomena are predicted to become more frequent and intense [1,2]. In addition, periods of drought and heat waves repeated at short intervals will exacerbate the economic crisis and threaten the natural ecosystems [3]. These phenomena may not only lead to habitat degradation but also directly and indirectly contribute to species extinction, especially for those species with poor adaptive capacity [4,5].

As sedentary organisms, plants are directly exposed to changes in the environmental conditions and have to adapt to stressful stimuli quickly. Temperature, drought, salinity, or intense lighting affect plant growth and development. When any of these factors exceed the optimum tolerance value for a given species, plants activate a series of complex defense responses that include changes in gene expression and morphogenesis processes [6,7]. Mild stress, although it may slow plant growth, contributes to the activation of adaptive mechanisms. Intense and sudden stress, however, often leads to severe damage, even death [8].

Environmental stress induces the formation of reactive oxygen species (ROS) in plants, such as singlet oxygen ($^1\text{O}_2$), superoxide anion (O_2^-), and the most common hydrogen peroxide (H_2O_2). ROS disrupt plant homeostasis, can damage practically all cellular macromolecules, including DNA, and inhibit *de novo* protein synthesis [6]. Stress induced by both low and high temperatures also leads to multistage damage to the photosynthetic apparatus, particularly photosystem II (PS II), which results in reduced photosynthetic capacity, inhibited growth, and reduced productivity [9,10]. Enzymes such as superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPOX), or ascorbate peroxidase (APX) play a key role in protecting plants from the effects of heat stress. They are involved in neutralizing ROS, reducing cell damage, and supporting plant survival [11].

There are lot of data on plant responses to stress factors, such as temperature changes, water availability, or pollution, but most of them, however, focus on cultivated or commercial plants [8,12]. In contrast, few data have been published on this topic for wildlife species, which represent ecological rather than economic value and are important for the biodiversity and the stability of natural ecosystems.

Active conservation of plant species threatened with extinction using translocation methods encounters many challenges in the conditions of current climate change. Many authors discuss the factors that may have a significant impact on the effects of reintroduction or population strengthening (knowledge of the biology and ecology of the species, selection of appropriate starting plant material, selection of appropriate habitat, etc.) and describe how to measure this effect, taking into account not only the survival of individuals introduced into the environment but also their conditions and reproductive ability [13–19]. There are also opinions that phenomena related to global warming will require a change in the approach to nature conservation, necessitating the use of completely new and unconventional methods [20].

Poland is located in a warm, temperate, transitional climate. The average annual temperature ranges from 6 °C to 8 °C, while the annual precipitation ranges from 630 mm to 700 mm [21]. The analysis of data from meteorological surveys carried out since the 1960s shows a significant increase in the number of hot days, the duration of heat waves, and the number of tropical nights. An increase in the annual average temperature has also been

observed. Climate models predict an increase of 1 °C in the mean annual temperature in the years 2021–2050 and as much as 2 °C to 4 °C in the years 2071–2100. More frequent periods of drought are also predicted, as well as more days with intense precipitation. Progressive climate change and the accompanying sudden atmospheric phenomena can have serious consequences and lead to several disruptions in the natural ecosystems [22,23].

There are approximately 30 species of willow in the vascular flora of Poland, of which the downy willow (*Salix lapponum* L.) is particularly noteworthy. The natural range of this species includes the Arctic Circle in northern Eurasia and the Scandinavian Peninsula. In Poland, Scotland, as well as in some mountainous regions of central Europe, the species occurs outside its geographical range and is considered a glacial relict [24]. Natural populations of this species have been declining drastically since the 1950s, as confirmed by research carried out in the Polesie Lubelskie region, where up to 80% of the individuals have disappeared [25]. In the Krkonoše Mountains (on both the Czech and Polish sides), 21 *Salix lapponum* sites were recorded, and the populations studied were relatively stable [26]. In Poland, the Czech Republic, Ukraine, and Lithuania, the downy willow has the status of threatened with extinction [24]. One of the reasons for the decline of *Salix lapponum* populations is the changes occurring in natural ecosystems. Increased anthropopressure and changes in water relations have contributed to the degradation of peatlands, with which the species is closely associated. Downy willow is a small shrub, growing up to 1 m in height, and is distinguished by its elliptical gray–green leaves, which are covered with dense tomentum. It is mainly found in peatlands, preferring sunny and partly shaded sites with an acidic substrate (pH 4–6), rich in organic matter [27,28].

Due to the threat of complete extinction of the species in its isolated locations outside the center of the continuous range, since the early 2000s, several studies have been conducted on the biology [29–31] and ecology of the species [25,32–36], which helped collect the necessary information to prepare a procedure and carry out the first active protection measure for the species [37–39]. During their implementation, further questions arose, primarily concerning the prospects and conditions for the success of the species translocation in the context of global climate warming. Therefore, it has become important to check whether plants developed under ex situ conditions can adequately respond physiologically to environmental stresses, especially at early stages of growth. Interference in the resources of the populations of species threatened with extinction, e.g., collecting fragments of plant material, its propagation, and ex situ cultivation, requires the use of methods that eliminate the loss of valuable plant material both in the laboratory and after planting in natural sites [40,41]. This is extremely important due to the fact that these species exist in small and often dispersed populations.

In 2023, experiments were carried out to assess the condition of *Salix lapponum* individuals and their physiological response to short-term thermal stress during plant acclimatization in laboratory conditions. To verify this, controlled conditions were used to simulate phenomena that intensify in the natural environment due to climate change, i.e., sudden, short-term, and extreme decrease or increase in ambient temperature, which may affect the plants reintroduced to their natural sites.

2. Materials and Methods

2.1. Plant Material and Experimental Design

The plant material used in the present experiment was *Salix lapponum* plantlets obtained by micropropagation from vegetative parts of mother plants growing in natural populations in eastern Poland [18].

A total of 300 plantlets were transferred from in vitro conditions into soil substrate (peat–deacidified peat–sand–perlite 1:1:1:1 v/v) and placed in a phytotron, where they

further grew under the same conditions of temperature (day/night 22 °C/20 °C), humidity (60%), and light (35 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with the 16/8 h light/dark cycle).

After 1 week (Term 1), 5 weeks (Term 2), or 9 weeks (Term 3), 90 plants were selected randomly and placed for 12 h in a climatic chamber (MLR-352H-PE, PHCbi, PHC Europe, Breda, The Netherlands) under conditions of constant humidity (75%) and light intensity (53 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) but at different temperatures. Thirty plants were placed at a temperature of 0 °C (Variant 1) and thirty plants at 30 °C (Variant 2). The control group comprised 30 plants exposed to a temperature of 22 °C (Variant 3). Therefore, there were 9 different combinations of periods of growing plants in substrate (called terms) and temperature.

Immediately after 12 h, the plant material was subjected to laboratory analyses to determine the values of selected morphophysiological indicators and to assess the condition and response of the plants to thermal stress (Figure 1).

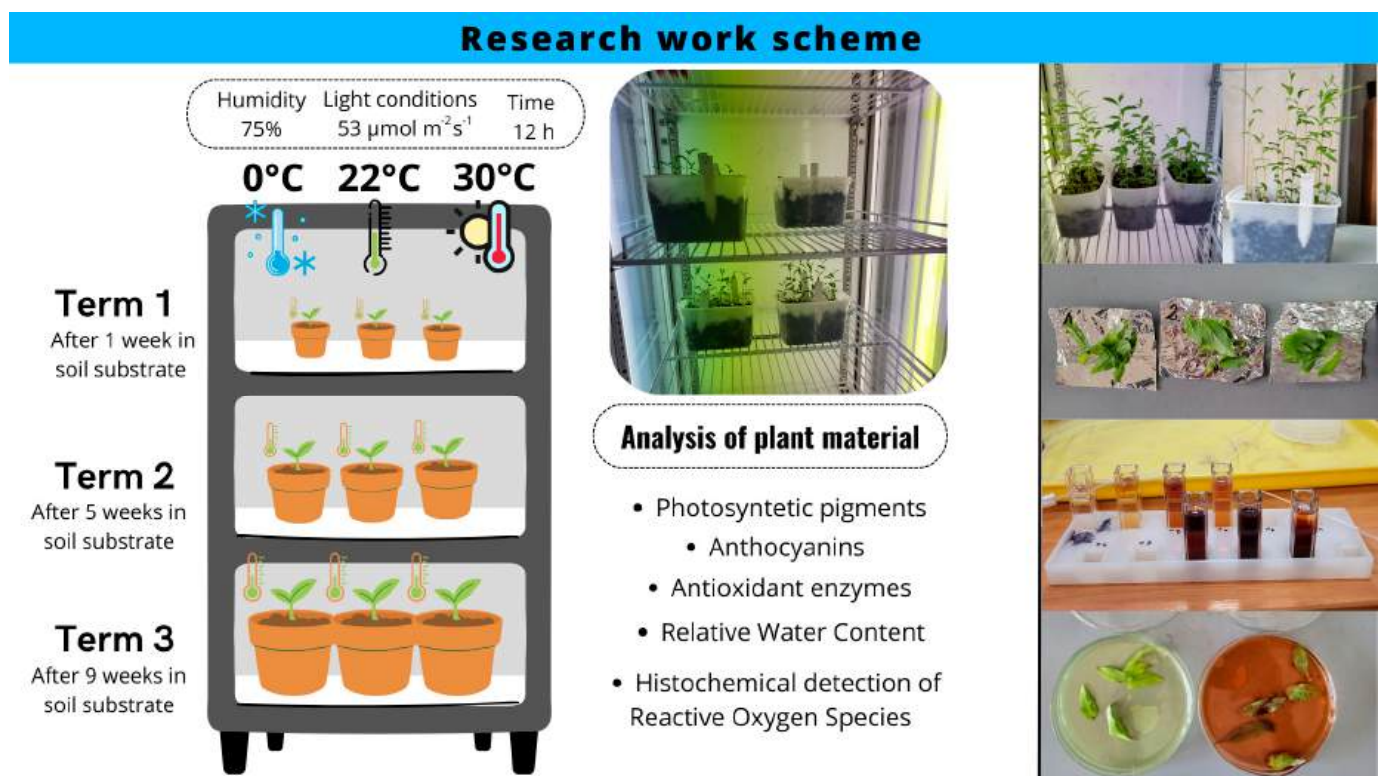


Figure 1. Study scheme, including timing, conditions of experiments, and analysis of plant material.

2.2. Determination of the Photosynthetic Pigment Content

The content of photosynthetic pigments, such as chlorophyll a, chlorophyll b, and carotenoids, was determined in fresh leaves collected from the middle part of the shoots from each combination. For the extraction of photosynthetic pigments, the leaves were homogenized in a 96% ethanol solution and then transferred to test tubes. The samples were placed in a water bath at 70 °C for 5 min and centrifuged (5 min, 10,000 rpm) to separate the liquid and solid phases. The absorbance of the obtained extracts was measured using a spectrophotometer (SPECORD 40, Analytik Jena, GmbH, Jena, Germany) at wavelengths of 470, 649, and 665 nm. The concentrations of the assimilatory pigments were calculated using a modified method proposed by Lichtenthaler and Wellburn [42].

2.3. Determination of the Anthocyanin Content

The anthocyanin content in the plant material was determined using the method described by Martinez and Favret [43]. An amount of 100 mg of leaves from each combination

were cut into pieces and placed in test tubes containing methanol and HCl (in a 99:1 ratio). Then, the samples were stored in a refrigerator at 4 °C for 24 h. After the extraction process, the mixture was centrifuged (5 min, 10,000 rpm), and the obtained supernatant was used for spectrophotometric analyses. The absorbance of the extracts was measured at wavelengths of 527 and 652 nm.

2.4. Oxidative Stress Indicators

2.4.1. Antioxidant Enzyme Activity

Leaf samples were collected from the middle part of the shoots. An amount of 100 mg of leaves from each combination was homogenized in 1 mL of 0.05 M phosphate buffer at pH 7.0 to determine the activity of guaiacol peroxidase (GPOX, EC 1.11.1.7) and catalase (CAT, EC 1.11.1.6). The prepared homogenates were centrifuged (10 min, 10,000 rpm, 4 °C). Enzyme activities were determined spectrophotometrically using a SPECORD 40 spectrophotometer (Analytik Jena GmbH, Jena, Germany). Catalase activity was measured at a wavelength of 240 nm, following the method described by Aebi [44]. For GPOX activity, the procedure of Małoplesza and Urbanek [45] was applied, with absorbance measured at 480 nm over 4 min at 1 min intervals.

2.4.2. Histochemical Detection of Reactive Oxygen Species (ROS)

The accumulation of hydrogen peroxide (H_2O_2) and superoxide anion (O_2^-) in the leaf tissues of *Salix lapponum* was assessed using the qualitative method described by Kumar et al. [46]. Leaves were collected from the middle part of the shoots. H_2O_2 detection was performed using 3,3'-diaminobenzidine (DAB). The leaves were immersed in a DAB solution (1 mg/mL) for 10–12 h and then incubated in darkness at room temperature. After incubation, the leaves were briefly treated with boiling water, followed by hot 96% ethanol to remove chlorophyll from the tissues and reveal the brown polymerization product of DAB formed in the presence of H_2O_2 .

To visualize the superoxide anion (O_2^-), nitroblue tetrazolium chloride (NBT) was used. The oxidized form of NBT (yellow) is reduced in the presence of endogenous O_2^- to form blue–purple diformazan. Leaves were incubated in a 0.2% NBT solution in 50 mM phosphate buffer (pH 7.5) for 10–12 h in darkness. Subsequently, the tissues were decolorized in hot 80% ethanol until the blue–purple reduction product of NBT became distinctly visible.

The above-described analyses for ROS detection were performed only on *Salix lapponum* Term 3 plants (grown in soil substrate for 9 weeks). Those analyses could not be conducted on plants at earlier growth stages (Terms 1 and 2) due to the small surface area and delicate structure of their leaves.

2.5. Determination of Relative Water Content

The relative water content (RWC) in leaf tissues was determined according to the method described by González and González-Vilar [47]. Whole leaves were collected immediately after the treatment to measure the fresh weight (FW). They were placed in distilled water for 24 h to achieve full turgor. After that, the leaves were weighed, with excess surface water gently removed using blotting paper, to determine the turgor weight (TW).

In the final step, the leaves were placed in a drying oven (SML 32/250, Zalmed, Warsaw, Poland) at a temperature of 60 °C for 24 h and then weighed to determine the dry weight (DW). The relative water content was calculated using the following formula:

$$\text{RWC} = (\text{FW} - \text{DW}) / (\text{TW} - \text{DW}) \times 100\%$$

2.6. Statistical Analysis

Statistical analysis was performed for the photosynthetic pigments, anthocyanins, RWC, and antioxidant enzymes using two-way analysis of variance followed by Tukey's multiple comparisons. All significant values are reported at $p < 0.05$. Principal Component Analysis (PCA) was employed to describe the structure of the data and identify the main factors differentiating the analyzed parameters. All computations were carried out in R environment, version 4.3.3 [48].

3. Results

3.1. Photosynthetic Pigment Content

The content of photosynthetic pigments (chlorophyll a and b) in the leaves of the analyzed *Salix lapponum* plants did not differ statistically between the experimental variants on any of the three experimental terms. However, significant changes were observed in the content of total photosynthetic pigments in the plant materials between Term 1 and Term 2 and between Term 1 and Term 3 (Figure 2a,b). The analysis of the content of carotenoids in *Salix lapponum* tissues also revealed no significant statistical differences between their concentrations in plants exposed to temperature extremes for 12 h within each of the three experimental terms. The total carotenoid content of the plant material was statistically different between Terms 2 and 3 and between Term 1 and Term 3 (Figure 2c). The mean values of the photosynthetic pigments are presented in Supplementary Materials (Table S1).

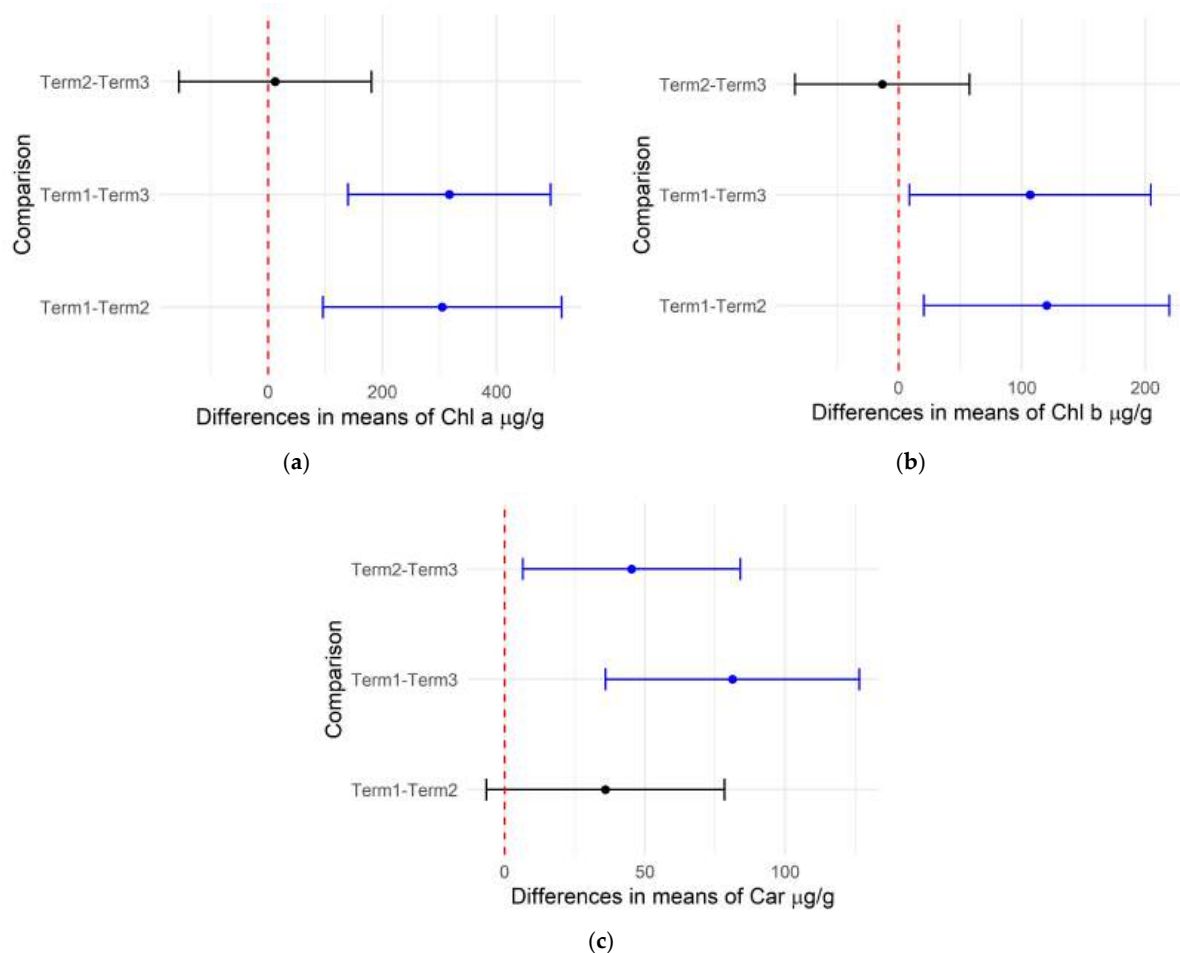


Figure 2. Differences in mean photosynthetic pigment contents: chlorophyll a (a), chlorophyll b (b), and carotenoids (c) in *Salix lapponum* tissues across three growth terms. Statistically significant differences ($p < 0.05$) are highlighted in blue, and non-significant differences are shown in black.

3.2. Content of Anthocyanins

In the case of plants grown in soil substrate for 1 week (Term 1), higher anthocyanin levels were observed after 12 h of exposure to 0 °C compared to the control sample maintained at 22 °C. For plants exposed to 30 °C, significantly lower anthocyanin concentrations were noted in plant tissues, both compared to the control sample and to plants exposed to low temperatures. For plants cultivated in the phytotron for 5 weeks, statistically significant differences were observed between anthocyanin content in plants exposed to 0 °C and 30 °C. Concerning the control sample, the plants reached lower values for this indicator after 12 h at low temperature, while high temperature resulted in an increase in the anthocyanin content in the tissues of the tested specimens. A similar trend to that of plants from Term 2 was demonstrated by plants after 9 weeks of growth (Term 3), except that in this case, there were no significant differences between the anthocyanin content of the plants exposed to the temperature extremes (Figure 3). The mean values of the anthocyanin content in leaves are presented in Supplementary Materials (Table S1).

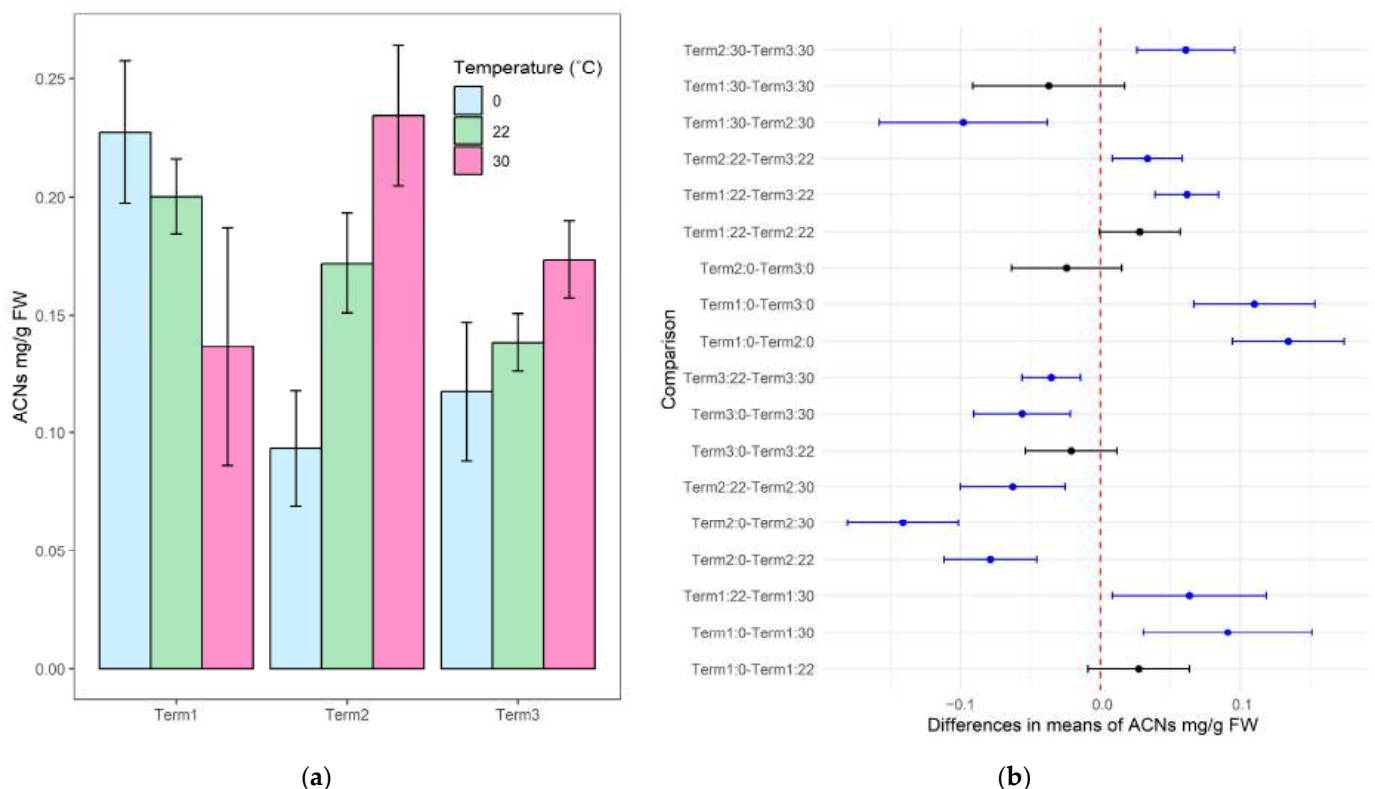


Figure 3. Anthocyanin content (ACNs) in the tissues of *Salix lapponum* exposed to different temperature extremes across three growth stages (Terms 1–3). Data are presented as mean $\pm$ standard deviation with a sample size of $n = 4$ (a). Pairwise comparison of ACNs between experimental conditions for *Salix lapponum*, showing statistically significant differences between term-temperature treatments across growth stages. Blue points indicate significant differences ($p < 0.05$), and black points indicate non-significant differences (b).

3.3. Antioxidant Enzyme Activity

The results of the guaiacol peroxidase activity analyses in plants grown for 1 week in the soil substrate indicated a significant increase in the content of that enzyme after 12 h of exposure to low temperature in comparison to the control (22 °C), in which the enzyme remained virtually inactive (Figure 4a). High temperature exposure resulted in a slight increase in guaiacol peroxidase activity. Experiments conducted on plants grown for 5 weeks revealed that high temperature induced a significant increase in peroxidase

activity. The highest enzymatic activity of guaiacol peroxidase was observed in the last term of the study. Both low- and high-temperature treatments caused an increase in its activity. However, it was not significantly higher compared to the control sample at that experimental term. Comparing with the first and second test terms, the enzymatic activity of peroxidase in the third term was statistically higher in each experimental variant tested (Figure 4b). Mean values for the guaiacol peroxidase activity are presented in Supplementary Materials (Table S1).

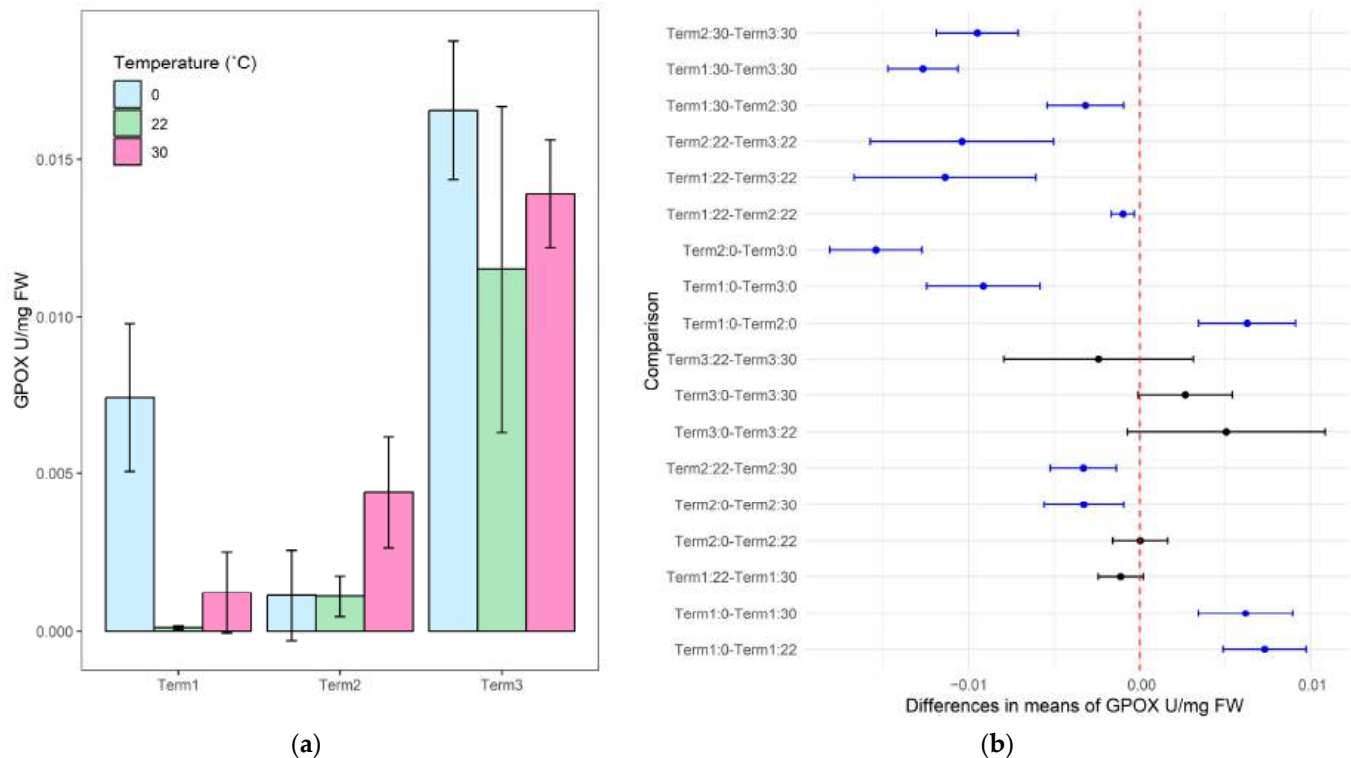


Figure 4. Guaiacol peroxidase activity (GPOX) in the tissues of *Salix lapponum* exposed to different temperature extremes across three growth stages (Terms 1–3). Data are presented as mean $\pm$ standard deviation with a sample size of $n = 4$ (a). Pairwise comparison of GPOX between experimental conditions for *Salix lapponum*, showing statistically significant differences between term-temperature treatments across growth stages. Blue points indicate significant differences ($p < 0.05$), and black points indicate non-significant differences (b).

Catalase activity was not detected in any of the combinations during the three study dates.

3.4. Histochemical Detection of Reactive Oxygen Species

Salix lapponum leaf tissues were subjected to histochemical detection of hydrogen peroxide and superoxide anion after nine weeks of growth in soil substrate. Plants in response to temperature extremes (0 °C and 30 °C) showed differential accumulation of reactive oxygen species (Figure 5). Plants exposed to low temperatures (0 °C) revealed only trace amounts of H_2O_2 staining product visible on the leaves. In the control sample (22 °C), the staining intensity of the plant material was moderate, with a brown staining product appearing on the entire leaf surface. When the plants were exposed to high temperatures (30 °C), the leaf staining was the strongest, and it was visible on most of the surface of the tested leaves. Visualization using the NBT reagent indicated an increased superoxide anion content after exposure of plants to 30 °C, which was evident by the intense coloring of the entire leaf blade surface. In the low-temperature treatment and control, the dark color,

which is a product of the reaction, indicates the accumulation of superoxide anion only within the petioles.

Temperature	$O_2^{\bullet-}$	H_2O_2
0°C		
22°C		
30°C		

Figure 5. Visualization of reactive oxygen species (ROS) in *Salix lapponum* tissues exposed to different temperatures in the third term of the experiment.

3.5. Relative Water Content (RWC)

The relative water content of the tissues of *Salix lapponum* plants that had been exposed to temperature extremes for 12 h was investigated. Regarding Term 1, there was an increase in the RWC after both 0 °C and 30 °C exposure compared to the control (22 °C), although those differences were not statistically significant. While conducting tests on plants growing for 5 weeks in soil, the relative water content was high in all combinations, but the changes induced by both low and high temperatures compared to the control sample were minimal. Only after 9 weeks of growth in soil substrate, there was a significant decrease in RWC in tissues of plants exposed to low temperature, compared to the control sample. A significant increase in the relative water content was also found between Term 1 and Term 3 after exposure to 30 °C, between Term 1 and Term 2 after exposure to 0 °C, and between Term 1 and Terms 2 and 3 after exposure to 22 °C. In Term 2, a significantly higher RWC value was recorded at low temperature compared to Terms 1 and 3 (Figure 6). Mean values of the RWC content are presented in Supplementary Materials (Table S1).

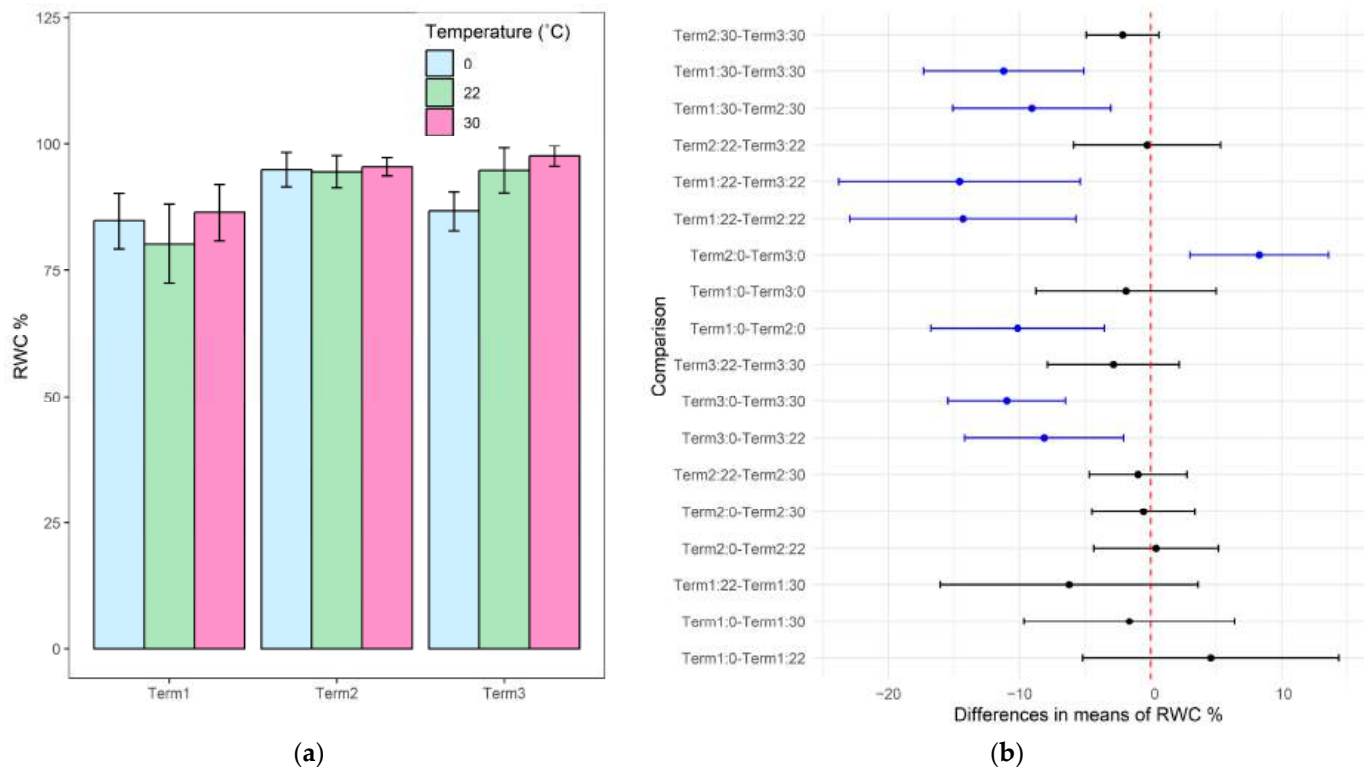


Figure 6. Relative water content (RWC) in the tissues of *Salix lapponum* exposed to different temperature extremes across three growth stages (Terms 1–3). Data are presented as mean $\pm$ standard deviation, with a sample size of $n = 4$ (a). Pairwise comparison of RWC between experimental conditions for *Salix lapponum*, showing statistically significant differences between term-temperature treatments across growth stages. Blue points indicate significant differences ($p < 0.05$), and black points indicate non-significant differences (b).

3.6. Principal Component Analysis (PCA)

Principal component analysis (PCA) was used to determine the relationship between the studied indicators of the physiological response of *Salix lapponum* to temperature extremes. All variants of the experiment carried out were included; these included the following parameters: Chl a, Chl b, Car, ACNs, GPOX, and RWC. The first three components explain 91.2% of the total variation in the data. The first component, PC1, explains 52.3% of the total variance; the second, PC2, 20.3%; and the third, PC3, 18.6%. Since the two components together explain 72.6% of the total variance, it can be concluded that the PCA plot reflects the variability of the data well and that the two components are suitable for understanding the main patterns of plant responses to heat stress simulated in the experiments. The first axis, PC1, is strongly associated with Chl a, Chl b, and carotenoids, suggesting that these variables have the greatest influence on the differences in the data. The second axis, PC2, is related to GPOX and RWC. The third axis, PC3, is associated with ACNs, suggesting that this variable also has a significant, although smaller, effect on the differences in the data.

We observe a very strong positive correlation for Chl a, Chl b, and Car, a weaker but still positive correlation between this group of variables and GPOX, and a negative correlation between GPOX and ACNs. It can also be noted that the correlation between RWC and both GPOX and ACNs is close to zero.

The spatial distribution of the analyzed experimental cases (Term) in the PCA plot provides further insight into the physiological responses of *Salix lapponum* to temperature extremes. Plants exposed to low temperatures (0 °C, such as Term1_0 and Term3_0) showed higher than average levels of Chl a, Chl b, Car, and GPOX. These results suggest that these

parameters play a key role in plant adaptation to cold stress. In contrast, plants exposed to higher temperatures (30 °C, e.g., Term1_30 and Term3_30) showed higher average RWC and ACN values. This indicates a change in the physiological priorities of the plant under heat stress, with increased water content (RWC) and anthocyanin production (ACNs) potentially contributing to the tolerance to higher temperatures (Figure 7).

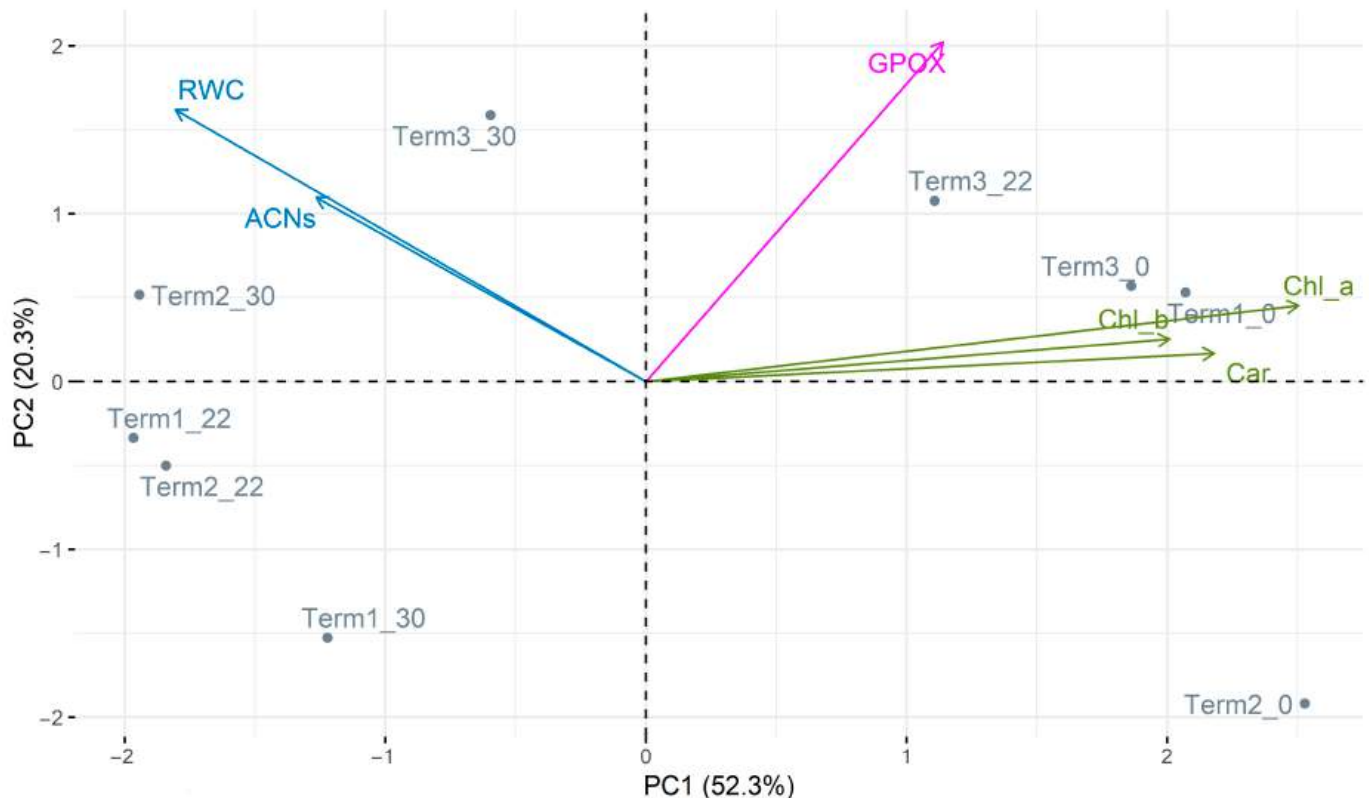


Figure 7. Results of the principal component analysis (PCA) of selected parameters of *Salix lapponum*'s physiological response to temperature extremes (ACNs—anthocyanins, GPOX—guaiacol peroxidase, Chl a—chlorophyll a, Chl b—chlorophyll b, Car—carotenoids, RWC—Relative Water Content).

4. Discussion

Thermal stress associated with global warming and heat waves is one of the main factors limiting crop production, leading to reduced plant biomass. In response to unfavorable environmental conditions, plants have developed several defense mechanisms to protect themselves from the effects of stress [6,9]. There is a wide range of biochemical and physiological indicators of plant condition that can be monitored in the context of response to environmental stresses, which allows for the assessment of plant adaptive strategies at different stages of growth and development [49].

Photosynthetic pigments are sensitive to high temperature stress, which can lead to their degradation and reduced photosynthetic efficiency [50]. Under the influence of thermal stress caused by high temperatures, chloroplasts undergo serious changes leading to structural damage [51]. In the current study conducted on *Salix lapponum*, no significant changes in chlorophyll content were observed after 12 h exposure to temperatures of 30 °C and 0 °C. A short exposure to elevated temperatures may not have been sufficient to induce visible changes in the levels of photosynthetic pigments in the plant tissues, as in the study conducted by Wassie et al. [52] on alfalfa (*Medicago sativa* L.), it was shown that after a 7-day exposure to high temperatures, the content of photosynthetic pigments decreased significantly, suggesting a strong stress response in that species. Furthermore, in the study conducted on *Salix lapponum*, the only significant changes observed were the differences in

pigment content between subsequent study dates, which most likely reflect natural changes related to the plant's development and not a response to short-term thermal stress.

Anthocyanins play an important antioxidant function and have a crucial role in scavenging reactive oxygen species, protecting plants from a wide range of damages caused by environmental stress [53]. Studies conducted on *Fagopyrum tataricum* showed an increase in anthocyanin synthesis in response to low-temperature stress. A positive correlation was also demonstrated between the antioxidant capacity and anthocyanin accumulation in *Fagopyrum tataricum* tissues, which potentially protected sprouts from low temperatures [54]. The significant role of anthocyanins in neutralizing ROS and acclimatization to low temperatures may be evidenced by the fact that plants deprived of these compounds show a higher level of reactive oxygen species accumulation in tissues under oxidative stress induced by low temperature [55,56]. The protective role of anthocyanins was also demonstrated in the case of high-temperature stress. Studies conducted in *Arabidopsis* showed severe damage to cell membranes in anthocyanin-deficient individuals, while in wild-type individuals, the damage was much lower [57]. With regard to the experiments presented, *Salix lapponum* plantlets demonstrated similar responses to the stress induced by temperature extremes. Plants exposed to extreme temperatures at the initial stage of growth in the phytotron (Term 1) contained significantly more anthocyanins when exposed to low temperatures compared to the control (22 °C) or increased temperatures (30 °C), which indicated the intensification response to stress in low temperature conditions [58]. The opposite trend in the second and third terms of the experiment may indicate the activation of defense mechanisms in response to heat stress [57]. In the last term of the research, after 9 weeks of plant growth in the soil substrate, the fluctuations in the anthocyanin levels concerning the control samples were lower than at the earlier stages of growth, which might indicate that in this particular phase of growth, the plant has achieved a certain level of adaptation to heat and cold stress. This adaptation may also be the result of other protective mechanisms that were activated over time. This may be indicated by a significant increase in guaiacol peroxidase activity after the exposure of downy willow plants to both low and high temperatures in the last term of the study (Term 3).

In addition to non-enzymatic antioxidants, which include, among others, anthocyanins, specialized antioxidant enzymes play an important role in protecting the plants from the adverse effects of stress. These enzymes contribute to maintaining plant homeostasis by reducing excess ROS [59]. In the case of plants from the in vitro cultures, lower levels of antioxidant enzymes, such as catalase and peroxidase, are observed due to constant, controlled growth conditions. Unlike the natural environment, where plants are exposed to variable abiotic stresses, phytotron conditions are stable and devoid of stress factors, such as temperature fluctuations or variable humidity, which can naturally stimulate the activity of defense mechanisms. Consequently, the lack of environmental stressors leads to lower activation of antioxidant enzymes in the in vitro cultures [60,61]. This may be reflected in the results of guaiacol peroxidase activity in downy willow tissues exposed to extreme temperatures obtained in Terms 1 and 2 of the experiment. Despite statistically significant differences in the GPOX activity (compared to the control sample), in the case of low temperature in Term 1 and high temperature in Term 2, its content in *S. lapponum* tissues was relatively low. In the conducted experiment, no catalase activity was detected in response to thermal stress. However, this enzyme is widely used as an indicator of antioxidant responses in studies on the response of plants to abiotic stress because it plays a key role in neutralizing hydrogen peroxide, one of the main reactive oxygen species formed during stress. Numerous reports suggest that the presence and activity of catalase can be regulated by other peroxidases, such as ascorbate peroxidase and guaiacol peroxidase,

which may limit or change its activity depending on the type of stress and the specific need of the plant for hydrogen peroxide detoxification [6,62,63].

Considering that the histochemical method of detecting hydrogen peroxide and superoxide anion is a qualitative technique that does not provide detailed quantitative data on the concentration of these reactive oxygen species [64] and the fact that in the study on *Salix lapponum* individuals presented in this article, it was possible to visualize ROS only in the third experimental term, the presented images should be regarded as a supplementary, indicative tool. The analyses of ROS accumulation in *Salix lapponum* tissues were also carried out in a separate study by Pogorzelec et al. [38], which aimed at assessing the condition of the plant in different stages of acclimatization. Significant differences in the intensity of leaf color were found depending on the stage of acclimatization, from laboratory conditions to the harsh conditions of natural ecosystems. In laboratory conditions where environmental stress was absent, no clear ROS staining products were observed. However, when the plants were transferred to a field acclimatization station, where they were exposed to rapid changes in environmental conditions, intense staining indicated a high level of oxidative stress. In the studies described in this article, the most pronounced staining was observed as a result of exposing the plants to high temperatures. However, short-term stress under controlled phytotron conditions did not cause such a pronounced accumulation of ROS as seen in the long-term exposure to various stress factors during acclimatization in a field station [38].

Relative water content (RWC) is a commonly used indicator reflecting hydration and general plant condition, especially important in abiotic stress, such as extreme temperatures or drought [65]. The results of the experiment conducted on *Salix lapponum* plants reveal a tendency of the plants to increase the RWC in response to high temperatures in all experimental terms, especially in Term 3, where RWC reached almost 98%, indicating the ability of the tested plants to retain water under high temperature conditions. The decrease in RWC in *S. lapponum* tissues in the last term after exposure to 0 °C compared to the control suggests a reduced capacity for water retention in older plants under cold stress [66,67]. However, the consistently high RWC values throughout the experiment indicate the good physiological condition of the plants, and the observed differences in the RWC values may come from individual traits.

To sum up, the results of the conducted research demonstrated that *Salix lapponum* individuals exhibit different physiological reactions to thermal stress depending on the duration of their growth in soil substrate. In general, plants at early growth stages responded to stress induced by low temperatures, while older plants were more sensitive to high temperatures, as evidenced by higher anthocyanin levels and increased activity of guaiacol peroxidase. Short-term exposure to extreme temperatures did change the content of chlorophyll a and b in plant tissues. It was found that catalase activity is a non-indicative marker of stress in the case of the species studied in the conducted experiments.

5. Conclusions

The obtained results presented suggest that *S. lapponum* plants from ex situ cultivation can survive periods of short-term thermal stress because they are capable of activating a number of physiological defense mechanisms. The studied indicators of plant response to stress (e.g., anthocyanins, guaiacol peroxidase, or RWC) can be used to monitor the plant condition throughout the entire process of their translocation. However, it is still unclear how the frequent occurrence of such stress factors will affect the plants' condition and acclimation capacity. Undoubtedly, there is a need for continued research that will take into account longer periods of exposure to extreme temperatures, as well as the impact of low and high temperature fluctuations on the plants of *S. lapponum*.

The conservation of endangered plant species, such as *S. lapponum*, in the face of global warming and climate change will soon need to rely not only on standard active conservation measures but also on a thorough understanding of the plant physiological response patterns under stress conditions. Such knowledge will enable the modeling and optimizing of methods using species translocations, taking into account possible scenarios of changes occurring in the substitute habitats.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/biology14010019/s1>: Table S1: Mean values of the tested parameters of the physiological response of *Salix lapponum*.

Author Contributions: Conceptualization, M.A. and M.P. (Magdalena Pogorzelec); methodology, M.A., M.P. (Magdalena Pogorzelec) and M.P. (Marzena Parzymies); validation, M.A., U.B.-M. and T.M.; formal analysis, M.A., M.P. (Magdalena Pogorzelec) and U.B.-M.; investigation, M.A. and M.P. (Magdalena Pogorzelec); resources, M.A., M.P. (Magdalena Pogorzelec) and M.P. (Marzena Parzymies); data curation, M.A.; writing—original draft preparation, M.A.; writing—review and editing, M.A., M.P. (Magdalena Pogorzelec), M.P. (Marzena Parzymies) and T.M.; visualization, M.A. and U.B.-M.; supervision, M.A. All authors have read and agreed to the published version of the manuscript.

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Article

Eutrophication and Salinization as Risk Factors in the Translocation Process of an Endangered Glacial Relict Species

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Abstract

Species translocation is an increasingly used method in active plant conservation, but its high costs and risk of failure highlight the need for prior research to support its effectiveness. *Salix lapponum* plantlets obtained through micropropagation were subjected to two biological experiments under laboratory conditions. The plants were watered with aqueous solutions of NaCl (Experiment 1) and N-NO₃ (Experiment 2) for a period of four weeks. The experiments were designed to simulate processes occurring in the natural habitats of the species- increased substrate salinity and eutrophication. To determine the plant response to the presence of NaCl and N-NO₃ in the soil substrate, various morpho-physiological traits were examined, including selected growth parameters, relative water content (RWC), photosynthetic pigment content, selected chlorophyll fluorescence parameters, reactive oxygen species (ROS) accumulation, antioxidant enzyme activity, and anthocyanin content. The results showed that both tested factors acted as abiotic stressors. Exposure to NaCl solutions of various concentrations led to a significant deterioration in morpho-physiological parameters, whereas low concentrations of nitrate nitrogen stimulated the growth of *S. lapponum*. In response to stress, the plants activated defense mechanisms such as increased anthocyanin synthesis, elevated antioxidant enzyme activity, and maintenance of a high relative water content.

Keywords: *Salix lapponum*; downy willow; biodiversity conservation; abiotic stress; salt stress



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1. Introduction

The condition of the flora of natural wetlands is affected by a multitude of stress factors. Of particular importance is the process of eutrophication, which is caused by the excessive influx of biogenic elements that primarily originate from surface runoff from intensively fertilized agricultural areas, municipal wastewater, and industrial waste, especially detergents containing phosphates [1,2]. The phenomenon of eutrophication is a multifactorial process, the course of which is influenced by temperature or groundwater levels, among other factors, and can result in habitat disturbances of varying severity and nature [3,4].

Peatlands play a pivotal role in the environment by mitigating the repercussions of excessive nutrient inputs. Operating as biological filters, peatlands effectively retain and accumulate substantial quantities of nitrogen and phosphorus [5]. This phenomenon can be attributed to the presence of humic compounds formed during the decomposition of organic matter, which form permanent complexes with nitrogen and phosphorus compounds, thereby limiting their availability to plants [6,7]. Peatlands are among the most threatened habitats on a global scale today [8]. The degradation of their ecosystems is the result of a number of factors, including, among others, intentional anthropogenic transformations as well as progressive climate change, which has the strongest impact on the hydrological conditions of these ecosystems [9]. Degradation of peatlands through drainage has the potential to exacerbate the process of eutrophication by releasing bound nutrients [10].

Swindles et al. [11] reported that over the past 300 years, about 70% of the studied European peatlands have shifted towards drainage. Restoration efforts, including re-wetting, have not fully returned them to their original state, leaving them less self-regulating and more vulnerable to extreme weather. Rewetted peatlands also show altered species composition, with reduced biodiversity, helophytization, and dominance of tall grasses that outcompete low-growing species [12].

Peatland drying can also have other negative consequences. The ongoing desiccation of peatland decreases hydrostatic pressure, thereby enabling saline groundwater (secondary salinity resulting from crop irrigation, industrial activities, mining, and de-icing salts) to infiltrate the drying ground. This process precipitates accelerated salinization, which can have deleterious effects on the peatland's capacity to retain water and accumulate organic matter [13]. The presence of salts in soil water can impede the capacity of plants to absorb water, thereby resulting in diminished growth rates. Salinity can also induce impairment to the cells of the photosynthetic apparatus. Salinity exerts two primary effects on plants: osmotic stress and ionic toxicity. These effects impact the vital processes of germination, growth, photosynthesis, osmotic balance, and yield [14,15]. There are numerous reports in the literature on the effects of salinity on plants. However, the majority of these studies focus on economically significant crops [16–20] or plants typical for saline habitats [21,22]. However, there is little information regarding the effects of salinity on plant functioning in natural ecosystems.

Given the degradation of wetlands caused by climate change and human activity, the survival and adaptation of plant species with specific requirements for new, different habitat conditions are in question. Another interesting issue is the rate and effectiveness with which these plants will be able to acclimatize to changes in trophic status or non-natural levels of soil salinity. A more profound comprehension of the manner in which plants react to environmental factors that impede their growth and diminish their physiological state may prove to be of considerable benefit in the development of effective conservation methods for particularly vulnerable and endangered species. Consequently, this enhanced understanding may result in improved outcomes [23,24].

In the context of the flora of European wetlands, there is a need to incorporate relics in various forms of active conservation. These species frequently exhibit limited distribution patterns, often occupying small, isolated populations that fall outside the typical range of their dense occurrence. The presence of plant glacial relicts, defined as remnants of glaciation periods, has been observed at elevated geographic altitudes and within wetland environments. These locations are particularly vulnerable to the progressive climate change and increased habitat fragmentation, which substantially elevate the risk of their extinction [25,26].

A notable example of a glacial relic in eastern Poland that needs prompt conservation efforts is *Salix lapponum*. The plant is a small shrub that typically reaches a height of ap-

proximately one meter. It occurs mainly in wetland habitats, including low and transitional bogs. The optimal growth conditions for this species include acidic soil (pH 4–6), abundant organic matter, and sufficient sunlight or partial shade. *S. lapponum* is characterized by elliptical, gray-green leaves, densely covered with tomentum [27,28]. Downy willow is a subarctic-boreal species with a continuous distribution range extending across northeastern Europe and Siberia. In Poland, as in the British Isles, the Sudetes, the Pyrenees, or the mountains of Bulgaria, it occurs in isolated sites that are relics from glacial periods [29]. The majority of the over 60 populations that were previously documented in Poland have become extinct, primarily as a result of alterations in habitat conditions, particularly the drying of wetlands [30]. Presently, *S. lapponum* is classified as a threatened or critically endangered plant in Poland, Ukraine, the Czech Republic, and Lithuania [29].

In order to ensure the viability of *S. lapponum* populations at the periphery of the species' geographic distribution, research has been conducted for many years to elucidate the biological [31–34] and ecological [35–39] mechanisms that govern their proper functioning. Active protection measures consisting of reintroduction and re-stocking of small populations of *S. lapponum* in eastern Poland are also being undertaken [40–42]. The accelerating changes in habitat conditions that are observed in the natural and replacement sites of the species in relation to the high cost of conducting species translocations generate questions about the rationale for undertaking such actions. It is imperative to determine whether the introduced individuals possess the capacity to acclimatize and, in the long term, adapt and survive in the transformed and even degraded habitat.

In order to assess the effectiveness of active conservation methods for *Salix lapponum* based on translocation, a series of experiments was conducted under controlled laboratory conditions. This study simulated changes in habitat conditions observed in the wild that may arise from anthropogenic pressure and climate change, such as progressive eutrophication and soil salinization. The aim was to determine the extent to which these factors could limit the growth and condition of *S. lapponum* individuals.

2. Materials and Methods

2.1. Plant Material

The plant material used for the experimental procedures was *Salix lapponum* plants obtained from the tissue culture laboratory through the micropropagation methods. The initial material comprised shoot fragments from individuals functioning in natural populations of the species in eastern Poland. Previously developed and optimized protocols [32] were the basis for obtaining 120 plants that were subjected to a biological experiment.

The plants were transferred from in vitro conditions into 1 dm³ containers filled with a mixture of peat (pH 3.5–4.5, Aura, Agaris Poland Sp. z o.o., Pasłęk, Poland), deacidified peat (pH 5.5–6.5, Aura, Agaris Poland Sp. z o.o., Pasłęk, Poland), washed river sand (local supplier), and perlite (Agro-Perlit 2–6 mm, Global Perlita, Warsaw, Poland) (1:1:1:1 v/v). There were 10 plants per container. The containers were placed in glass tanks and covered with plastic foil to keep the high humidity. After two weeks, the hardening process began with the gradual removal of the plastic foil. Four weeks later, plants were individually transferred into P9 pots (9 × 9 × 9 cm) filled with the same substrate mixture. During the whole cultivation and experimental period, plants were kept in a growing room, under controlled conditions, at a temperature of 22 °C during the day and 20 °C during the night, and a light intensity of 35 µmol·m⁻²·s⁻¹ with the 16/8 h light/dark cycle. The source of light was white daylight fluorescent lamps (Philips, Colorado Springs, CO, USA). After a 3-month growth period in the phytotron, the plants were divided into two groups of 60 individuals each.

2.2. Experimental Design

Two independent experiments were conducted to determine the effects of aqueous sodium chloride solution at different concentrations (Experiment 1) and varying doses of nitrate nitrogen (Experiment 2) on selected morpho-physiological parameters of *Salix lapponum* specimens. The two experiments involved the indirect application of NaCl and N-NO₃ solutions at three-day intervals for a period of four weeks. The solutions were prepared in advance and replenished in trays that contained pots with plants (15 plants per treatment in each experiment) (Table 1; Figure 1).

Table 1. Experimental variants with applied concentrations and electrical conductivity (EC) values for NaCl treatments.

Experiment 1		Experiment 2	
Variant		Variant	
NaCl20	20 mmol/L NaCl, EC = 3230 $\mu\text{S}/\text{cm}$	N1	1 mg/L N-NO ₃
NaCl40	40 mmol/L NaCl, EC = 4200 $\mu\text{S}/\text{cm}$	N10	10 mg/L N-NO ₃
NaCl80	80 mmol/L NaCl, EC < 8000 $\mu\text{S}/\text{cm}$	N100	100 mg/L N-NO ₃
C	Control (distilled water)	C	Control (distilled water)

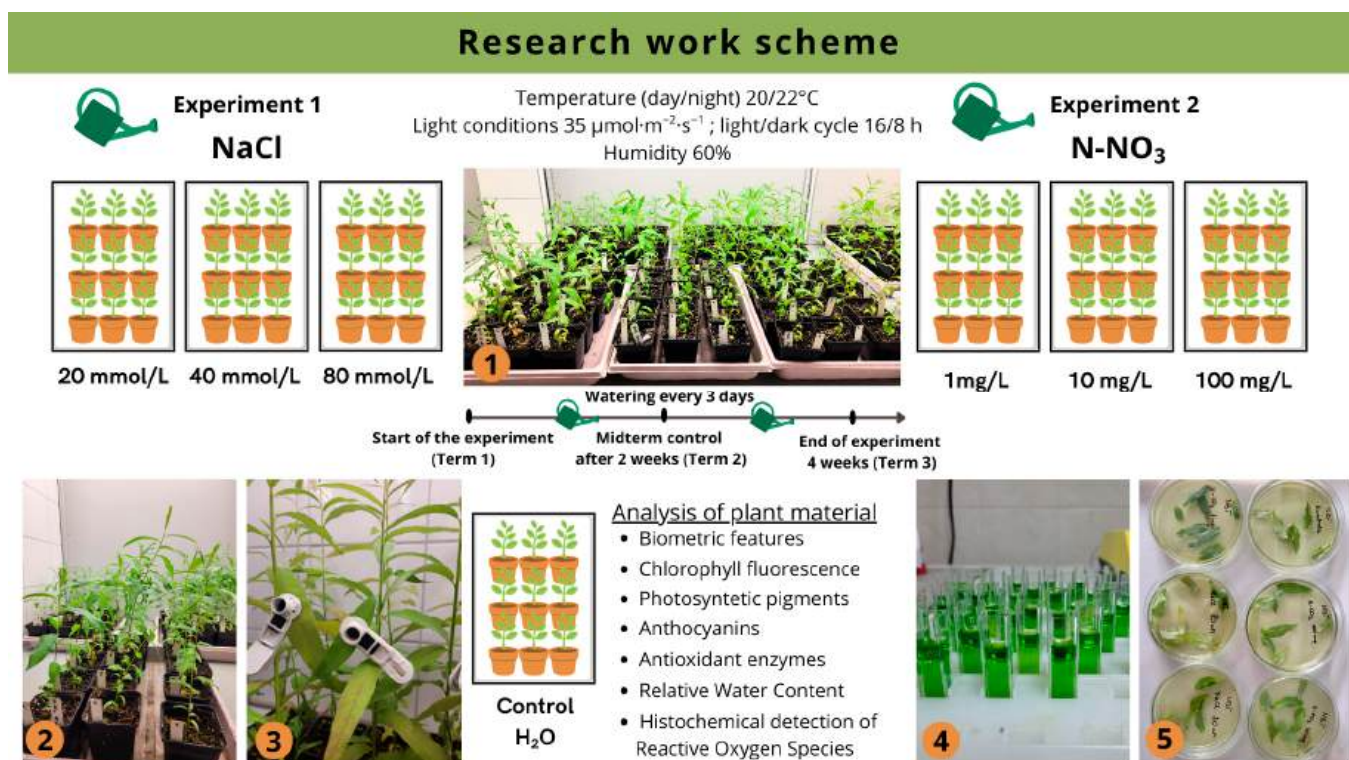


Figure 1. Study scheme, including timing, conditions of experiments, and analysis of plant material. 1,2—*Salix lapponum* specimens in the experimental setup; 3—measurement of chlorophyll fluorescence; 4—determination of assimilation pigment content; 5—histochemical detection of reactive oxygen species.

Diagnostic assessments were conducted three times during the course of the experiment: on the first day (Term 1), after two weeks (Term 2), and after four weeks

(Term 3), to determine selected morphophysiological traits of the plants exposed to the treatment solutions.

2.3. Analysis of Plant Material

2.3.1. Selected Biometric Features and Plant Growth

During the course of the experiment, selected biometric traits of the plants were measured, including the length of the main shoot (cm), the number of leaves, and the number of lateral shoots of each individual. The length of the main shoot was measured with a ruler with an accuracy of 1 mm, from the base of the stem to the apex. The mean daily growth rate of the primary shoot was calculated based on the data obtained. Biometric measurements were repeated on the same individuals within each variant of both experiments, at all experimental terms.

2.3.2. Relative Water Content

The relative water content (RWC) of leaf tissue was examined according to the method described by González and González-Vilar [43]. Plant material in the form of whole leaf blades was collected, their fresh weight (FW) was determined, and they were then immersed in distilled water for 24 h to reach maximum turgor. After that time, the leaves were subjected to a second weighing, during which their full turgor weight (TW) was determined. The final step was to place the leaves in a drying oven (SML 32/250, Zalmed, Warsaw, Poland) at 60 °C for 24 h. To determine dry weight (DW), the leaves were weighed prior to and following the drying process. The relative water content was calculated according to the following formula:

$$\text{RWC} = (\text{FW} - \text{DW}) / (\text{TW} - \text{DW}) \times 100\% \quad (1)$$

2.3.3. Photosynthetic Pigments Content

The content of photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoids) was measured in the fresh weight of leaves collected from the middle parts of the plant shoot. To extract the pigments, the leaves were homogenized in a 96% ethanol solution. Then, the samples were transferred to a 70 °C water bath for 5 min, followed by centrifugation (10,000 rpm, 10 min) to separate the solid phase from the liquid one. The degree of absorption of the extracts was measured using a spectrophotometer (SPECORD 40, Analytik Jena, GmbH, Jena, Germany) at wavelengths of 470, 649, and 665 nm. The calculation of dye concentration was executed by means of a modified Lichtenthaler and Welburn [44] method, as follows:

$$\text{Chl a} = (13.95 \times A_{665}) - (6.88 \times A_{649}) \quad (2)$$

$$\text{Chl b} = (24.96 \times A_{649}) - (8.12 \times A_{665}) \quad (3)$$

$$\text{Carotenoids} = ((1000 \times A_{470}) - (2.05 \times \text{Chl a}) - (114.8 \times \text{Chl b})) / 245 \quad (4)$$

2.3.4. Selected Chlorophyll a Fluorescence Parameters

Chlorophyll a fluorescence parameters, such as minimum fluorescence (F_0), maximum fluorescence (F_m), and maximum quantum yield of PS II (F_v/F_m), were measured on leaves located in the middle part of the shoot. The measurements were taken from 10 individuals who were randomly selected for this study. The measurement of chlorophyll fluorescence was conducted using a fluorimeter (OS5p+, Opti-Sciences, Hudson, NH, USA). Prior to measurement, the leaves were subjected to dark adaptation by blocking light access for 15 min. Measurements of selected chlorophyll fluorescence parameters were repeated on the same individuals within each variant of both experiments, across all experimental terms.

2.3.5. Anthocyanin Content

The anthocyanin content of the plant material was determined using the method described by Martinez and Favret [45]. To extract anthocyanins, 100 mg of cut leaf blade fragments were used (mixed sample), which were placed in tubes containing methanol and HCl (in a ratio of 99:1). The samples were placed in a refrigerator at 4 °C for 24 h. After that time, the samples were centrifuged (10,000 rpm, 10 min), and the resulting supernatant was subjected to spectrophotometric analyses. The absorbance of the samples was measured at wavelengths of 527 and 652 nm.

2.3.6. Guaiacol Peroxidase Activity

Leaf samples were obtained from the middle segment of the shoots. For the analysis of guaiacol peroxidase (GPOX, EC 1.11.1.7) activity, 100 mg of leaves (mixed sample) were used, which were homogenized in 1 mL of 0.05 M phosphate buffer, pH 7.0. Subsequently, the resulting homogenates were centrifuged for 10 min (10,000 rpm) at 4 °C.

Enzyme activity was determined spectrophotometrically using a SPECORD 40 spectrophotometer (Analytik Jena GmbH, Jena, Germany). The procedure for determining GPOX activity was carried out according to the method described by Małolepsza and Urbanek [46]. Absorbance was measured at 480 nm for 4 min at 1-min intervals.

2.3.7. Histochemical Detection of Reactive Oxygen Species (ROS)

The accumulation of hydrogen peroxide (H_2O_2) and superoxide anion (O_2^-) in leaf tissues was assessed using a qualitative method described by Kumar et al. [47] after 2 and 4 weeks of the experiment. Leaves for analysis were collected from the middle part of the shoots.

The detection of hydrogen peroxide (H_2O_2) was carried out using 3,3'-diaminobenzidine (DAB). The leaves were immersed in a DAB solution (1 mg/mL) for 12 h and then incubated in the dark at room temperature. Following the incubation period, the leaves were briefly treated with hot water, then treated with hot 96% ethanol to remove chlorophyll and to facilitate the visualization of the brown polymerization product of DAB, formed in the presence of H_2O_2 .

Nitro blue tetrazolium chloride (NBT) was used to visualize the superoxide anion (O_2^-). The oxidized (yellow) form of NBT is reduced in the presence of endogenous O_2^- , forming a blue-purple diformazan. The leaves were incubated in a 0.2% solution of NBT in 50 mM phosphate buffer (pH 7.5) for 12 h in the dark. The tissues were then decolorized with hot 96% ethanol until a clear visible blue-purple NBT reduction product was observed.

2.4. Statistical Analyses

Statistical analyses were conducted using non-parametric tests. The Kruskal–Wallis test was employed to compare the effects of NaCl and N- NO_3 solutions at varying concentrations on guaiacol peroxidase (GPOX) activity, relative water content (RWC), anthocyanin content (ACNs), and photosynthetic pigment content in *S. lapponum* tissues. Following the identification of significant differences, a post-hoc analysis was conducted employing the Dunn test with Bonferroni correction for multiple comparisons. In order to make a comparison between the chlorophyll fluorescence parameters (F_0 , F_m , F_v/F_m), an analysis of variance (ANOVA) of aligned rank-transformed data was used, including post-hoc pairwise comparisons. The model employed for the analysis was the one with repeated measures. This method was also used for the analysis of selected morphological traits. The R computing environment (R version 4.3.1) was used to perform the analyses and visualizations.

3. Results

3.1. Experiment 1. Effect of Substrate Salinity

3.1.1. Plant Growth

In the first half of the experiment (Term 1–Term 2), the plants of the control sample exhibited a significantly higher average daily growth rate (approximately 0.47 cm) in comparison to the plants treated with NaCl solutions (ranging from 0.30 to 0.37 cm). In the second half of the experiment (Term 2–Term 3), the lowest growth rate was observed in plants exposed to the NaCl80 treatment (approximately 0.37 cm per day). The results of the experiment demonstrated that the growth rate of plants treated with NaCl solutions of 20 mM and 40 mM, as well as those watered with distilled water, was found to be statistically significantly higher during the second stage of the experiment (Term 2–Term 3) in comparison to the first stage (Term 1–Term 2) (Figure 2a).

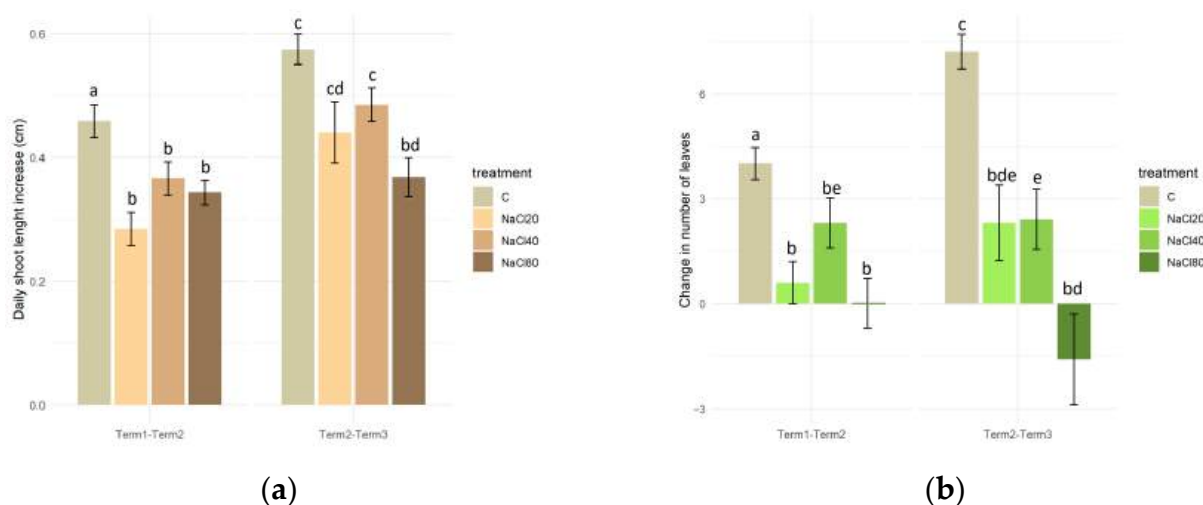


Figure 2. Daily growth rate of the main shoot (a) and change in the number of leaves (b) of *Salix lapponum* under different conditions of substrate salinity ($n = 10$; $\pm$ standard deviation). Statistically significant differences ($p < 0.05$) are indicated by different letters. Comparisons were made (1) between variants within the same experimental phase, and (2) between corresponding variants across the two phases.

From the beginning of the experiment to the end of the second week (Term 1–Term 2), plants watered with water devoid of NaCl addition exhibited a significantly greater increase in the number of leaves (4 on average) in comparison to all variants treated with NaCl solutions (0 and 2 leaves on average). During the second stage of the experiment (Term 2–Term 3), the trend persisted, with plants of the control variant producing a greater number of leaves compared to those growing in the saline substrate. Despite minor variations in the number of leaves of plants exposed to differing levels of salinity, it was observed that only those plants that were watered with 80 mM NaCl solution exhibited a negative value for that particular parameter (Figure 2b).

3.1.2. Relative Water Content

At the beginning of the experiment, the plants were distinguished by a significantly elevated relative water content in their tissues (approximately 97%). After 2 weeks of experimentation in Term 2, the RWC in all cases exhibited a consistent oscillation around 88%. The results of tests conducted after a four-week exposure period (Term 3) indicated a further decrease in RWC values. The lowest values of that parameter were recorded in plants watered with NaCl solutions of 20 mM (66%) and 80 mM (69%). Statistical analysis revealed a substantial decline in RWC values of plant tissues across all salinity variants at Term 3, in comparison to the initial value of this parameter (Term 1; Figure 3).

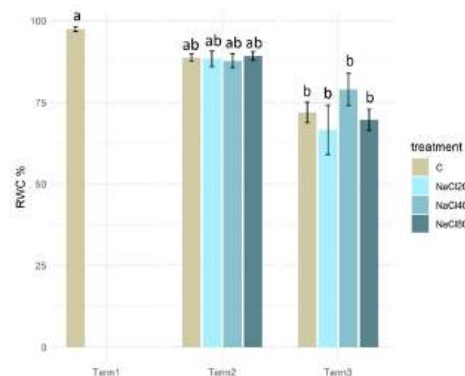


Figure 3. Relative water content (RWC) in the tissues of *Salix lapponum* exposed to different substrate salinity conditions ($n = 5$; $\pm$ standard deviation). Statistically significant differences ($p < 0.05$) are indicated by different letters. Comparisons were made (1) between variants at each measurement term; (2) between corresponding variants at different terms. The control group from Term 1 was additionally used as a reference for comparison with each variant in subsequent terms.

3.1.3. Photosynthetic Pigments Content

The content of chlorophyll a in plant tissues exhibited no significant variation among individuals treated with solutions of varying NaCl concentrations (20, 40, and 80 mM) at both Term 2 and Term 3. Irrespective of the concentration of NaCl solution used for watering, a significant decrease in chlorophyll a content was evident in all plant groups at the final experimental date in comparison to the initial value (Term 1; Figure 4a).

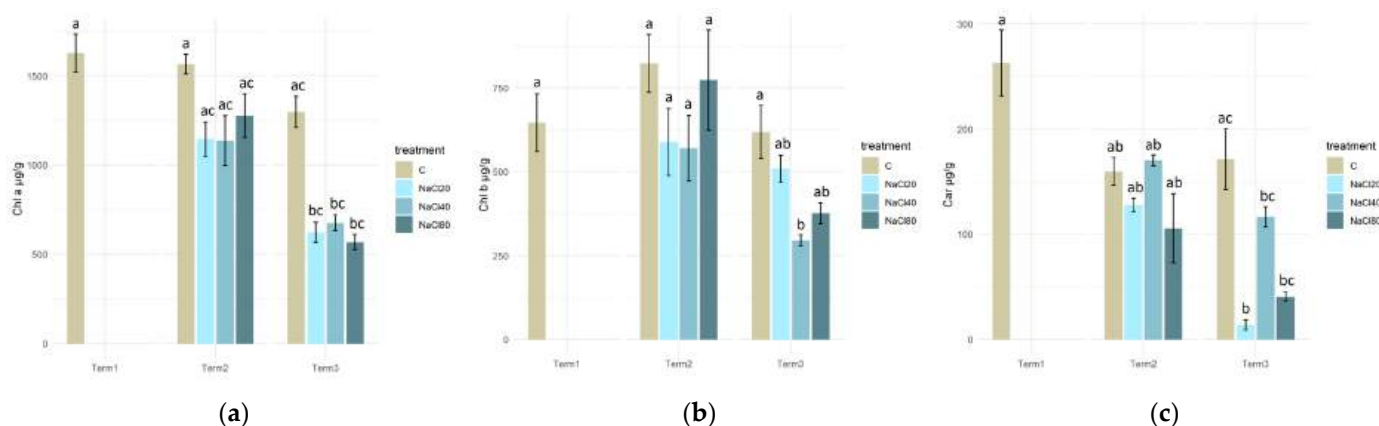


Figure 4. Concentrations of photosynthetic pigments. Chlorophyll a (a), chlorophyll b (b), and carotenoids (c) in *Salix lapponum* leaves exposed to different substrate salinity conditions ($n = 5$; $\pm$ standard deviation). Statistically significant differences ($p < 0.05$) are indicated by different letters. Comparisons were made (1) between variants at each measurement term; (2) between corresponding variants at different terms. The control group from Term 1 was additionally used as a reference for comparison with each variant in subsequent terms.

In the case of chlorophyll b content in the *S. lapponum* tissues, no significant differences were observed between the test specimens from the different salinity variants at Term 2. After 4 weeks of the experiment, it was evident that the application of a 40 mM NaCl solution to the plants had a substantial impact on the chlorophyll b content in tissues. A significant decline in chlorophyll b was observed when compared to the control. There was a significant decrease in the content of chlorophyll b in the tissues of plants treated with a 40 mM NaCl solution compared to the control sample at that time, as well as to the initial values recorded during Term 1 (Figure 4b).

After two weeks of the experiment, no statistically significant differences were observed in the carotenoid content of the tested plants' tissues across all the experimental variants. A significant decrease in carotenoid levels was observed in plants of all salinity variants, relative to the initial level recorded in Term 1, only after four weeks (Figure 4c).

3.1.4. Selected Chlorophyll Fluorescence Parameters

After two weeks of the experiment, no significant differences were observed in F_0 and F_m values between plants treated with different solutions of varying NaCl concentrations. Significant reductions in both F_0 and F_m were recorded in *S. lapponum* individuals after four weeks of exposure to 40 mM and 80 mM NaCl solutions, compared to the control group. In the case of F_0 , following a two-week exposure to varying concentrations of NaCl, a decline in the parameter value was observed in all the plant groups that were analyzed. Following a further two-week period (Term 3), a substantial increase in F_0 values was observed in each salinity variant tested. The exposure of plants to varying levels of substrate salinity resulted in a gradual decline in F_m values. The exceptions were found in plants watered with a 20 mM NaCl solution. In these cases, an increase in the F_m parameter was observed in Term 2, and in Term 3, the value was found to be close to the result obtained at the beginning of the experiment (Table 2).

Table 2. Selected parameters of chlorophyll a fluorescence. Minimum fluorescence (F_0), maximum fluorescence (F_m) value, and maximum quantum yield of PSII photochemistry (F_v/F_m) in *Salix lapponum* leaves under different conditions of substrate salinity ($n = 10$; $\pm$ standard deviation).

Term	Variant	F_0	F_m	F_v/F_m
Term 1	C	207.3 $\pm$ 15.9	885.6 $\pm$ 60.0	0.765 $\pm$ 0.014
	NaCl20	217.7 $\pm$ 57.1	747.9 $\pm$ 234.0	0.733 $\pm$ 0.076
	NaCl40	203.6 $\pm$ 14.3	845.8 $\pm$ 49.7	0.758 $\pm$ 0.018
	NaCl80	196.8 $\pm$ 19.6	857.7 $\pm$ 51.1	0.770 $\pm$ 0.018
Term 2	C	173.8 $\pm$ 19.3	839.5 $\pm$ 64.2	0.793 $\pm$ 0.016
	NaCl20	169.2 $\pm$ 24.7	843.8 $\pm$ 38.4	0.800 $\pm$ 0.020
	NaCl40	177.9 $\pm$ 12.0	832.7 $\pm$ 56.1	0.785 $\pm$ 0.021
	NaCl80	179.8 $\pm$ 31.0	850.1 $\pm$ 61.8	0.788 $\pm$ 0.033
Term 3	C	178.6 $\pm$ 8.4	806.9 $\pm$ 60.5	0.777 $\pm$ 0.013
	NaCl20	204.5 $\pm$ 48.6	757.7 $\pm$ 69.8	0.724 $\pm$ 0.083
	NaCl40	246.9 $\pm$ 39.3	742.7 $\pm$ 55.5	0.668 $\pm$ 0.040
	NaCl80	221.3 $\pm$ 40.6	695.2 $\pm$ 110.6	0.670 $\pm$ 0.092

In the case of the F_v/F_m parameter, which is related to the maximum efficiency of PSII, its value at the start of the experiment ranged between 0.733 and 0.770. Following a fortnight of exposure to NaCl solutions of varying concentrations, a significant increase in the values of the indicator was observed in all experimental variants, ranging from 0.785 to 0.800. No statistically significant differences were observed among the plants treated with different NaCl concentrations at that stage. After four weeks, a decline in the F_v/F_m value was observed in all variants of the experiment. Furthermore, significant differences were noted in the value of F_v/F_m among plants from different experimental variants. Plants exposed to NaCl solutions with concentrations of 40 mM and 80 mM NaCl were subjected to statistical analysis, which revealed that the lowest F_v/F_m values recorded throughout the experiment were approximately 0.670 (Table 2).

3.1.5. Anthocyanin Content

The plant material of the tested *S. lapponum* specimens exhibited a low anthocyanin content of approximately 0.04 mg/g FW prior to the application of NaCl solutions. A statistically significant increase in anthocyanin content was observed in plants treated with

NaCl solutions at concentrations of 20 and 80 mM during Term 2. In Term 3, the level of anthocyanin in plant tissues remained consistent and was only significantly higher only in the NaCl20 and NaCl40 salinity variants (Figure 5).

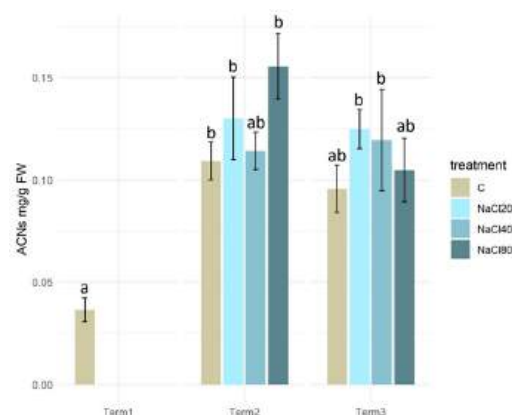


Figure 5. Anthocyanin content in tissues of *Salix lapponum* exposed to different substrate salinity conditions ($n = 5$; $\pm$ standard deviation). Statistically significant differences ($p < 0.05$) are indicated by different letters. Comparisons were made (1) between variants at each measurement term; (2) between corresponding variants at different terms. The control group from Term 1 was additionally used as a reference for comparison with each variant in subsequent terms.

3.1.6. Antioxidant Enzyme Activity

Analysis of changes in guaiacol peroxidase activity in *S. lapponum* tissues revealed no statistically significant differences between groups of plants from different variants of the experiment at both Term 2 and Term 3. Following a two-week exposure to NaCl solutions, guaiacol peroxidase activity in *S. lapponum* tissues was relatively low. After 4 weeks of exposure of plants to NaCl solutions, there was an increase in GPOX activity in the tissues, with the highest levels being observed in the NaCl20 and NaCl80 mM salinity variants. As a consequence of exposure to NaCl solution at a concentration of 20 mM, a statistically significant increase in GPOX levels was observed in plants at Term 3 in comparison with those at Term 2. Conversely, the group of plants that were watered with NaCl 80 mM solution exhibited a statistically significant increase in GPOX activity at Term 3 in comparison with Term 1 (Figure 6).

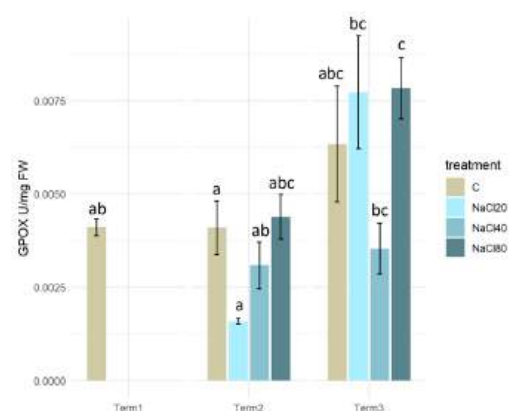


Figure 6. Guaiacol peroxidase activity (GPOX) in the tissues of *Salix lapponum* exposed to different substrate salinity conditions ($n = 5$; $\pm$ standard deviation). Statistically significant differences ($p < 0.05$) are indicated by different letters. Comparisons were made (1) between variants at each measurement term; (2) between corresponding variants at different terms. The control group from Term 1 was additionally used as a reference for comparison with each variant in subsequent terms.

3.1.7. Histochemical Detection of Reactive Oxygen Species (ROS)

Histochemical analysis employing NBT and DAB staining revealed the level of reactive oxygen species (ROS) in the leaf tissues of NaCl-treated *S. lapponum* specimens.

In the case of superoxide anion ($O_2^{\bullet-}$), detected with NBT, the intensity of staining exhibited a marked enhancement after a period of two weeks (Term 2), while after four weeks (Term 3), a decline in the intensity of the blue reaction product was discernible, thereby suggesting a reduction in $O_2^{\bullet-}$ content. At concentrations of 20 and 40 mM NaCl, ROS accumulated was predominantly observed in the petioles and as a spot in the leaf blade. However, at a concentration of 80 mM, there was additionally intense staining within the leaf nerves (Figure 7).

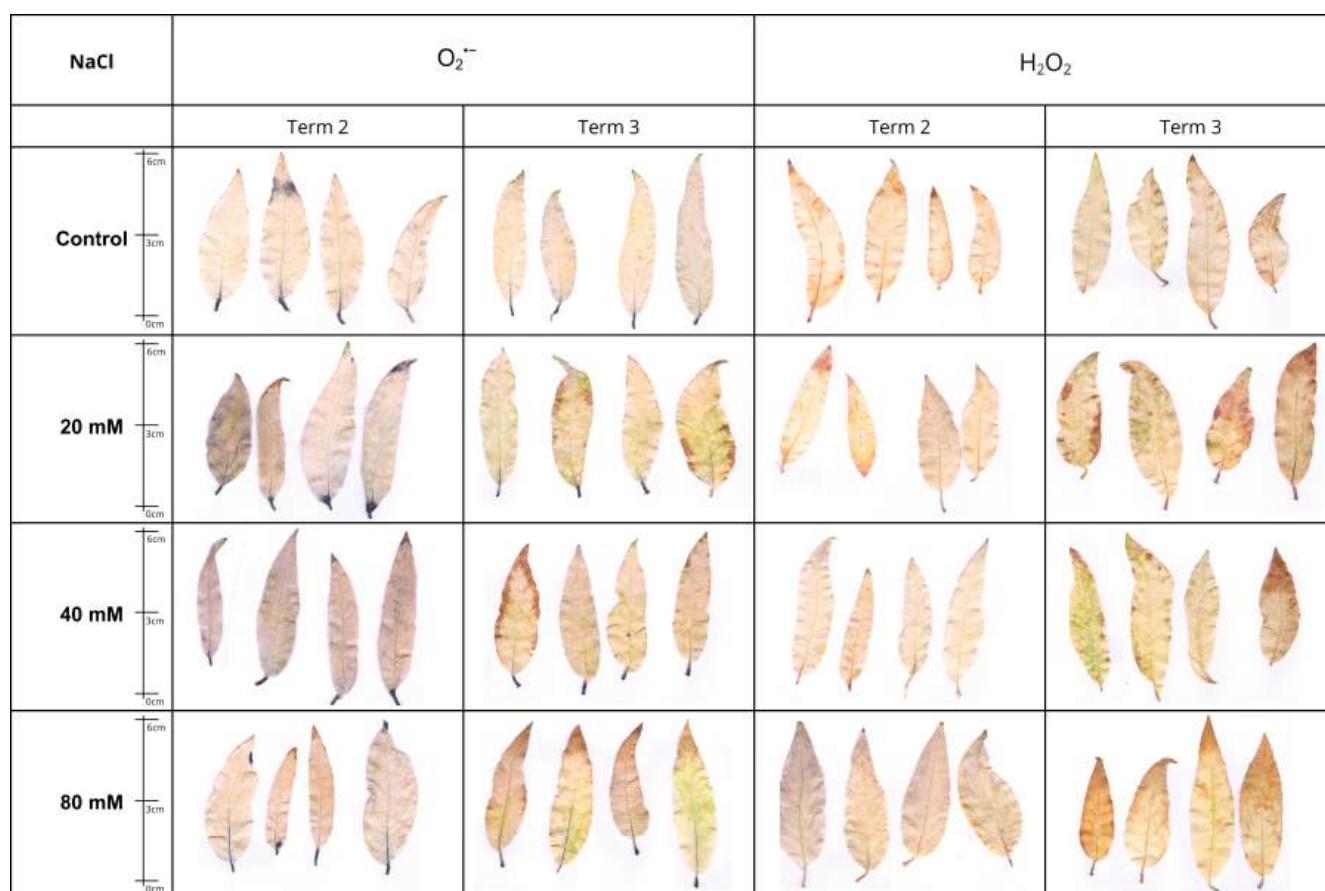


Figure 7. Visualization of histochemical detection of superoxide anion ($O_2^{\bullet-}$, NBT staining) and hydrogen peroxide (H_2O_2 , DAB staining) in *Salix lapponum* leaves exposed to 0, 20, 40, and 80 mM NaCl at two sampling times (Term 2, Term 3). Dark blue indicates $O_2^{\bullet-}$ accumulation; brown indicates H_2O_2 accumulation. Scale in cm.

A divergent trend was observed in the detection of hydrogen peroxide (H_2O_2) with DAB. After four weeks, the staining was found to be more intense in comparison to the result obtained after two weeks, indicating the accumulation of H_2O_2 in plant tissues during the experiment. The highest ROS content was recorded in *S. lapponum* leaves in the NaCl80 variant after four weeks, where the dark brown staining product covered almost the entire leaf blade. In the presence of reduced concentrations of NaCl (20 and 40 mM), the ROS were predominantly localized at the periphery of the leaf blade (Figure 7).

3.2. Experiment 2. The Effect of Nitrate Nitrogen

3.2.1. Plant Growth

During the initial period of the experiment (Term 1–Term 2), a significant difference in the mean daily rate of shoot growth was observed, exclusively between the control plants (0.47 cm) and those treated with the solution with the highest N-NO₃ concentration (0.38 cm). In the second half of the experiment (Term 2–Term 3), plants watered with solutions of 1 mg/L and 10 mg/L N-NO₃ concentrations exhibited significantly higher growth (0.72–0.8 cm per day) in comparison to the control plants and those treated with the 100 mg/L N-NO₃ solution (approximately 0.6 cm per day on average). A comparison of the mean daily growth rate of the plants across both experimental terms revealed that the plants in all of the experimental variants intensified their growth rate in the second half of the experiment (Figure 8a).

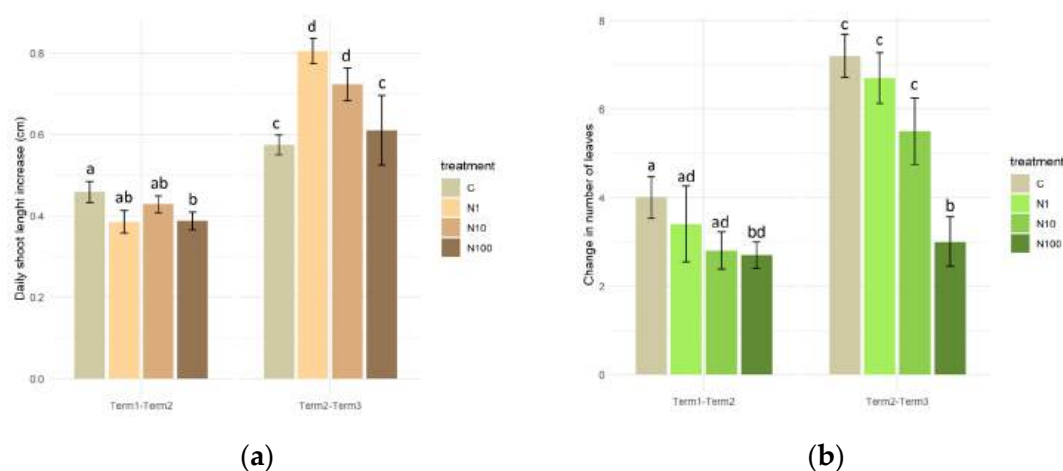


Figure 8. Daily growth rate of the main shoot (a) and change in the number of leaves (b) of *Salix lapponum* under the influence of different concentrations of nitrate nitrogen ($n = 10$; $\pm$ standard deviation). Statistically significant differences ($p < 0.05$) are indicated by different letters. Comparisons were made (1) between variants within the same experimental phase, and (2) between corresponding variants across the two phases.

An analysis of the change in the number of leaves in *S. lapponum* specimens revealed that after 4 weeks of the experiment (Term 2–Term 3), plants that were watered with distilled water and solutions containing N-NO₃ at concentrations of 1 and 10 mg/L exhibited a significant intensification in leaf production compared to the initial measurement period. Despite the absence of statistically significant differences in the number of newly formed leaves between the control plants and those from the N1 and N10 variants, a trend was observed in both measurement periods, indicating that as the concentration of N-NO₃ increased, the production of leaves was slower (Figure 8b).

3.2.2. Relative Water Content

Initially, *S. lapponum* individuals were characterized by very high values of relative water content (RWC) (approximately 97%), which decreased during the course of the experiment. At Term 2, the RWC value in each treatment was approximately 89%. Following a period of four weeks, it was observed that the RWC values exhibited variation in the plants, depending on the N-NO₃ concentration, with a range from 72% to 84%. In Term 3, a significant decline in RWC was evident in all experimental variants in comparison to Term 1 (Figure 9).

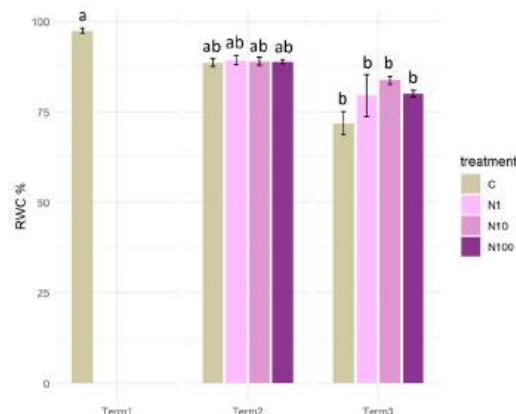


Figure 9. Relative water content (RWC) in the tissues of *Salix lapponum* under the influence of different concentrations of nitrate nitrogen ($n = 5$; $\pm$ standard deviation). Statistically significant differences ($p < 0.05$) are indicated by different letters. Comparisons were made (1) between variants at each measurement term; (2) between corresponding variants at different terms. The control group from Term 1 was additionally used as a reference for comparison with each variant in subsequent terms.

3.2.3. Photosynthetic Pigments Content

An analysis was conducted of the photosynthetic pigments present in plants exposed to varying concentrations of nitrate nitrogen, which revealed no significant differences in the content of chlorophyll a, chlorophyll b, and carotenoids between plants at each measurement date.

After 4 weeks of the experiment, it was evident that the application of N-NO₃ solution to the plants resulted in a substantial decline in the levels of chlorophyll a and carotenoids in the tissues, as compared to the initial values recorded at the beginning of the experiment. With regard to the content of chlorophyll b in the tissues of *S. lapponum*, the results obtained did not differ significantly between the experimental terms (Figure 10a–c).

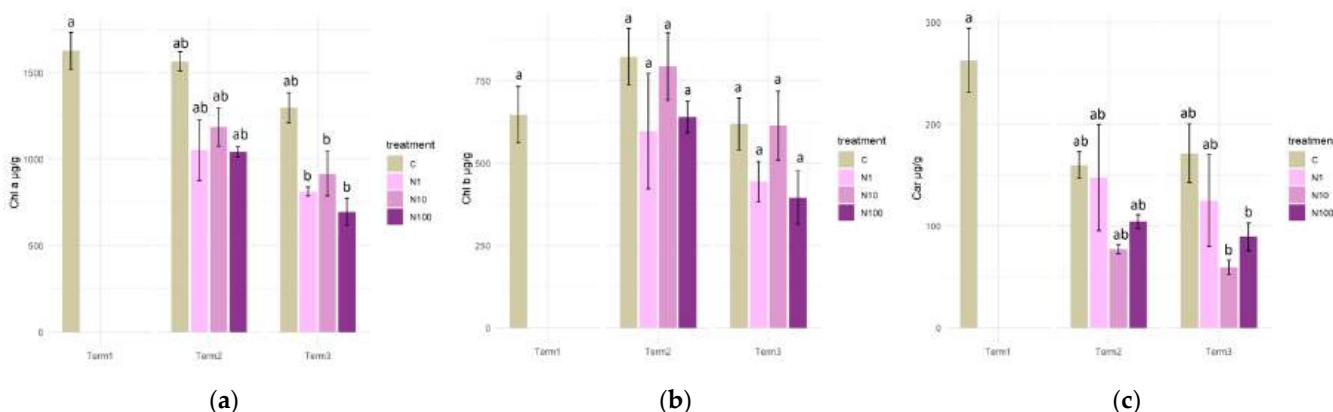


Figure 10. Concentrations of photosynthetic pigments. Chlorophyll a (a), chlorophyll b (b), and carotenoids (c) in *Salix lapponum* leaves under the influence of different concentrations of nitrate nitrogen ($n = 5$; $\pm$ standard deviation). Statistically significant differences ($p < 0.05$) are indicated by different letters. Comparisons were made (1) between variants at each measurement term; (2) between corresponding variants at different terms. The control group from Term 1 was additionally used as a reference for comparison with each variant in subsequent terms.

3.2.4. Selected Chlorophyll Fluorescence Parameters

An analysis of chlorophyll fluorescence parameters in plants treated with solutions containing different concentrations of nitrate nitrogen revealed no statistically significant differences in F_0 and F_m values either between groups of plants within each measurement term or between terms. The values of F_0 exhibited a consistent tendency to approximate

180 throughout the experiment, while the value of F_m remained at around 830. The values of the maximum efficiency of photosystem II (F_v/F_m) in each of the three terms of measurement oscillated around 0.8. Nevertheless, the findings of the statistical analyses demonstrated a significant increase in the values of this parameter after 2 weeks of conducting the experimental variants that were examined (Table 3).

Table 3. Selected parameters of chlorophyll a fluorescence. Minimum fluorescence (F_0), maximum fluorescence (F_m) value, and maximum quantum yield of PSII photochemistry (F_v/F_m) in *Salix lapponum* leaves under the influence of different concentrations of nitrate nitrogen ($n = 10$; $\pm$ standard deviation).

Term	Variant	F_0	F_m	F_v/F_m
Term 1	C	207.3 $\pm$ 15.9	885.6 $\pm$ 60.0	0.765 $\pm$ 0.014
	N1	180.6 $\pm$ 28.8	834.7 $\pm$ 65.8	0.733 $\pm$ 0.022
	N10	181.4 $\pm$ 23.6	806.4 $\pm$ 70.3	0.758 $\pm$ 0.020
	N100	180.8 $\pm$ 12.9	823.6 $\pm$ 48.5	0.770 $\pm$ 0.008
Term 2	C	173.8 $\pm$ 19.3	839.5 $\pm$ 64.2	0.793 $\pm$ 0.016
	N1	156.2 $\pm$ 9.2	837.7 $\pm$ 26.0	0.800 $\pm$ 0.008
	N10	167.3 $\pm$ 21.9	840.8 $\pm$ 72.8	0.785 $\pm$ 0.016
	N100	170 $\pm$ 19.1	827.1 $\pm$ 32.9	0.788 $\pm$ 0.017
Term 3	C	178.6 $\pm$ 8.4	806.9 $\pm$ 60.5	0.777 $\pm$ 0.013
	N1	167.8 $\pm$ 11.4	837 $\pm$ 37.9	0.724 $\pm$ 0.011
	N10	174.3 $\pm$ 17.4	822.6 $\pm$ 48.2	0.668 $\pm$ 0.013
	N100	180.6 $\pm$ 21.2	823.9 $\pm$ 32.7	0.670 $\pm$ 0.021

3.2.5. Anthocyanin Content

The results of the analysis demonstrated that the initial levels of anthocyanins in the plant tissues were minimal. However, after 2 weeks of exposure to N-NO₃ solutions, a significant increase in the content of these compounds was observed in plants in all variants of the experiment. A decline in the anthocyanin content of plant tissues was observed at Term 3. Statistical analysis revealed that there were no significant differences in anthocyanin content either between plants exposed to N-NO₃ at Term 2 and Term 3 of the analyses, nor between the two terms (Figure 11).

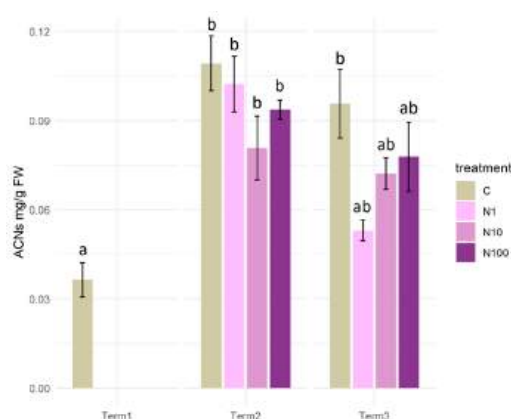


Figure 11. Anthocyanin content in tissues of *Salix lapponum* under the influence of different concentrations of nitrate nitrogen ($n = 5$; $\pm$ standard deviation). Statistically significant differences ($p < 0.05$) are indicated by different letters. Comparisons were made (1) between variants at each measurement term; (2) between corresponding variants at different terms. The control group from Term 1 was additionally used as a reference for comparison with each variant in subsequent terms.

3.2.6. Antioxidant Enzyme Activity

After two weeks of the experiment, a statistically significant decrease in GPOX activity was observed in plants of the N1 and N100 variants in comparison to the control and N10 plants. The situation changed after four weeks, when an increase in GPOX activity was observed in all variants of the experiment. In the case of plants that were watered with solutions of 1 and 100 mg/L of N-NO₃, there was a statistically significant increase in comparison to the results obtained in Term 2 (Figure 12).

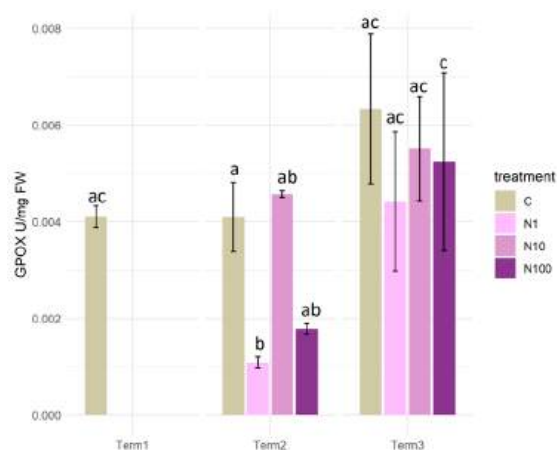


Figure 12. Guaiacol peroxidase activity (GPOX) in the tissues of *Salix lapponum* under the influence of different concentrations of nitrate nitrogen ($n = 5$; $\pm$ standard deviation). Statistically significant differences ($p < 0.05$) are indicated by different letters. Comparisons were made (1) between variants at each measurement term; (2) between corresponding variants at different terms. The control group from Term 1 was additionally used as a reference for comparison with each variant in subsequent terms.

3.2.7. Histochemical Detection of Reactive Oxygen Species (ROS)

Plants treated with aqueous solutions of N-NO₃ demonstrated differential accumulation of ROS in response to this stress factor. Following a period of 2 weeks, a slight accumulation of superoxide anion was observed in the N1 and N10 variants. The staining product accumulated primarily within the petioles and at the base of the leaf blade. In the N100 variant, the staining was more intense. The control sample exhibited minimal staining. In Term 3, the intensity of NBT staining was lower than in Term 2.

It was established that hydrogen peroxide was detected in response to the action of N-NO₃ solutions. ROS accumulation was observed at the margins of leaf blades after 2 weeks. As the concentration of the solution increased, the staining became more intense. After 4 weeks of experimentation, it was determined that there were no statistically significant differences in DAB staining (Figure 13).

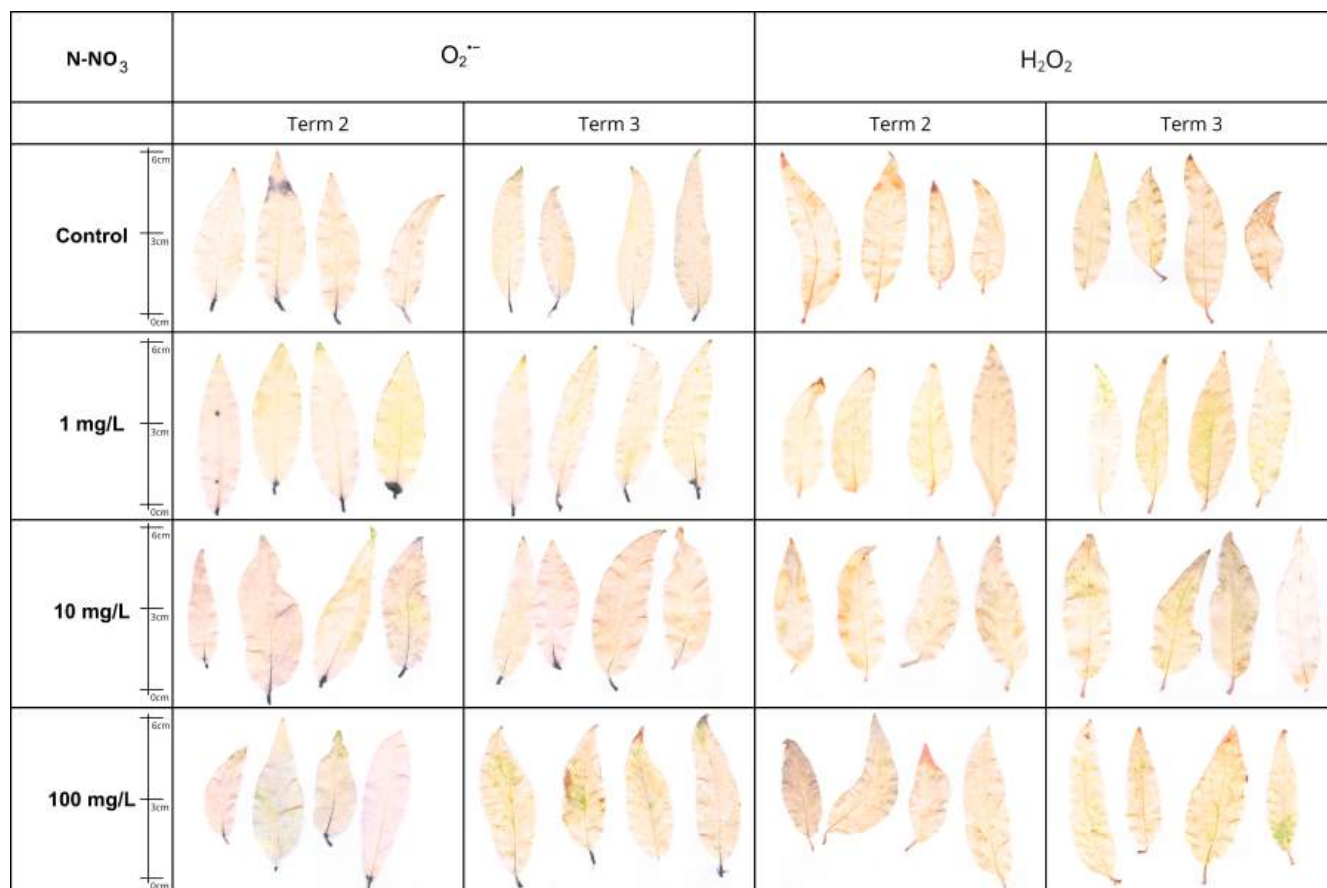


Figure 13. Visualization of histochemical detection of superoxide anion ($\text{O}_2^{\bullet-}$, NBT staining) and hydrogen peroxide (H_2O_2 , DAB staining) in *Salix lapponum* leaves exposed to 0, 1, 10, and 100 mg/L N-NO_3 solutions at two sampling times (Term 2, Term 3). Dark blue indicates $\text{O}_2^{\bullet-}$ accumulation; brown indicates H_2O_2 accumulation. Scale in cm.

4. Discussion

In the era of ongoing climate change, the conservation of biodiversity is confronted with numerous new challenges. The efficacy of conventional in situ methods employed for the protection of plant species may be inadequate [48]. As Twardek et al. [49] note, there is an increasing tendency to consider actions based on the so-called assisted migrations and translocations to new, relatively stable, and climate-resilient areas [49]. The effectiveness of these measures depends on their adaptation to the specific species concerned. The findings of physiological studies provide many answers to questions about the range of ecological tolerance and stress responses, while empirical data provide insight into the mechanisms that influence the efficiency of plant acclimatization to fluctuating environmental conditions. This, in turn, has resulted in the development of dedicated methods for the protection of species, thereby increasing their chances for long-term survival in substitute habitats [50].

Although stress factors rarely occur in isolation and usually act synergistically or antagonistically, with different intensities in time and space, conducting laboratory experiments using single stressors remains a fundamental tool for understanding the mechanisms of plant physiological response [51–53]. In the context of protected and endangered species, such studies assume particular significance, as they provide the knowledge necessary to formulate effective active conservation measures. At the same time, these species impose considerable demands, primarily due to the restricted availability of plant material resulting from low population size and the legal and ethical requirements [54].

Studies conducted in order to gain a more detailed understanding of the response of *Salix lapponum* specimens to different concentrations of NaCl and N-NO₃ in the soil solution demonstrated that both factors act as abiotic stressors. The effect of these substances on plants is dependent on various morpho-physiological parameters, which are determined through analysis. The parameters vary depending on the dose and time of exposure.

The rate of shoot growth, as well as the development of new leaves, reflects the cumulative effect of environmental factors on plant metabolism. These phenomena are also the first phenotypically apparent signs of abiotic stress [55]. It is evident that the alterations in biometric parameters, as observed at the morphological level, are the consequence of disturbances at the physiological level. Consequently, further analysis of parameters such as the content of photosynthetic pigments and anthocyanins, the efficiency of the photosynthetic apparatus, the relative water content, and the activity of antioxidant enzymes enables a more profound comprehension of intracellular adaptations to stress conditions [56,57].

In the experiments conducted, salt stress had a more profound effect on *S. lapponum* specimens than the effect of aqueous solutions containing N-NO₃. Irrespective of the concentration of the solution and the duration of exposure, the salinity of the substrate in each case led to a decline in the growth rate and negatively affected the foliage condition of plants, resulting in leaf necrosis. Conversely, nitrate nitrogen solution administered at low doses (1 and 10 mg/L) with prolonged exposure had a stimulatory effect on the growth of *S. lapponum* specimens. Bayat et al. [58] conducted a series of experiments on *Salvia lavandulifolia*, irrigating the plants with solutions of varying salinity levels. The findings indicated that as the concentration of the solutions increased, there was a substantial decline in the growth rate of the plants. Additionally, the number, size, and shape of leaves were smaller in comparison to plants cultivated in an unsalted medium. This study also documented a reduction in the length of the roots. Furthermore, elevated nitrogen levels in the soil solution can also inhibit growth rates, as evidenced by studies undertaken by Chen et al. [59] on three species of leafy vegetables.

The findings of the experiments conducted on *S. lapponum* specimens demonstrated that osmotic stress induced by aqueous solutions of NaCl and N-NO₃ significantly affects the relative water content (RWC) of plant tissues. Following a 14-day experimental period, the RWC in the examined plants remained high (about 88%) irrespective of the salinity variant. However, only prolonged exposure (4 weeks) to substrate salinity resulted in a significant decrease in RWC values (up to 66% in the NaCl20 variant), indicating an impaired ability of plants to maintain water homeostasis under long-term salt stress. A comparable, though less severe, decline in RWC was observed in plants exposed to nitrate-nitrogen-containing solutions, with RWC values decreasing to 89% after 2 weeks, and reaching values of 72 to 84% after 4 weeks. Despite its relatively mild course, this stress factor also led to disturbances in plant water management. The results of the present study demonstrate that *S. lapponum* is capable of counteracting short-term osmotic stress; however, its long-term effect results in the disruption of plant water management. A comparable phenomenon was described by Yazici et al. [60] in a study conducted on *Portulaca oleracea* L., where, after 18 days of exposure to salinity (70 and 140 mM NaCl), the relative water content of the leaves was about 80%. After 30 days of exposure, a decline in the RWC was observed, with oscillations reaching approximately 65%. The authors suggest that the high RWC value in short-term exposure to stress factors results from osmoregulatory mechanisms, including the synthesis of osmoprotective proline. Nevertheless, longer exposure to both salinity and nitrogen compounds may lead to ionic imbalance, which, in turn, may result in a decline in RWC values [60–62].

Conducted experiment revealed that elevated levels of salinity and nitrate nitrogen in the substrate led to a decrease in the content of photosynthetic pigments present in

the tissues of the plants studied, even at low concentrations of the solutions used for watering. Salt stress has been shown to result in numerous ion imbalances and toxic effects of Na^+ and Cl^- ions, which directly disrupt chlorophyll synthesis [63]. Reductions in chlorophyll a and b content were also observed in studies on *Salvia lavandulifolia*, in which an increase in salinity levels correlated with a decrease in photosynthetic pigments [58]. As Bertamini et al. [64] demonstrate, measurements of chlorophyll fluorescence and, in particular, the parameter of maximum photosystem II efficiency (F_v/F_m) allow for the early detection of plant responses to environmental stresses. The results on *Salix lapponum* are consistent with those reported by Ran et al. [65] for *Salix alba*, where salt stress significantly impaired photosynthetic efficiency through both stomatal and extra-stomatal mechanisms, and induced oxidative stress at the PSII level, leading to more frequent inactivation of the PSII reaction center. In the study conducted by Yousefi and Karamian [66], *Satureia mutica* was treated with salt solutions at concentrations ranging from 0 to 150 mM. Their study revealed a significant decline in chlorophyll fluorescence parameters in the absence of salinity. The value of the F_v/F_m parameter was recorded as 0.81, while at 150 mM NaCl, it decreased to 0.66.

A conducted study indicates that in salt stress situations, *S. lapponum* plants accumulate significantly higher amounts of reactive oxygen species than when nitrate nitrogen availability in soil is increased. When exposed to N-NO_3 , the staining products of DAB and NBT solutions were less pronounced than in plants exposed to salt stress. Ashraf [67] indicates that salt stress disrupts gas exchange in plants by limiting CO_2 uptake, which in turn contributes to the production of ROS such as hydrogen peroxide, singlet oxygen, and superoxide anion. Studies previously conducted on *S. lapponum* and *Salix myrtilloides* specimens acclimated to ex vitro conditions demonstrated that, in circumstances of a drastic change in environmental conditions (i.e., transfer of plants from the phytotron to field acclimation), there is a significant accumulation of ROS in the plants' tissues, in comparison to the exposure of plants to single stress factors [42,68]. The histochemical detection of ROS has confirmed the intensification of oxidative stress in response to NaCl solutions, as observed in *Sphaerophysa kotschyana*. After 14 days of exposure to salt stress, strong staining of reaction products with NBT and DAB was observed. The most significant accumulation of ROS was observed at a concentration of 300 mM NaCl, resulting in the complete coverage of the entire leaf blade. In contrast, at lower concentrations (150 mM), the staining was mainly limited to the areas of the conductive bundles [69].

It has been proven that plants, through specialized enzymatic mechanisms, are capable of eliminating ROS from the body and mitigating the effects of oxidative stress. The findings of the two experiments presented in this paper demonstrated an increase in GPOX activity in *S. lapponum* tissues following prolonged exposure to stress factors. However, this value was statistically significant only when the highest concentration of NaCl was applied. Short-term application of NaCl solutions and N-NO_3 to the substrate resulted in a decline in guaiacol peroxidase activity in the plants studied. This phenomenon was previously observed in the study of Yildiztugay et al. [69] on *Sphaerophysa kotschyana*, where a decline in peroxidase activity (POX) was documented in plant tissues in response to salt stress. The authors suggest that reduced POX activity may impede the lignification process, thereby facilitating sustained water uptake and diminished water loss. This phenomenon is hypothesized to be an adaptive mechanism to maintain elevated levels of RWC.

Non-enzymatic defense systems, in which anthocyanins play a pivotal role, have also been identified as a means of protecting plants from the negative effects of environmental stress [70]. A study conducted on *S. lapponum* revealed that elevated levels of anthocyanins were particularly evident at the initial stage of the occurrence of salt stress. An increase in anthocyanin content in response to salt stress is also described by Eryilmaz [71] in studies

conducted on tomatoes and red cabbage. The author observed elevated anthocyanin concentrations after a single week of exposure of plants to 50 and 100 mM NaCl solutions. In the course of research conducted on a range of tomato varieties, Napar et al. [72] demonstrated that a tomato genotype exhibiting increased levels of anthocyanins showed enhanced tolerance to drought and salinity in comparison to genotypes characterized by lower levels of these compounds. Consequently, the concentration of anthocyanins in plants may serve as a sensitive indicator expressing plant tolerance to salinity stress.

It is well known that appropriate doses of nitrogen have a stimulatory effect on plant growth, whereas its excess, in both nitrate (NO_3^-) and ammonium (NH_4^+) forms, can result in stunted plant growth [73,74]. Research undertaken in the natural habitats of *S. lapponum* in eastern Poland has demonstrated that the concentrations of nitrogen compounds in plant-accessible groundwater are minimal [27]. The findings of these studies indicate that the downy willow is capable of functioning properly in nitrogen-poor habitats. However, the upper limit of the species' ecological tolerance to this factor remains to be determined. The results of the experiment presented in this study showed that *S. lapponum* specimens do not react with a strong stress to increased nitrogen doses or to the salinity of the substrate. Given that nitrogen is one of several biogenic elements that cause eutrophication, and that in the present study only the nitrate form was taken into account, there is a need for further research, especially taking into account other forms of nitrogen. The results of studies conducted on *Aldrovanda vesiculosa* demonstrate that the form in which nitrogen is present in the water is more important for optimal plant growth and development than its content in solution alone. Ammonium ions (NH_4^+) had a detrimental effect on turion development and inhibited the growth of *A. vesiculosa* plants. Conversely, nitrate ions (NO_3^-), which were present in the solution in which the plants were kept, had a stimulatory effect on their growth [75].

5. Conclusions

The experimental results have expanded the current understanding of the response of *Salix lapponum* individuals to short-term abiotic stress associated with changes in the chemical composition of the soil.

S. lapponum individuals activate defense mechanisms in the form of antioxidant enzymes, anthocyanin synthesis, and maintain high RWC values when exposed to environmental stress factors such as increased soil salinity.

The presence of low concentrations of nitrate nitrogen in the soil solution stimulates plant growth, but the use of solutions with high concentrations of this form of nitrogen causes a deterioration of the studied morphophysiological parameters of *S. lapponum*.

Further research is necessary to determine both the impact of long-term exposure of plants to the studied factors and the impact of other nutrients on the growth and condition of the studied species. This will allow to determine the feasibility of conservation measures through reintroduction into habitats potentially vulnerable to eutrophication or salinity.

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Abbreviations

The following abbreviations are used in this manuscript:

ROS Reactive Oxygen Species
RWC Relative Water Content

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Could species translocation be an effective conservation method for *Salix lapponum* populations in wetlands affected by anthropogenic changes?

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ABSTRACT

Wetlands, and consequently the fauna and flora associated with these habitats, represent some of the most threatened natural ecosystems worldwide. In Poland, a long-term decline in the population size of *Salix lapponum*, a glacial relict species associated with peatland habitats, has been observed. In response to the limited effectiveness of passive habitat protection and the difficulty of predicting environmental change, active conservation measures are increasingly implemented, including the introduction of species into substitute habitats. In 2023, a translocation experiment involving individuals of *S. lapponum* was conducted at five alternative sites in eastern Poland. The aim of the study was to assess the effectiveness of active conservation measures in peatland habitats undergoing both natural and anthropogenic transformations. Two years after translocation, the lowest plant survival rate was recorded at the drained peatland site (32.5%), whereas the highest survival was observed at the reference site located on a transitional mire (77.5%). Analysis of plant community composition revealed significant differentiation among sites, correlated with physicochemical water parameters. Habitat characteristics, particularly the stability of hydrological conditions, proved to be a key factor determining the success of *Salix lapponum* translocation.

1. Introduction

Peatlands are waterlogged ecosystems in which oxygen limitation reduces organic matter decomposition and enables long-term peat accumulation. Their ecological functioning is therefore strongly dependent on the maintenance of specific hydrological and physicochemical conditions (Gorham, 1991). At present, peatlands are among the most extensively transformed ecosystems globally. Drainage, peat extraction and land-use conversion disrupt their hydrological and biogeochemical functioning, while climate change may further intensify degradation, particularly in previously drained systems (Bu et al., 2011). Approximately 25% of European peatland area is currently considered degraded (Tanneberger et al., 2021). Rising temperatures, more frequent droughts and altered precipitation regimes can lower water-table levels, increase peat aeration and accelerate organic matter decomposition (Dise, 2009; Macrae et al., 2013).

Many specialised peatland plant species exhibit narrow ecological

amplitudes and depend on relatively stable hydrological and physicochemical conditions, including high water tables, acidic substrates and limited nutrient availability (Minayeva & Sirin, 2012; Wheeler & Proctor, 2000). Among them are boreal taxa that persist in parts of temperate Europe as glacial relicts, where peatlands may provide local hydrological and microclimatic refugia beyond the core areas of their distribution (Dítě et al., 2018). Climate-driven changes in temperature and water availability may reduce the local persistence of these cold-adapted taxa and alter peatland community composition. Lower water tables and progressively drier conditions are commonly associated with declining *Sphagnum* cover and increasing dominance of graminoids, trees and shrubs, potentially reducing habitat suitability for specialised peatland species (Dieleman et al., 2015; Gunnarsson, 2005; Hájková et al., 2011; Hedwall et al., 2017; Weltzin et al., 2000).

Peatland degradation and climate change may reduce the effectiveness of passive conservation, particularly where hydrological conditions have been substantially altered. In such cases, conservation

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translocation may provide an active means of reducing extinction risk in threatened plant taxa (Bellis et al., 2024). Depending on the conservation objective, it may involve reintroduction into formerly occupied sites, reinforcement of existing populations or introduction to suitable sites outside the indigenous range when restoration of the original habitat is not feasible (Plein et al., 2016). Conservation translocation does not guarantee the successful establishment of a self-sustaining population, and its outcomes depend on interactions among habitat quality, founder population characteristics and subsequent management (Abeli & Dixon, 2016; Batson et al., 2015; Bellis et al., 2024). Experimental comparisons across translocation sites are needed to determine whether pre-translocation assessments of habitat suitability reliably predict the establishment and longer-term persistence of ex situ-propagated plants (Guerrant and Kaye, 2007; Maschinski et al., 2012; Maschinski & Albrecht, 2017; Wendelberger & Maschinski, 2016). This issue is particularly relevant because a recent synthesis of European plant translocations identified appropriate recipient-site selection as the most frequently reported explanation for success, whereas unsuitable microsites, plant competition and stochastic weather events were recurrently associated with failure. Moreover, among cases for which medium-term outcomes were available, almost half of the translocated populations did not persist for more than five years, and only 28% were considered self-sustaining (Godefroid et al., 2025). Post-translocation monitoring should therefore extend to growth, reproduction, recruitment and long-term population persistence. Because conservation translocations require substantial financial, logistical and temporal investment, they should be preceded by a systematic assessment of habitat suitability and plant-material quality and followed by sufficiently long monitoring to minimise the risk of establishment failure (Godefroid et al., 2011, 2025).

Salix lapponum, a glacial relict reaching its southern range in Poland, was selected as the model species in this study. This species has a predominantly boreal distribution, whereas its Central European populations are geographically isolated and occur near the southern margin of the species' range (Kłosowski & Kłosowski, 2006; Kruszelnicki, 2014). *S. lapponum* occurs in open or partially shaded wet habitats, including peatlands, mires, marshes, and wet meadows located near lakes and streams (Urbaniak et al., 2021). In Poland, this species is found primarily in peatlands with acidic substrates, with pH values ranging from 4 to 6 (Pogorzelec, 2008a). *S. lapponum* is classified as Critically Endangered (CR) both in the Polish Red Book of Plants (Kruszelnicki, 2014) and in the Polish Red List of Pteridophytes and Flowering Plants (Każmierczakowa et al., 2016). Since the 1950s, its population size has declined dramatically, and the remaining extant populations can be found in only a few locations in Poland (Kruszelnicki, 2014).

Previous studies on *Salix lapponum* have addressed its habitat requirements, population structure, genetic diversity and ex situ propagation potential (Finger et al., 2022; Hroneš et al., 2011, 2018, 2019; Parzymies et al., 2020; Pogorzelec, 2008a, 2008b, 2010; Stamati et al., 2007). In addition, several attempts have been undertaken to reinforce or restore populations of the species in eastern Poland (Arciszewski et al., 2023; Pogorzelec et al., 2022; Pogorzelec et al., 2020). However, evidence regarding the establishment and persistence of ex situ-propagated individuals across peatland habitats differing in hydrological condition, vegetation structure and degree of transformation remains limited.

The aim of the study was to assess the potential of translocation as a tool used in the active conservation of the endangered species *Salix lapponum* in peatland habitats, where changes in abiotic and biocenotic conditions are caused directly and indirectly by anthropogenic pressure and global climate change.

The following research hypotheses were formulated: (1) the success of active conservation measures for *S. lapponum* based on translocation is lower at substitute habitats where conditions have been altered as a result of natural or anthropogenic transformations (the greater the deviation of habitat parameters from species-specific reference conditions,

the lower the survival rate of translocated individuals); (2) translocation using individuals produced under ex situ conditions may represent an effective tool of active conservation for *S. lapponum*, but only at sites that strictly meet the species' specific habitat requirements.

2. Materials and methods

2.1. Overview of the experimental design

The field experiment was conducted from September 2023 to June 2025. Its aim was to examine the acclimatization process of introduced plants to substitute sites differing in abiotic and biotic conditions. The planned activities included plant translocation to experimental sites, regular monitoring of habitat conditions, and observations of selected plant morphological parameters. The subsequent stages of the experiment, together with their duration, are presented in the scheme (Fig. 1).

Stage I – Pre-experimental phase – Preparation of plant material and selection of sites for the translocation experiment:

The plant material used in the translocation experiment consisted of *Salix lapponum* plantlets obtained using micropropagation methods from vegetative parts of mother plants. The use of developed and optimised micropropagation procedures made it possible to obtain approximately 200 plants constituting clones of individuals originating from natural populations in eastern Poland (Parzymies et al., 2020). After transfer to a solid substrate, the plantlets were placed in a phytotron, where they continued to grow under controlled conditions: day/night temperature 22/20°C, relative humidity 60%, light intensity 35 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and photoperiod 16/8h (day/night). The growth period in the phytotron lasted three months. The plants were then transferred for two months to a field acclimatization facility located in the immediate vicinity of the translocation sites. This allowed the seedlings to gradually adapt to natural habitat conditions before their introduction into the target habitats (methodology according to Pogorzelec et al., 2022).

The locations of the experimental sites were determined based on an analysis of the available literature and preliminary field observations in the Łęczna-Włodawa Lake District (Lublin Province, eastern Poland). The sites for the species translocation experiment were selected to represent (I) habitats characteristic of the region's peatlands, diverse in terms of abiotic and biocenotic conditions, (II) both undisturbed habitats and those undergoing accelerated ecological succession and changes caused by deliberate human activity (e.g. drainage); (III) sites occupied by *S. lapponum* populations and those where the species has not been recorded in the past (Pogorzelec et al., 2014).

Five sites were selected for the translocation experiment, located in: 1) a transitional mire (Bikcze, B) – a reference site with one of the largest populations of *S. lapponum* in the studied region (Łęczna-Włodawa Lake District) (Głębocka & Pogorzelec, 2017; Pogorzelec, 2008b); 2) a raised bog (Durne Bagno, DB), 3) transitional mire undergoing accelerated ecological succession (Splawy, S), 4) drained peatland transformed into mesic meadow on muck soil (Krowie Bagno, KB) and 5) degraded peatland (Piaseczno, P) (Table 1, Fig. 2).

Stage II – Planting procedure:

In September 2023, 40 individuals of *S. lapponum* were introduced at each experimental site. Plants were planted manually with intact root balls, either into the peat substrate or directly into the soil, depending on habitat characteristics. The saplings were randomly distributed within an area of approximately 25 m² at each site.

Stage III – Monitoring of plant establishment and performance:

The proportion of *S. lapponum* individuals that survived the winter period at the experimental sites and resumed growth (survival rate), as well as the proportion of flowering individuals (flowering rate) in the newly established populations, was assessed during two consecutive growing seasons in 2024 and 2025. Survival was evaluated each year in early spring, following the onset of the growing season.

Survival rate (SR) = number of surviving plantlets / number of translocated plantlets × 100%.

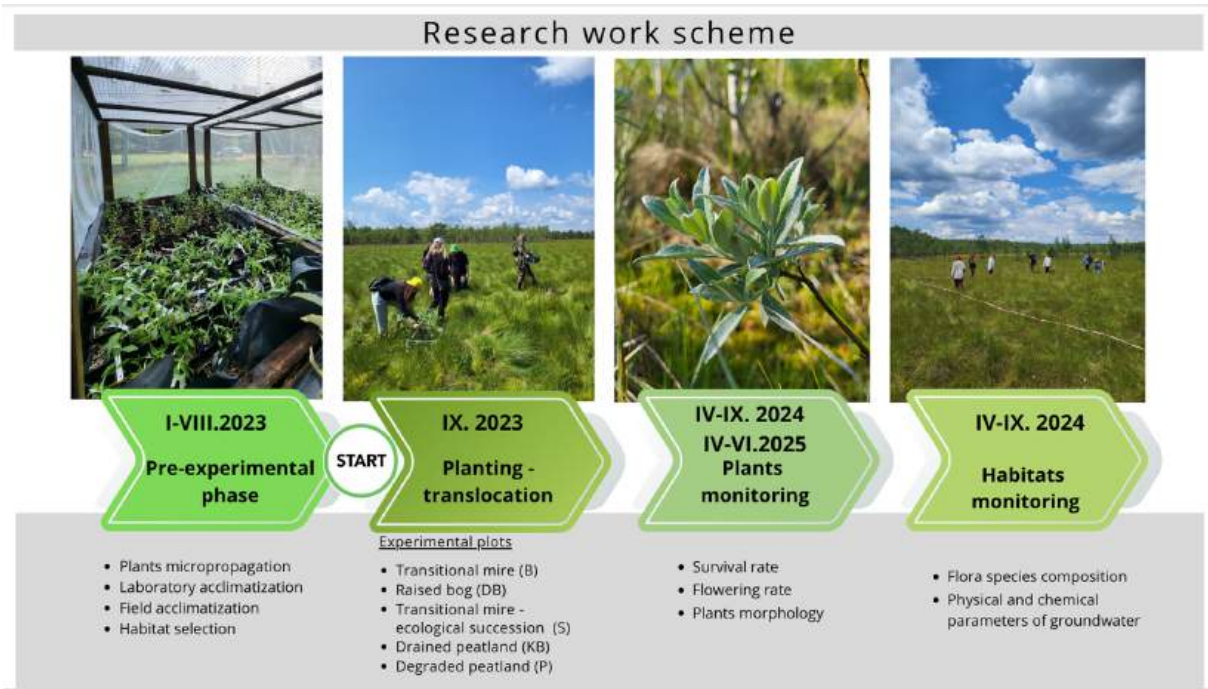


Fig. 1. A schematic representation of the translocation process, showing the successive stages, time frames and main activities performed at each stage.

Table 1
Characteristics of experimental plots.

Site	Bikcze (B)	Durne Bagno (DB)	Splawy (S)	Krowie Bagno (KB)	Piaseczno (P)
Habitat type	Transitional mire	Continental raised bog	Transitional mire	Mesic meadow	Peatland
Habitat condition/ status	Natural	Natural	Natural, ecological succession occurs	Transformed – drained, used for agriculture	Degraded
Location in the natural landscape	The shore of an eutrophic lake	A complex peatlands surrounded by forest	A complex of wetlands adjacent to the forest and lake	Wetland complex partially covered with meadow and scrub habitats	The shore of a mesotrophic lake
Dominant water source	Surface runoff	Precipitation	Precipitation and ground water	Precipitation	Surface runoff
Habitat water availability during the growing season	Water table above the acrotelm layer	Fluctuating water level	Fluctuating water level	Fluctuating water level	Fluctuating water table above ground level
Main threats	Ecological succession and woody vegetation encroachment	Reduced precipitation and drying of the moss layer	Accelerated ecological succession and encroachment of competitive woody species	Invasive species, agricultural chemical pollution and drainage	Progressive peat mineralization, encroachment of expansive and invasive species, recreational use of the lake
Conservation status	Natura 2000 SAC Jeziora Uściwierskie PLH060009	Polesie National Park	Polesie National Park	–	Landscape Park Pojezierze Łęczyńskie
<i>S. lapponum</i> population	currently functioning	known in the past	known in the past	never recorded	never recorded

Flowering rate (FR) = number of flowering plantlets / number of surviving plantlets × 100%.

Observations of *S. lapponum* individuals introduced into the environment were conducted regularly, at monthly intervals at each site. The overall condition of the plants was assessed on the basis of selected qualitative morphological characteristics and changes occurring during the field experiment. Plant morphology was assessed based on shoot architecture (main shoot growth and lateral shoots development), leaf blades morphology, the presence and density of tomentum, leaf coloration and occurrence of leaf deformations. Photographic documentation was collected at all observation dates.

Stage IV – Monitoring of abiotic and biocenotic habitat parameters:

From the beginning of the growing season (April 2024), measurements of parameters of shallow groundwater available to plants were

conducted over a five-month period. Measurements were performed in situ, at the experimental sites. Water pH and electrical conductivity (EC; μS/cm) were measured using a portable multiparameter field meter (YSI 556 MPS; YSI Inc., USA). In addition, from April to August 2024, water samples (1 dm³) were collected to determine physicochemical parameters in the laboratory. Water samples at the peatland sites (B, DB, and S) were collected from the acrotelm layer, whereas samples at the mesic meadow (site KB) were taken from a drainage ditch and those at the degraded peatland (site P) from the littoral zone of the lake. Due to low groundwater levels, measurements could not be performed at site KB in July 2024, nor at sites KB and DB in August 2024.

Standard laboratory methods were used to determine the content of: total nitrogen (NT; mg N/dm³. Photometric detection of generated nitrate after UV and thermal digestion via reduction to nitrite and azo dye

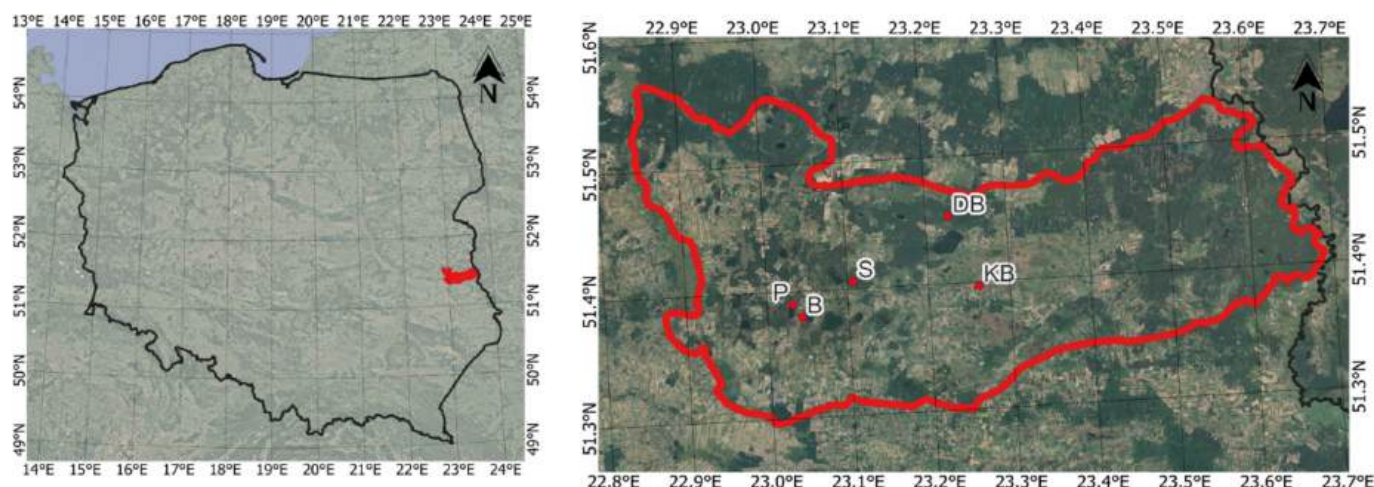


Fig. 2. Location of the experimental translocation sites in Poland. Site abbreviations correspond to experimental locations described in the text.

formation. Flow analysis method according to DIN EN ISO 29441), nitrate content in water (N-NO_3 ; mg N/dm^3 , Photometric via azo dye formation. Flow analysis method according to DIN EN ISO 13395). The concentration of total phosphorus (PT; mg P/dm^3) and phosphates (P-PO_4 ; mg P/dm^3) was determined by spectrophotometry (SPECORD40 spectrophotometer, Analytik Jena, GmbH, Jena, Germany) with ammonium molybdate. The content of total organic carbon (TOC; mg C/dm^3), chemical oxygen demand (COD; $\text{mg O}_2/\text{dm}^3$) and biochemical oxygen demand (BOD; $\text{mg O}_2/\text{dm}^3$) was determined using an automatic PASTEL UV analyser (Secoman, France).

Floristic composition, as well as qualitative and quantitative structure of vegetation at the experimental sites, was assessed using the Braun–Blanquet phytosociological relevé method (Braun–Blanquet, 1932). At each site, three relevés were recorded during the 2024

growing season: spring (April), summer (June), and late summer (August), in order to capture seasonal variability in species composition. Relevés were conducted on plots of a fixed area of 25 m^2 . Plant species were identified using the taxonomic keys of Rutkowski (2008) and Kłosowski & Kłosowski (2006). The cover of individual plant species was estimated using the seven-grade Braun–Blanquet scale. Based on the collected data, plant communities were classified into appropriate syn-taxonomic units following Matuszkiewicz et al. (2012).

Data on atmospheric conditions at plant translocation sites were obtained from the nearest meteorological station with complete and continuous data records, located in Włodawa ($\text{N } 51^\circ 32.97'$, $\text{E } 23^\circ 32.25'$). The meteorological data was used as the environmental background for the study. The average monthly air temperatures and total precipitation from January 2024 to June 2025 were taken into account (Fig. 3).

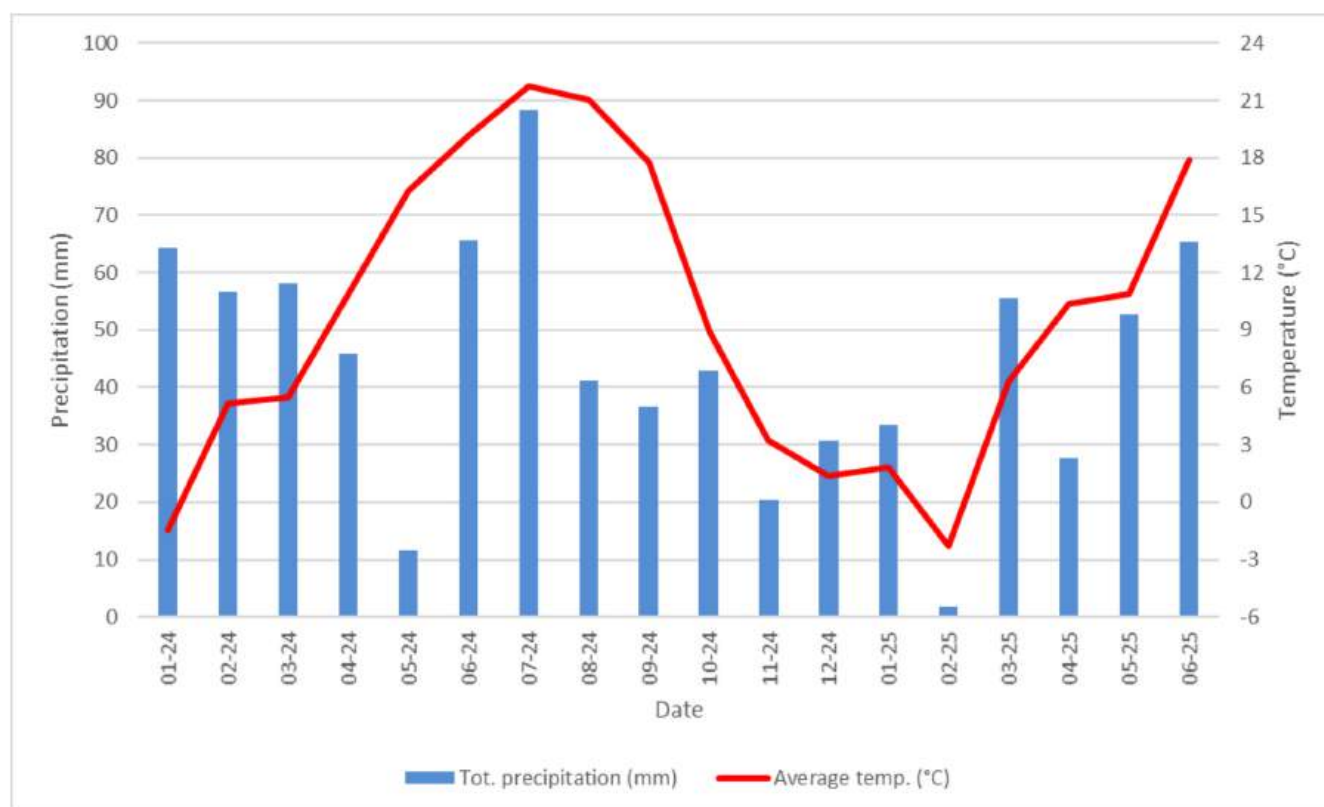


Fig. 3. Mean monthly air temperature and total monthly precipitation recorded at the Włodawa meteorological station from January 2024 to June 2025.

2.2. Statistical analyses

The Kruskal–Wallis non-parametric test was used to assess the significance of differences in the physical and chemical parameters of water between stations. The analysis was performed separately for each parameter. If the global test yielded a significant result, pairwise comparisons between sites were performed using Dunn's post hoc test, with the significance level corrected using the Bonferroni method.

Data on the species composition of the flora of the experimental sites were subjected to Hellinger transformation. Environmental variables (pH, EC, TOC, COD, BOD, PO₄, PT, NT) were transformed by logarithmisation (for variables with a skewed distribution) and standardisation to ensure comparability of scale. Principal component analysis (PCA) was performed on environmental data aggregated at the site level, using mean values calculated from monthly measurements for each site, to identify the main gradients and reduce collinearity. The first two PCA components (PC1 and PC2) were used as orthogonal predictors in canonical RDA (Redundancy Analysis). The significance of the model was assessed using a permutation test, and the strength of the explanation of variability was determined based on the R² and adjusted R² coefficients. The results are presented in the form of PCA and RDA biplots, allowing for the interpretation of the distribution of sites in relation to the main environmental gradients. All statistical analyses were performed in the R computing environment using functions from the *stats*, *rstatix*, and *vegan* packages (Kassambara, 2025; Oksanen et al., 2026), along with base R functions (R Core Team, 2023). Visualisations were supported by the *factoextra* (Kassambara & Mundt, 2026) and *ggplot2* packages (Wickham, 2016).

3. Results and discussion

The translocation experiment conducted in 2023–2025 enabled the

assessment of survival, flowering and morphological response of *Salix lapponum* individuals introduced into habitats differing in abiotic and biocenotic conditions, and degree of anthropogenic transformation.

3.1. Assessment of plant survival and reproduction

In the present study, *S. lapponum* specimens were planted in locations where habitat conditions were supposed to reflect the progressive changes taking place under the influence of anthropogenic pressure and global warming. Monitoring carried out in the spring of the following year showed that the highest survival rate (SR) was found in experimental populations of *S. lapponum* in drained peatland, transformed into a mesic meadow habitat (site KB). In 2025, however, the number of individuals in this site decreased significantly (only 13 individuals survived). In the remaining populations, the SR value ranged from 75% to 77.5% in 2024, and in 2025 it decreased slightly in two sites (raised bog – DB and transitional mire – S). In the transitional mire (B) and degraded peatland (P), the number of individuals in the population remained stable with a SR of 77.5% and 75% respectively (Fig. 4).

Data presented by Godefroid et al. (2011), analysing the outcomes of more than 200 reintroduction experiments conducted worldwide, indicate that the SR for the studied species was 52%, which is lower than the values obtained during both years of monitoring of the newly established *S. lapponum* populations. Nevertheless, the hypothesis that success rates decline over time is supported. This trend has also been reported by other authors presenting the results of active conservation measures applied to various plant species worldwide (Drayton & Primack, 2012; Haskins & Keel, 2012; Segovia-Rivas et al., 2018).

One of the indicators of successful reintroduction is the ability of plants to begin the generative reproduction cycle, the first stage of which is flowering. In 2024, *S. lapponum* individuals that produced inflorescences were recorded in all experimental populations. The highest

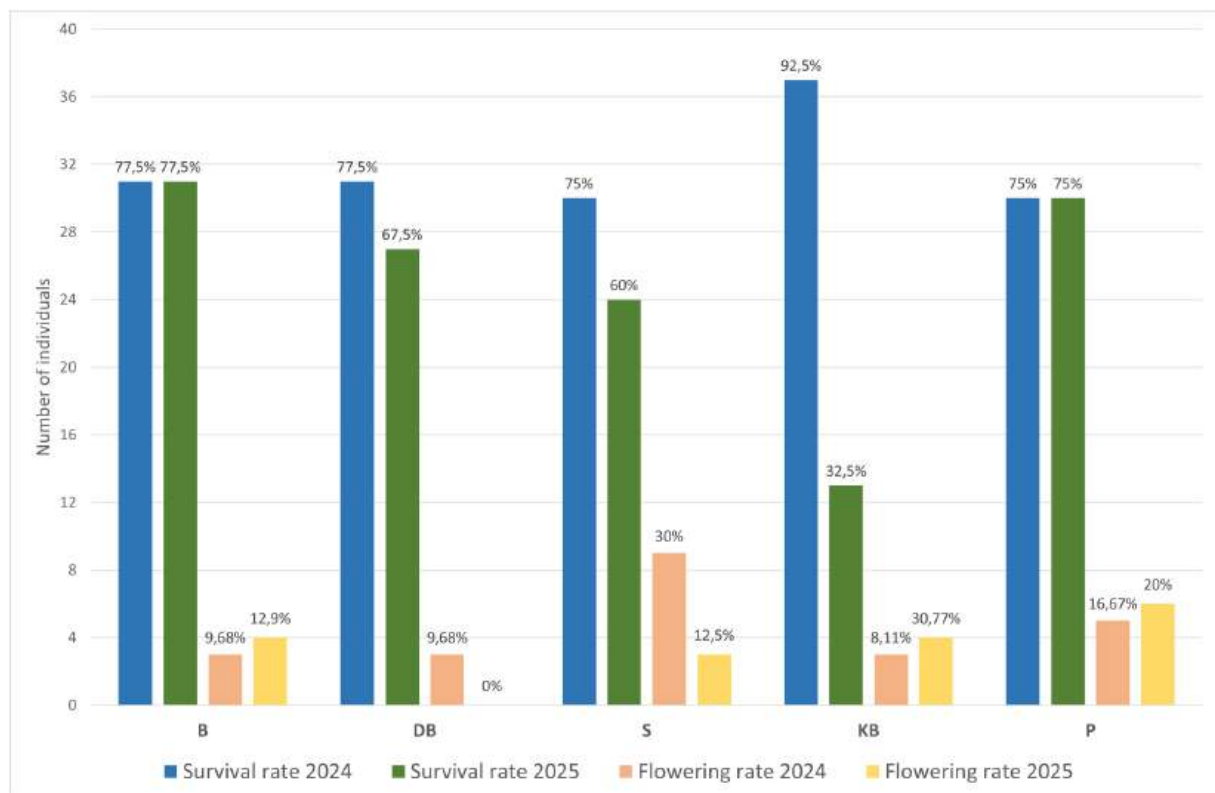


Fig. 4. Survival and flowering rates of plants on experimental plots at the beginning of two growing seasons. The values above the bars represent the SR and FR ratios, expressed as a percentage (%). B – transitional mire, reference site, DB – raised bog, S – transitional mire undergoing accelerated ecological succession, KB – drained peatland transformed into mesic meadow, P – degraded peatland.

flowering rate in 2024 (FR = 30%) was found in the population on a transitional mire undergoing accelerated ecological succession (S), but unfortunately, the following year the rate decreased to 12% (Fig. 4). In all experimental sites, between 8% and 30% of individuals flowered in both years following translocation. According to the meta-analysis by Godefroid et al. (2011), the average flowering rate of reintroduced plant species is low, around 19%. In woody species or clonal lineages, this rate can be largely unpredictable due to extended vegetative phases (Sun et al., 2022) and marked variation in fecundity within populations, with less than a quarter of clones typically accounting for almost half of the total flower production (Kang & Lindgren, 1998).

3.2. Variability of morphological features in relation to habitat conditions

Plant observations conducted in 2024 as part of post-translocation monitoring allowed for the characterisation of their overall condition by identifying changes in the morphology of individual *S. lapponum* specimens. Most of these changes were the result of natural phenological processes (e.g. leafing, changes in leaf colour), but some were directly related to the conditions prevailing in the experimental sites (e.g. high water levels or water shortages in the habitat).

In spring 2024, *S. lapponum* individuals at the natural peatland sites (B, S, DB) as well as at the drained peatland (site KB) exhibited a characteristic shoot morphology. Plants that survived the winter period developed short internodes in the apical parts of the shoots, where numerous leaves densely covered with tomentum were concentrated. The overall growth form was typical of the species, particularly of the ecotype occurring in peatland ecosystems of eastern Poland (Pogorzelec,

2008a, 2010). Only at the degraded peatland (site P), where a persistently high water level was recorded in spring, plant shoots were exceptionally long, with leaves occurring at high density primarily in the apical portions of the shoots (Fig. 5).

At the reference site on transitional mire (B), typical phenological changes in *S. lapponum* individuals were observed during the summer period (July – September 2024), including the gradual disappearance of tomentum and the appearance of discolouration on the leaf blades. In the spring of 2025, the specimens were in good condition and resumed vegetative growth, forming the characteristic shape of shrubs, typical of the species (Fig. 5). In the phytocoenosis of the mire site B, the herbaceous layer was dominated by species of the genus *Carex* typical of mires, as well as *Comarum palustre* and *Menyanthes trifoliata*. The presence of shrubs of the genus *Salix* and trees (*Betula pubescens*, *B. pendula*) was noted, whose crowns were not dense and did not shade the surface of the mire (Table S1). The phytocenosis was classified within the *Caricion nigrae* alliance, with characteristics of the *Caricetum rostratae* and *Caricetum limosae* communities.

At the site located on raised bog (DB), at the beginning of the 2024 growing season, the main shoots of *S. lapponum* individuals were characterised by small leaves, significantly smaller than those of plants growing in other locations. During the summer, intensive growth of main shoots was observed, accompanied by the development of large leaf blades with a less developed tomentum layer, as well as the development of young shoots growing from the lower parts of the plants (Fig. 5). The morphological changes observed can be linked to the low amount of rainfall in early spring, which determined weaker plant growth, and to the increase in rainfall in summer, which favoured more



Fig. 5. Seasonal changes in the morphology of *Salix lapponum* at five experimental sites (B, DB, S, KB, and P) documented in spring and summer 2024 and spring 2025. B – transitional mire, reference site, DB – raised bog, S – transitional mire undergoing accelerated ecological succession, KB – drained peatland transformed into mesic meadow, P – degraded peatland.

intensive development (Fig. 3; Grossiord et al., 2017; Marcysiak, 2012a). The phytocenosis in the DB experimental site was characterised by low species richness and the absence of tree and shrub species. The moss layer was dominated by species of the genus *Sphagnum*, while the herbaceous layer was mainly composed of species characteristic of the *Oxycocco-Sphagnetum* class, such as *Oxycoccus palustris* and *Eriophorum vaginatum* (Table S1). Based on phytosociological relevés, the phytocenosis was classified as *Sphagnion magellanici* alliance.

S. lapponum individuals growing at the site on transitional mire (S) in 2024 and spring 2025 were characterised by the production of numerous young shoots at the base of the main stem, accompanied by the dieback of older shoots (Fig. 5). The phytocenosis structure of experimental site S was characterised by a large proportion of species that could potentially compete with *S. lapponum*, limiting its access to light and the space necessary for the growth of individual specimens. The phytocenosis had a very high proportion of tree and shrub species characteristic of the *Alnetea glutinosae* class, such as *Alnus glutinosa*, *Betula humilis* and *Salix cinerea*, which formed dense clumps in places. In the herbaceous layer, in addition to species characteristic of mires communities, the presence of rush species from the *Phragmitetea* class, such as *Typha latifolia* and *Thelypteris palustris*, was noted. The phytocenosis represented a transitional community between mire vegetation and shrub-swamp communities and was most closely related to the *Alnion glutinosae* alliance. (Table S1).

At the drained peatland (site KB), during the summer of 2024, a gradual thinning of the tomentum on leaf blades of *S. lapponum* was observed, accompanied by the appearance of numerous discolorations and leaf deformities, as well as the dieback of some shoots (Fig. 5). These symptoms were likely associated with the limited availability of water recorded at this site. In spring 2025, dieback of older shoots was observed in some individuals, accompanied by intensive production of young resprouting shoots. Analysis of phytosociological relevés recorded at the site KB revealed the presence of species characteristic of the class *Molinio-Arrhenatheretea*. The phytocenosis was most closely related to wet meadow communities of *Calthion palustris* alliance, although a substantial contribution of expansive species, *Phragmites australis*, *Eupatorium cannabinum* and *Salix cinerea*, indicates a transitional character with elements of reedbed and shrub vegetation (Table S1).

At the degraded peatland adjacent to Lake Piaseczno (site P), following a decrease in water level during the summer of 2024, some of the elongated shoots collapsed onto the substrate and became rooted through the formation of adventitious roots. At the same time, abundantly foliated lateral shoots began to grow upright. In spring 2025, in the absence of standing water, numerous young shoots emerged from the shoots that had rooted in the previous year. These shoots were straight and unbranched, bearing numerous leaves densely covered with tomentum (Fig. 5). The phytocenosis at experimental site P was dominated by *Phragmites australis* and was most closely related to communities of *Phragmition* alliance. The other helophyte species were also present, along with submerged aquatic plants of the genus *Potamogeton*. In the shrub layer, *Betula pendula* and *Salix cinerea* occurred (Table S1). Vegetation did not form compact stands and therefore did not pose a significant constraint on light availability or space for the introduced *S. lapponum* individuals.

In the case of species of the genus *Salix*, which are widespread in the northern hemisphere, enormous variability in the morphological characteristics of individuals is observed even within a single ecotype. *S. lapponum* is no exception, and the results of studies conducted in various European populations indicate that individual morphotypes are associated with the specific environmental conditions in which they function (Fijałkowski, 1958; Hroneš et al., 2018; Pogorzelec, 2008a). Kolos et al. (2015) also found that the competition for access to light determines the morphological structure of *S. lapponum* shoots. In shaded conditions, the shoots are less branched and significantly longer than those growing in full sunlight. Research conducted by (Marcysiak, 2012b, 2012a), Todeschini et al. (2011) and Castro-Díez et al. (1997)

confirm that environmental conditions, especially local climatic conditions, soil moisture, nutrient concentration and pH, modify the morphology of the leaves of various woody plant species of the genera *Salix*, *Betula*, *Populus* and *Quercus*. It is also worth noting that shoots of the same plant may differ in terms of hair density, leaf shape and leaf arrangement depending on their age (Rechinger, 1992).

Other factors may also be responsible for the morphological variability of the studied species. In the case of long-isolated or marginal populations, but also when phenomena such as hybridisation and introgression, genetic drift, and bottleneck effects occur, changes in the structure of individuals can be observed (Keller & Waller, 2002). In the case of the group of plants studied in our experiment in 2024–2025, the above-mentioned factors can be excluded, as all individuals were clones of representatives of the species originating from a single population functioning in eastern Poland.

Studies conducted in Polish and Czech populations of *S. lapponum* have shown that this species may occur in sites with diverse floristic composition (Hroneš et al., 2018; Pogorzelec, 2008a). However, as indicated by Hroneš et al. (2018), only a subset of plant communities provides conditions close to the species' ecological optimum, thereby supporting its proper functioning. The sites selected for the translocation experiment in 2023–2025, dominated by species characteristic of transitional mires (B and S) and wet habitats (P), proved to be the most suitable for the growth of *S. lapponum*. Hroneš et al. (2018) describe as conducive to the full functionality of the population, which is manifested, e.g., by a higher proportion of flowering individuals. Studies conducted by Kolos et al. (2015) and Serafin et al. (2015) indicate that even under stable hydrological conditions of the habitat, the species composition of phytocenoses, and in particular the stage of ecological succession and the accompanying competitive pressure from trees and shrubs, may be the main factor limiting the functioning of the *S. lapponum* population. This relationship was probably observed at transitional mire (site S), where the phytocenosis contains a significant number of shrub species that competed with *S. lapponum* for space and access to light. At this site, a significant decline in the number of individuals of the studied species was observed in the second year of monitoring.

3.3. Characteristics of habitat parameters

The functioning of wetland habitats is directly dependent on water conditions, and the physical and chemical factors of the substrate significantly shape the structure and development of plant communities. The key factor differentiating the species composition of vegetation is the chemical-trophic gradient, expressed primarily by pH values, EC and the availability of biogenic elements (Hájek et al., 2006; Wheeler & Proctor, 2000).

The results of statistical analyses confirmed significant differences in the pH values of water available to plants at the raised bog (site DB; lowest pH) and drained peatland (KB site; highest pH). Statistically significant differences in the values of parameters related to organic matter content (BOD, COD and TOC) were recorded between the raised bog (site DB), and the drained and degraded peatland (sites KB and P), which were characterised by lower organic matter content. The highest EC values were recorded at the KB site. Due to the very low content of various forms of phosphorus and nitrogen in the water at all studied sites, no statistical differences were found in this respect (Table 2).

Peatland ecosystems support species that are closely associated with the specific biotic and abiotic conditions prevailing in these habitats and are often highly sensitive to environmental change. Although natural populations of *S. lapponum* in eastern Poland are primarily located in wetland areas, previous studies indicate that the species exhibits a relatively broad ecological tolerance with respect to selected abiotic habitat parameters (Pogorzelec, 2008a; Serafin et al., 2015). Elevated EC values recorded at the drained peatland site (KB) may indicate increased water mineralisation and potentially higher salinity levels.

Table 2
Variation in physicochemical water parameters (mean ± SD) among the translocation sites. Different letters indicate statistically significant differences between sites based on the Kruskal–Wallis test followed by Dunn’s post hoc test with correction for multiple comparisons ($p < 0.05$). B – transitional mire, reference site, DB – raised bog, S – transitional mire undergoing accelerated ecological succession, KB – drained peatland transformed into mesic meadow, P – degraded peatland.

Parameter/ Site	pH	EC (μS/cm)	TOC (mg C/ dm ³)	COD (mg O ₂ / dm ³)	BOD (mg O ₂ / dm ³)	P-PO ₄ (mg P/ dm ³)	PT (mg P/ dm ³)	NT (mg N/ dm ³)	N-NO ₃ (mg N/ dm ³)
B	5.67 ± 0.79 ^{ab}	403.94 ± 288.41 ^a	40.04 ± 12.55 ^{ab}	95.10 ± 30.45 ^{ab}	48.00 ± 17.40 ^{ab}	0.808 ± 0.581 ^a	0.907 ± 0.429 ^a	3.854 ± 2.392 ^a	<0.2
DB	4.74 ± 0.21 ^a	312.25 ± 161.11 ^a	68.38 ± 17.47 ^a	156.75 ± 41.15 ^a	90.63 ± 22.77 ^a	0.775 ± 0.584 ^a	0.931 ± 0.436 ^a	2.640 ± 1.069 ^a	<0.2
S	6.84 ± 0.14 ^{ab}	313.60 ± 13.41 ^a	34.94 ± 10.55 ^{ab}	78.50 ± 27.35 ^{ab}	46.24 ± 14.39 ^a	0.290 ± 0.407 ^a	0.376 ± 0.392 ^a	3.435 ± 2.522 ^a	<0.2
KB	7.20 ± 0.00 ^b	946.67 ± 238.37 ^a	10.90 ± 3.79 ^b	25.43 ± 6.41 ^b	14.63 ± 5.29 ^b	0.033 ± 0.018 ^a	0.088 ± 0.054 ^a	1.584 ± 0.856 ^a	<0.2
P	6.66 ± 0.42 ^{ab}	106.44 ± 20.57 ^a	10.28 ± 2.19 ^b	24.72 ± 5.16 ^b	13.96 ± 2.80 ^b	0.065 ± 0.078 ^a	0.134 ± 0.084 ^a	1.294 ± 0.261 ^a	<0.2

Experimental studies conducted by Arciszewski et al. (2025) demonstrated that increased salinity negatively affects shoot growth and selected physiological parameters of *S. lapponum*, including photosynthetic efficiency.

Despite the low nitrogen content at the sites studied, *S. lapponum* is capable of functioning both in conditions of low nitrogen availability and in environments with higher levels of this nutrient, as confirmed by experimental studies conducted by Arciszewski et al. (2025). At the same time, it has been shown that with higher nitrogen availability, *S. lapponum* individuals are characterised by better condition, faster growth rate and more favourable physiological parameters (Arciszewski et al., 2025).

Considering the characteristics of habitats in experimental sites, as determined by the results of physical and chemical analyses of water, (based on mean values of environmental variables calculated for each site from monthly measurements) a principal component analysis (PCA) was performed, which indicated a division into natural peatland habitats and transformed habitats (Fig. 6a). Site DB, located on a raised bog, was clearly distant from the other natural peatland sites (S and B) and

most strongly associated with higher phosphate values and organic matter indicators.

Drained peatland (site KB) was strongly correlated with electrical conductivity, indicating a higher degree of water mineralisation. In contrast, degraded peatland (site P) was the most distant from the other sites and was associated with the lowest EC values and relatively high pH, highlighting its distinctly different character, related to peatland degradation.

The influence of the main environmental gradients identified by the PCA on plant community composition was assessed using Redundancy Analysis (RDA), with PC1 and PC2 axes used as explanatory variables. The resulting RDA model was marginally statistically significant (permutation test, $p \approx 0.05$) and explained approximately 61% of the total variance in the species composition of plant communities at the experimental sites. The adjusted coefficient of determination ($R^2_{adj} \approx 0.22$) indicates a moderate strength of the relationship between environmental variables and plant communities structure.

The similar arrangement of sites on the PCA and RDA biplots indicates that the structure of plant community composition reflects the

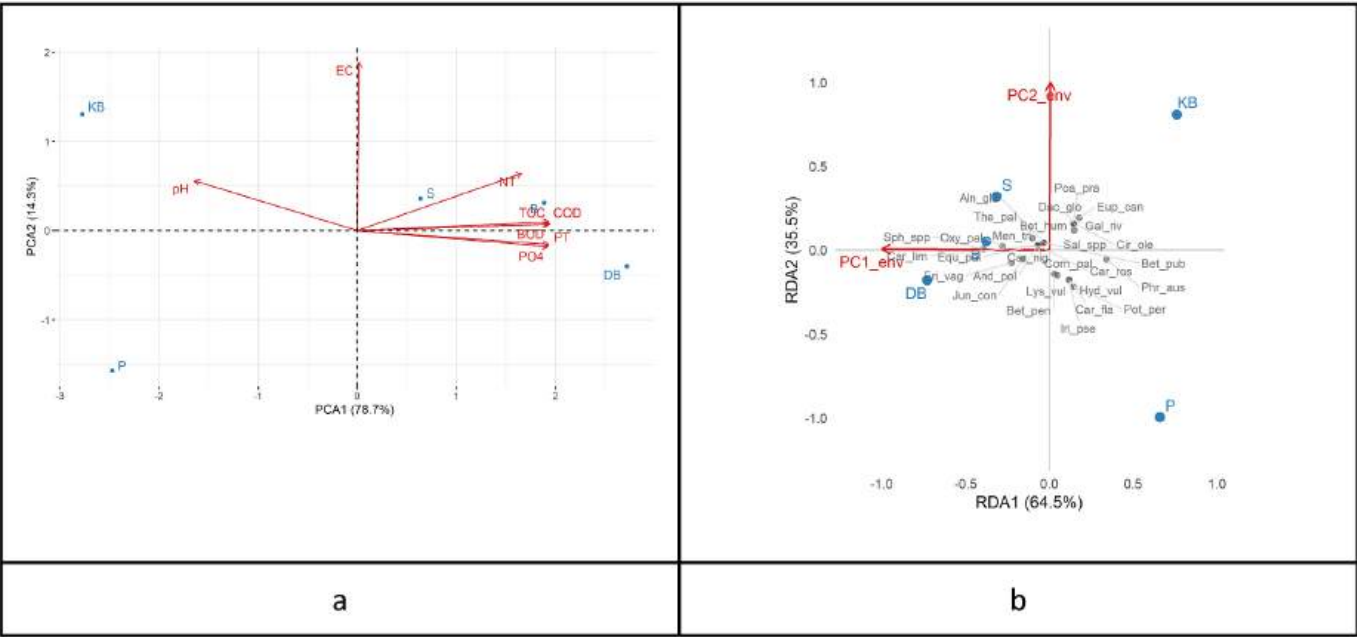


Fig. 6. Principal Component Analysis (PCA) of physicochemical water parameters at the sites selected for the translocation experiment conducted in 2023. Points represent the study sites (B, S, DB, KB, P), while vectors indicate environmental variables: pH, electrical conductivity (EC), total organic carbon (TOC), chemical oxygen demand (COD), biochemical oxygen demand (BOD), phosphates (PO₄), total phosphorus (PT), and total nitrogen (NT). The direction and length of vectors reflect the relationships between variables and the ordination axes (a); Redundancy Analysis (RDA) biplot illustrating relationships between plant species composition and environmental conditions at the translocation sites. The diagram is presented using scaling type 2. Species abbreviations corresponding to plant taxa are explained in Table S2 in the Supplementary material (b).

physicochemical properties of water, and that sites KB and P remain clearly separated from peatland habitats in both the environmental and species-based analyses (Fig. 6b).

Considering the indicative importance of plant species, it can be concluded that the habitat conditions in natural peatland (sites B, S and DB) are most similar and, at the same time, meet the requirements of *S. lapponum* (Pogorzelec et al., 2020b; Serafin et al., 2015). Despite clear differences in both the composition of the phytocenosis and the physical and chemical parameters of the water, the conditions at the site located on a degraded peatland (site P) also supported survival and establishment of *S. lapponum*, as indicated by the high survival and flowering rates. It is noteworthy that this site was located in the coastal zone of the lake and, despite changes in the water level, its presence itself seems to be a key factor here. The importance of hydrological conditions for the functioning of *S. lapponum* populations was also emphasised in studies by Hroneš et al. (2018), pointing to the important role of snow cover as a source of water during the spring thaw. In recent years, however, there has been a marked decline in the extent and persistence of snow cover in Poland, which may lead to water deficit at the beginning of the growing season (Wibig & Jędruszkiewicz, 2023). Meteorological data from the Włodawa station indicate lower total spring precipitation in 2025 compared with 2024 (Fig. 3). These differences may partially explain the variation in survival rates observed between the two study years. In 2024, which was characterised by higher spring precipitation, high survival of introduced individuals was recorded at all sites, whereas in 2025 a decline in survival was observed, most pronounced at the drained peatland (site KB), which is more susceptible to periodic drought, and at the raised bog site (DB), which is supplied primarily by precipitation.

Assessing the suitability of habitats for conservation measures such as translocations requires comprehensive consideration of both the structure and composition of plant communities and key abiotic parameters related to substrate or soil properties. These factors determine the ability of plants to survive and adapt in new locations. At the same time, in conditions of progressive and multidirectional environmental changes, predicting the long-term direction of peatland ecosystem transformations is fraught with considerable uncertainty. This emphasises the need for careful selection of sites for translocation, based on a detailed analysis of habitat parameters, as well as the need for experimental activities (Albrecht & Long, 2019; Pogorzelec et al., 2020b; Stadtmann & Seddon, 2020).

The results of this study should be interpreted within the specific ecological and geographical context of eastern Poland. Since the experiment was conducted within a specific region, the transferability of the results to other regions may be limited. It should also be noted that changes in peatland ecosystems may vary depending on local environmental conditions, however, the experimental sites were selected to reflect a clear gradient of habitat change. An additional limitation of the study is the relatively small number of *S. lapponum* individuals introduced into the experimental sites, resulting from the conservation status of the species and the technical limitations associated with the propagation of plant material. Moreover, the monitoring period covered only two growing seasons and therefore does not allow for assessment of the long-term stability of the newly established populations. Therefore, this study should be regarded as an experiment aimed at identifying possible scenarios for the survival of the *S. lapponum* population under conditions of ongoing habitat transformation at the edge of its range.

4. Conclusions

The results of the translocation experiment only partially support hypothesis 1. Although the highest survival rate of *S. lapponum* was recorded in the reference transitional mire, high survival and flowering rates were also observed in the degraded peatland, despite significant differences in vegetation composition and abiotic conditions of habitat. These results suggest that *S. lapponum* exhibits a relatively broad ecological tolerance within the environmental conditions represented

by the experimental sites and is capable of surviving, maintaining vegetative growth, and initiating flowering in substitute sites that differ from its typical peatland habitats.

Hypothesis 2 was not supported by the obtained results, as it was demonstrated that translocation can be an effective tool for the active conservation of *S. lapponum* not only in habitats with conditions close to the species' ecological optimum. Individuals generated ex situ can also survive and begin their reproductive cycle in transformed peatland habitats, provided that stable hydrological conditions are maintained. Peatland drainage and advanced ecological succession can pose a serious threat to the survival of newly established *S. lapponum* populations, as observed at the drained peatland (site KB) and transitional mire (site S) during the experiment.

CRediT authorship contribution statement

Michał Arciszewski: Writing – original draft, Visualization, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Magdalena Pogorzelec:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization. **Urszula Bronowicka-Mielniczuk:** Writing – review & editing, Visualization, Validation, Formal analysis. **Mateusz Piejak:** Writing – review & editing, Visualization, Resources.

Ethics declaration

The author(s) declare(s) that the study does not involve humans nor animal subjects.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jnc.2026.127403>.

Data availability

Data will be made available on request.

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Article

Physiological Indicators for Post-Translocation Monitoring of *Salix lapponum* in Natural vs. Degraded Peatlands

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Abstract

The progressive degradation of natural habitats, driven by anthropogenic pressures and climate change, constitutes one of the most serious threats to biodiversity. Peatland ecosystems, along with the valuable plant species associated with them, are particularly vulnerable to these processes. *Salix lapponum*, a glacial relict species, is undergoing a drastic decline in both its range and population size across Poland and Europe. This emphasizes the need for the implementation of conservation measures, including species translocation, as well as the development of effective methods for monitoring plant condition following introduction. The aim of this study was to evaluate the usefulness of selected physiological indicators for the rapid and reliable assessment of plant condition in active conservation efforts. The experimental material consisted of *S. lapponum* plantlets derived from tissue culture, which were introduced into five experimental sites in eastern Poland, differing in habitat conditions. Over two growing seasons, chlorophyll fluorescence parameters (F_0 , F_m , F_v/F_m), the content of photosynthetic pigments and anthocyanins, relative water content, guaiacol peroxidase activity, and the presence of reactive oxygen species were analyzed. The results revealed clear seasonal variability in most of the studied physiological parameters, as well as their differentiation across habitat conditions. The highest sensitivity to environmental changes was observed for indicators related to photosynthetic performance (F_v/F_m), tissue hydration status (RWC), and enzymatic activity. Declines in photosystem II efficiency at the beginning of the growing season, reflected in F_v/F_m values decreasing to 0.47–0.49 indicate transient stress conditions in plants. Simultaneously, variation in relative water content (52–90%) and peroxidase activity reflects differences in water availability and the intensity of environmental stress across habitats. The findings confirm that selected physiological indicators can serve as effective tools for the early monitoring of plant condition and for assessing the success of *S. lapponum* translocation.

Keywords: active conservation; biomarkers; chlorophyll fluorescence; downy willow; environmental stress; glacial relicts; peatlands; translocation success



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1. Introduction

As a result of the increasing transformation of natural habitats driven by human activity and climate change, environmental conditions in which populations of many plant species persist are undergoing significant alteration [1]. As demonstrated by Staudt et al. [2], the disruption of ecosystem functioning may result from factors such as landscape fragmentation, alterations in water regimes, eutrophication, heavy metal pollution, accelerated ecological succession, and biological invasions. Peatlands are among the habitats that have

undergone particularly significant transformation in recent decades. Despite their relatively small global coverage, their degradation leads to disproportionately large ecological losses, making them one of the most threatened ecosystems [3–5]. Plants in transformed habitats are exposed to complex environmental stressors, which significantly affect their long-term condition. The primary effects of stress include reduced photosynthetic efficiency, growth inhibition, and disturbances in reproduction, which may ultimately lead to population extinction [6,7].

Glacial relict species are particularly sensitive to ongoing changes in natural habitats. These species persist as remnants of the glacial era and occupy small, isolated climatic refugia, primarily located in mountain and peatland ecosystems. The small population sizes of relict species, combined with their specific habitat requirements, make them especially vulnerable to environmental changes and local extinction [8,9]. Among such species is *Salix lapponum*, whose population size is declining drastically. This species was once widespread across numerous sites in Poland and Europe, but is currently threatened with extinction. *S. lapponum* is a small shrub that occurs in fens and transitional mires with a moderately acidic pH (4 to 6.5) in both open and partially shaded habitats. The species grows up to 1–1.5 m in height and is characterized by elliptical, olive-green leaves covered with a dense layer of tomentum [10–13]. Research conducted in eastern Poland in habitats occupied by *S. lapponum* has shown that the species most frequently occurs in phytocoenoses representing the classes *Alnetea glutinosae*, *Oxycocco-Sphagnetum*, and *Scheuchzerio-Caricetum* [14]. In Poland, the species reaches the southern limit of its distribution range and is currently found only at a few locations, which has resulted in its classification as critically endangered. The decline in *S. lapponum* populations is primarily driven by habitat changes associated with the drainage of wetlands [15]. In order to protect populations of *S. lapponum* in Poland, reintroduction efforts have been undertaken at sites where the species had previously been recorded [16,17].

In order to protect valuable plant taxa at risk of extinction, species translocations are increasingly used as a method of active protection [18]. However, the results of plant translocations remain highly uncertain because their success depends on many interacting factors, including the selection of an appropriate target site, the quality and origin of the propagation material, the biology and ecology of the species, and appropriate post-translocation monitoring methods. Numerous studies indicate that plant translocation is a process that involves significant expenses and requires careful planning, technical preparation, and effective logistical coordination [19–21]. A recent study of translocations in Europe showed that almost half of the applications were lost after five years, and only 28% became self-sustaining. The early mortality was high, with 40% of the effects resulting in less than 20% survival after the first year [22].

The restoration of endangered species populations should be closely linked with the protection of the habitats in which active conservation measures are implemented. Otherwise, the risk of failure of such efforts increases significantly [23]. The instability and ongoing transformation of habitat conditions require verification of whether a given species is capable of surviving under rapidly changing environmental conditions. It is also essential to identify alternative habitats in which the species could find a suitable ecological niche, thereby enabling effective population recovery in new locations [24]. One of the key determinants of successful translocation is the ability of plants to complete their full life cycle, including growth, flowering, and the production of viable offspring [23]. However, such assessment is a long-term process, often requiring many years of field observations. Therefore, there is a need to identify additional, more rapidly responding indicators, particularly physiological and morphological ones, that would enable the early assessment of plant condition and the prediction of translocation success [25]. In the case of plant

species threatened with extinction, the development of physiological monitoring protocols requires not only methodological sensitivity but also consideration of nature conservation constraints. Small and fragmented populations often limit the possibility of extensive tissue sampling, making the level of invasiveness an important factor in the selection of monitoring tools. Therefore, conservation physiology is increasingly emphasizing the development of minimally invasive methods that provide reliable information while minimizing disturbance to both individuals and their natural habitats [26,27].

Previous studies on *Salix lapponum* have primarily focused on its ecology, habitat requirements, and the processes driving population decline, particularly in relation to peatland transformation [15]. Experimental conservation efforts have addressed ex situ propagation and reintroduction, and the effectiveness of these actions was mainly evaluated using plant survival rates and morphological traits [17]. More recent research has incorporated physiological parameters, including chlorophyll fluorescence, photosynthetic pigments, and ROS accumulation, but these have been applied mainly under controlled conditions or during early stages of acclimatization. Notably, Pogorzelec et al. [16] analyzed these indicators across successive stages of the reintroduction process, focusing on plant acclimation under conditions corresponding to suitable, reference habitats.

However, the use of physiological indicators for post-translocation monitoring under natural, heterogeneous field conditions remains limited. In particular, there is a lack of studies assessing plant responses across habitats representing different degrees of peatland transformation. Therefore, this study introduces a novel approach by applying a set of physiological biomarkers to monitor *S. lapponum* after translocation across a gradient of peatland habitats differing in transformation level. This experimental design reflects potential future scenarios of habitat change and enables the evaluation of plant responses under realistic, multifactorial environmental conditions.

The aim of this study was to evaluate the usefulness of physiological indicators as tools for monitoring the condition of *S. lapponum* individuals in active conservation using translocation. Additionally, we assessed which of the examined parameters allow for a rapid and reliable evaluation of the effectiveness of conservation measures.

2. Results

2.1. Selected Chlorophyll Fluorescence Parameters

In 2024, the maximum quantum efficiency of photosystem II (F_v/F_m) in plants at the experimental sites increased from May to September, reaching the highest values at the end of the growing season (0.81–0.84). During the first year of the study, F_v/F_m values did not differ significantly among most translocation sites. Only plants growing on the degraded peatland exhibited lower values (approx. 0.69). Clear differences among plants growing in the studied habitats were observed at the beginning of the following growing season (May 2025). The lowest F_v/F_m values (approx. 0.47–0.49) were recorded in plants introduced into the raised bog and the drained peatland. In the remaining habitats, the maximum PSII efficiency values were lower than those recorded at the end of the previous growing season, but similar to those observed during the same period in the first year of the study (Figure 1a).

For the remaining chlorophyll fluorescence parameters (F_0 and F_m), only minor statistical differences were observed among plants across the studied sites. Plants growing at the reference site (A) and at the raised bog site (B) were characterized by relatively stable values of both minimal and maximal fluorescence. The lowest values of these parameters, along with pronounced seasonal fluctuations, were recorded in plants inhabiting the degraded peatland site (Figure 1b,c).

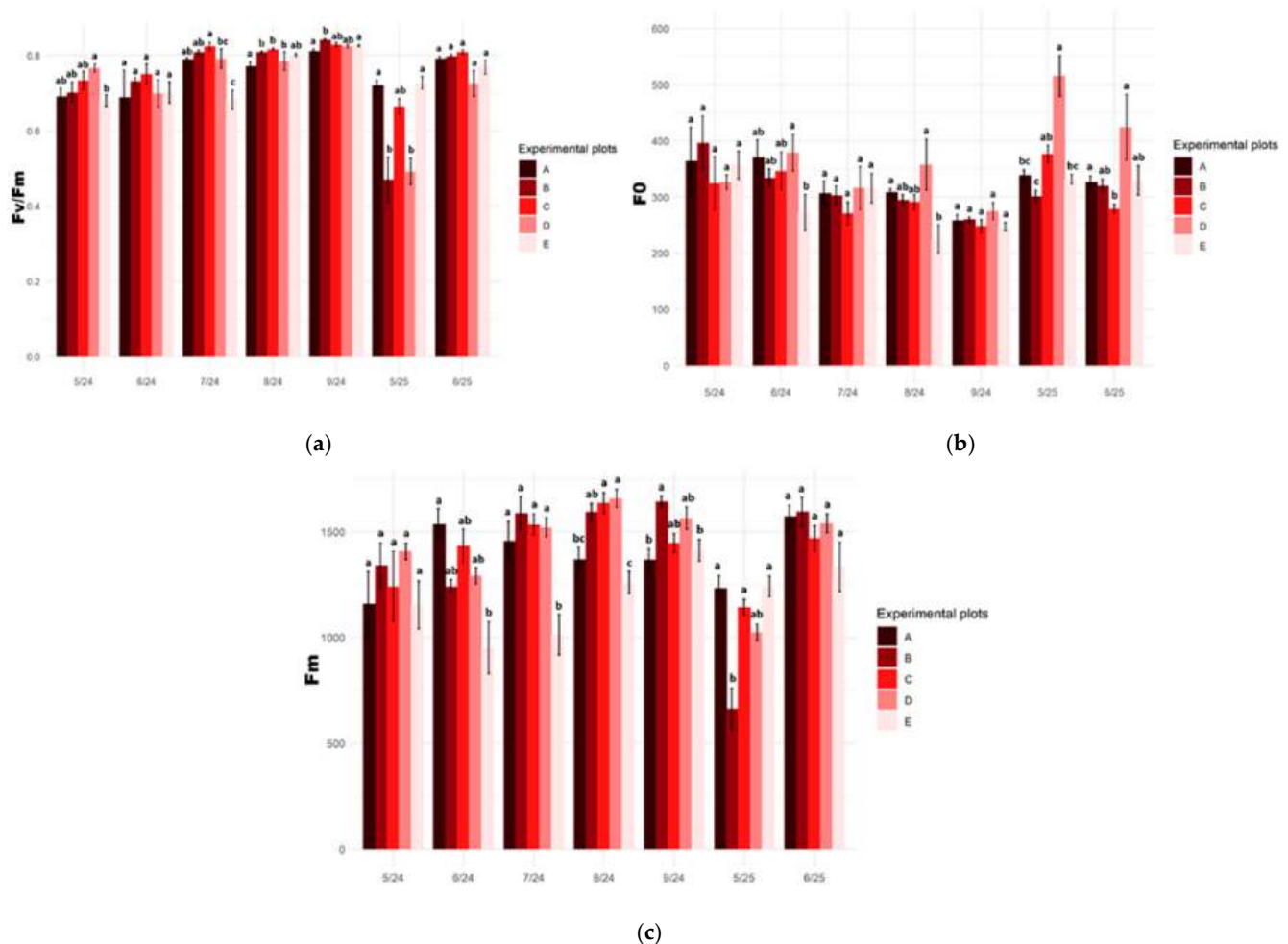


Figure 1. Chlorophyll fluorescence parameters in the leaves of *Salix lapponum* at five experimental sites (A–E) from May 2024 to June 2025: (a) maximum quantum efficiency of PSII (F_v/F_m); (b) minimal fluorescence (F_0); (c) maximal fluorescence (F_m). Bars represent mean values $\pm$ SD, $n = 10$. Different letters above the bars indicate significant differences among sites within a given sampling term (Kruskal–Wallis test, $p \leq 0.05$). Experimental plots: A—transitional mire (reference site), B—raised bog, C—transitional mire undergoing ecological succession, D—drained peatland, E—degraded peatland.

2.2. Photosynthetic Pigment Content

The chlorophyll *a* (Chl *a*) and chlorophyll *b* (Chl *b*) content in the tissues of *S. lapponum* plants growing at the reference site (A) showed relative stability during both growing seasons. Seasonal variation of the tested pigments in plant tissues was moderate, without sudden fluctuations. Compared to the reference site, the Chl *a* and Chl *b* content in plants growing in the other habitats revealed greater variability, particularly in the degraded peatland (E), where marked decreases and increases in the content of these pigments were observed (Figure 2a,b).

The Chl *a/b* ratio in the tissues of the studied plants ranged from approximately 1.5 to 3.5 and exhibited significant seasonal variation. In 2024, the highest values of this parameter were recorded in May (site A) and July (site E), whereas in August and September, a decline in the ratio was observed, particularly pronounced in plants at site E. At the beginning of the following growing season (May 2025), the Chl *a/b* ratio in plant tissues exceeded 2.5 at all sites (Figure 2c).

The carotenoid (Car) content in the tissues of plants introduced into the experimental sites varied dynamically throughout the growing season. However, no statistically signif-

ificant differences in the carotenoid content in the tissues of *Salix lapponum* individuals were observed within the individual sampling times (Figure 2d).

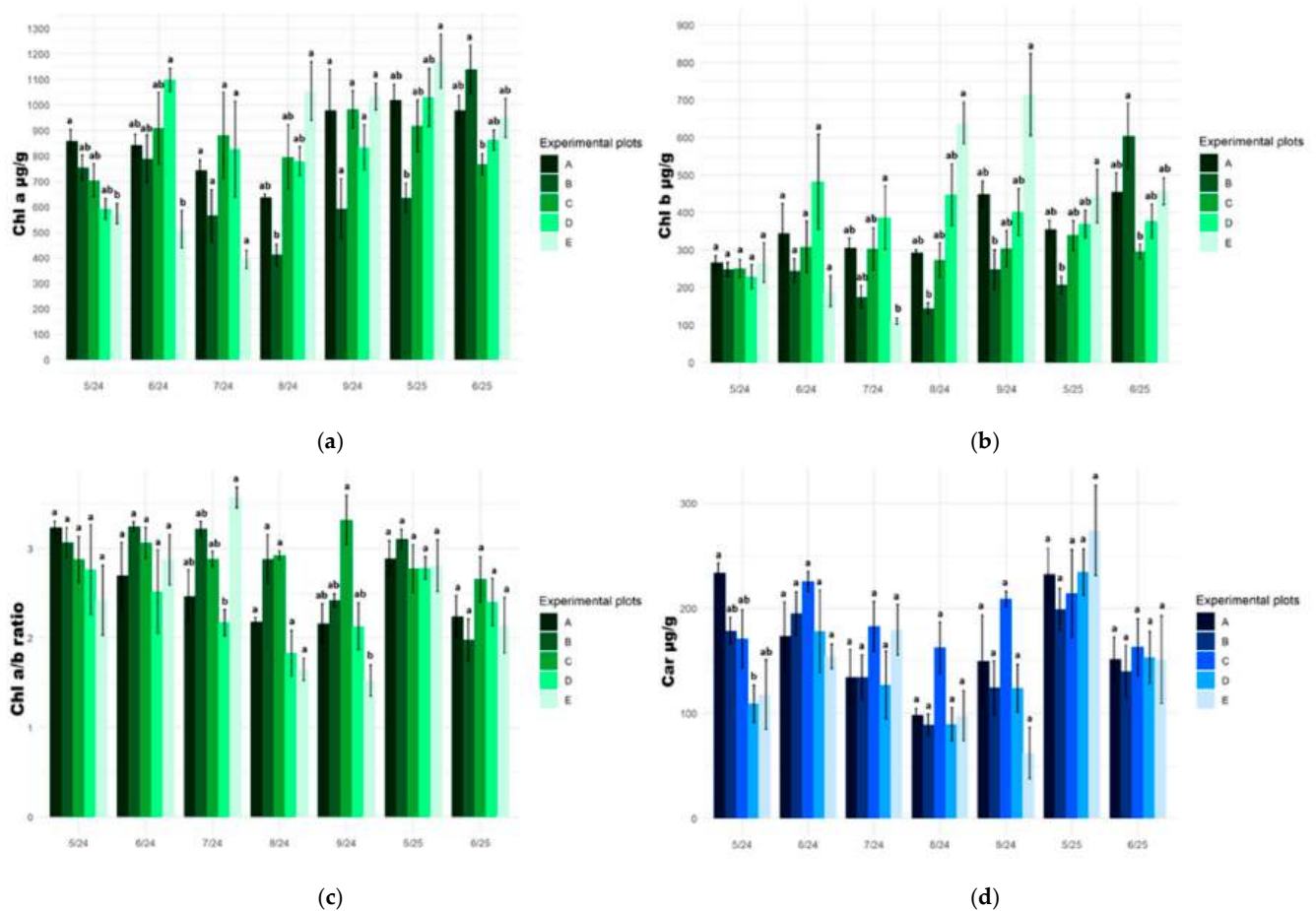


Figure 2. Photosynthetic pigment content in leaves of *Salix lapponum* at experimental sites in consecutive sampling periods (May–September 2024; May–June 2025): (a) chlorophyll *a* (Chl *a*; $\mu\text{g}\cdot\text{g}^{-1}$ FW); (b) chlorophyll *b* (Chl *b*; $\mu\text{g}\cdot\text{g}^{-1}$ FW); (c) chlorophyll *a/b* ratio (Chl *a/b*); (d) carotenoids (Car; $\mu\text{g}\cdot\text{g}^{-1}$ FW). Bars represent means $\pm$ SD, $n = 3$. Different letters above the bars indicate statistically significant differences among sites within a given sampling term (Kruskal–Wallis test, $p \leq 0.05$). Experimental plots: A—transitional mire (reference site), B—raised bog, C—transitional mire undergoing ecological succession, D—drained peatland, E—degraded peatland.

2.3. Anthocyanin Content

During the 2024 growing season, the anthocyanin (ACN) content in plant material increased at all transitional sites, with the highest values recorded in August (approximately $0.15\text{--}0.17\text{ mg}\cdot\text{g}^{-1}$ FW). In September, there was a decrease in anthocyanin content in plant tissues, particularly pronounced at the reference site (A). In the spring of 2025, the anthocyanin content in the studied plants was significantly lower compared to the first year of the study and was lowest in plants growing on the transitional mire (A—below $0.05\text{ mg}\cdot\text{g}^{-1}$ FW). At most sampling times, no statistically significant differences in anthocyanin content in the plant material were observed among the plots (Figure 3).

2.4. Relative Water Content

The relative water content (RWC) in the leaves of *Salix lapponum* exhibited significant variation both across seasons and among sites. During the 2024 growing season, from May to July, the relative water content in plant tissues fluctuated at all experimental sites and reached its lowest values in June (RWC = 52% in plants at the raised bog site—B).

In the following months, this value increased significantly in plants, and in most cases, was approximately 90%. In 2025, RWC in the *S. lapponum* tissues remained at a relatively stable, high level. Particularly high values were recorded in May 2025 at the reference site (Figure 4).

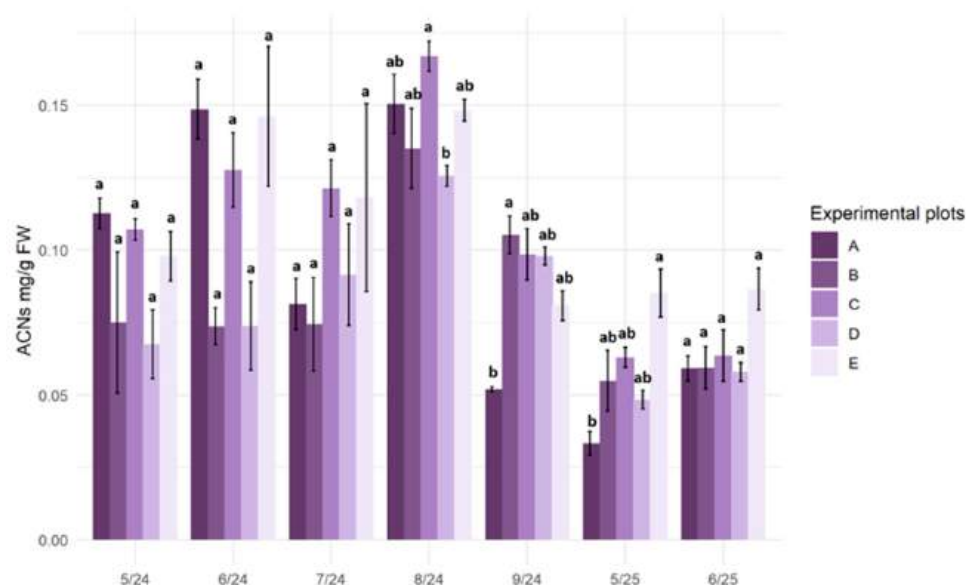


Figure 3. Anthocyanin content (ACNs; $\text{mg} \cdot \text{g}^{-1}$ FW) in the leaves of *Salix lapponum* at five experimental sites (A–E) from May 2024 to June 2025. Bars represent mean values $\pm$ SD, $n = 3$. Different letters above the bars indicate significant differences among sites within a given sampling term (Kruskal–Wallis test, $p \leq 0.05$). Experimental plots: A—transitional mire (reference site), B—raised bog, C—transitional mire undergoing ecological succession, D—drained peatland, E—degraded peatland.

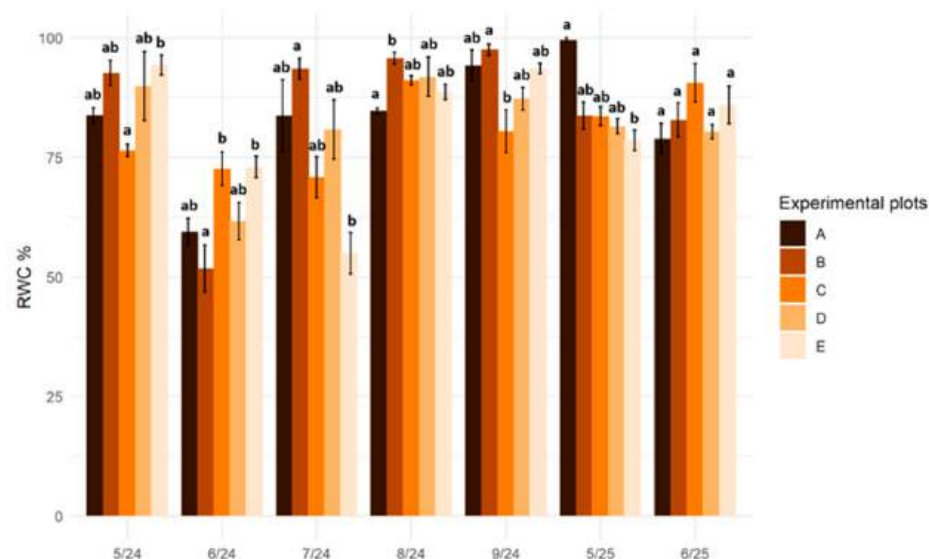


Figure 4. Relative water content (RWC; %) in the leaves of *Salix lapponum* at experimental sites in consecutive sampling periods (May–September 2024; May–June 2025). Bars represent means $\pm$ SD, $n = 5$. Different letters above the bars indicate statistically significant differences among sites within a given sampling term (Kruskal–Wallis test, $p \leq 0.05$). Experimental plots: A—transitional mire (reference site), B—raised bog, C—transitional mire undergoing ecological succession, D—drained peatland, E—degraded peatland.

2.5. Guaiacol Peroxidase Activity

At the reference site (A), located in a transitional mire, guaiacol peroxidase (GPOX) activity in plant tissues remained relatively stable throughout the experiment ($0.008\text{--}0.010\text{ U}\cdot\text{mg}^{-1}\text{ FW}$). At the raised bog site, a significant increase in enzymatic activity was recorded in May 2025 compared to the same period in 2024. At the remaining sites, guaiacol peroxidase activity in the plant tissues exhibited considerable seasonal fluctuations during the 2024 growing season. Significant differences were observed in plants from the drained peatland (D) and the degraded peatland (E), where activity was lowest in May 2024 and increased during the summer months (Figure 5).

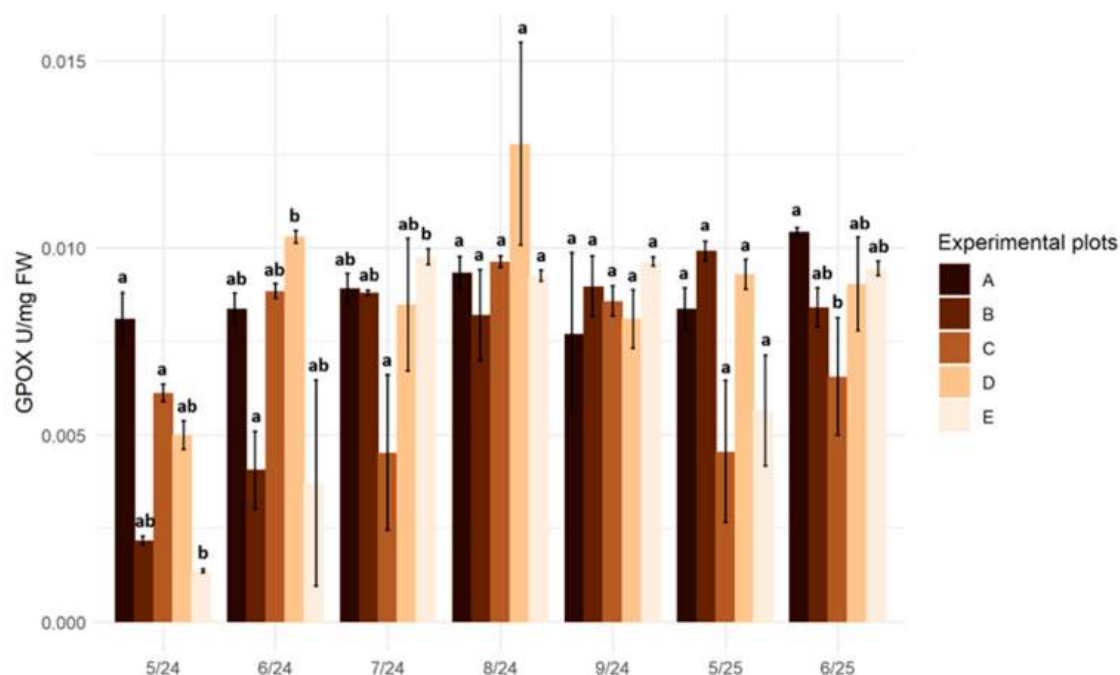


Figure 5. Guaiacol peroxidase activity (GPOX; $\text{U}\cdot\text{mg}^{-1}\text{ FW}$) in the leaves of *Salix lapponum* at experimental sites in consecutive sampling periods (May–September 2024; May–June 2025). Bars represent means $\pm$ SD, $n = 3$. Different letters above the bars indicate statistically significant differences among sites within a given sampling term (Kruskal–Wallis test, $p \leq 0.05$). Experimental plots: A—transitional mire (reference site), B—raised bog, C—transitional mire undergoing ecological succession, D—drained peatland, E—degraded peatland.

2.6. Histochemical Detection of Reactive Oxygen Species (ROS)

Histochemical staining using NBT and DAB revealed the presence of hydrogen peroxide and superoxide anions in plant tissues at all analyzed plots, both in the spring and summer of 2024. Staining intensity varied mainly between seasons and moderately between sites. In spring, significantly more intense tissue staining was observed at all experimental sites for both DAB and NBT reactions compared to the summer period. The most pronounced differences among plants were observed in transformed habitats (D and E), where more extensive and intense staining of the leaf blade occurred, particularly in the NBT reaction (Figure 6).

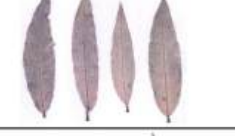
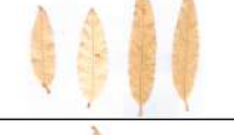
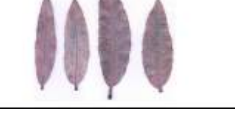
Experimental plot	SPRING 2024		SUMMER 2024	
	NBT ($O_2^{\bullet-}$)	DAB (H_2O_2)	NBT ($O_2^{\bullet-}$)	DAB (H_2O_2)
A				
B				
C				
D				
E				

Figure 6. Visualization of the histochemical detection of superoxide anion ($O_2^{\bullet-}$, NBT staining) and hydrogen peroxide (H_2O_2 , DAB staining) in *Salix lapponum* leaves collected from five peatland habitats in spring and summer 2024. Experimental plots: A—transitional mire (reference site), B—raised bog, C—transitional mire undergoing ecological succession, D—drained peatland, E—degraded peatland.

3. Discussion

The transformation and degradation of natural habitats lead to a decline in biodiversity at all levels. The extinction of entire plant populations or a drastic decline in their abundance is often associated with a reduction in the area of habitats to which species are strongly associated, due to their specific ecological requirements [28–30]. In such cases, active conservation measures involve not only increasing the population size, but above all, identifying suitable substitute habitats that enable successful establishment, acclimatization, and long-term persistence of self-sustaining populations. On the other hand, it is important to determine whether and to what extent plants can respond to environmental stresses that could potentially affect them under the conditions of new habitats [23,31,32].

The assessment of selected physiological parameters during the experimental translocation of *Salix lapponum* in 2024–2025 enabled the identification of reliable indicators for monitoring plant condition at the early stage of acclimatization in substitute habitats.

The efficiency of the photosynthetic process is determined by prevailing habitat conditions, particularly by light and water availability. A deficiency or excess of either of these factors can cause environmental stress [33–35]. Therefore, in degraded habitats or those exposed to frequent fluctuations in abiotic conditions, introduced plants show a greater susceptibility to disruptions in physiological processes.

Chlorophyll fluorescence analysis is a widely used, non-invasive method for assessing the functional status of the photosynthetic apparatus in plants. It enables the early detection of stress responses prior to the appearance of visible morphological symptoms. Maximum photochemical efficiency of photosystem II (F_v/F_m) values above 0.75 in dark-adapted

plants generally indicate proper photosynthetic performance, whereas lower values may reflect oxidative stress and damage to the photosynthetic apparatus [25,36,37].

The results of chlorophyll fluorescence measurements conducted during the field experiment indicate that the F_v/F_m parameter was the most sensitive diagnostic tool for assessing the condition of *S. lapponum*. Analysis of F_v/F_m values in plants from substitute habitats revealed habitat-dependent variation in physiological responses. It also demonstrated a pronounced stress response in all studied introduced plants in May 2025.

The potential usefulness of this indicator for monitoring the condition of plants introduced to new habitats is supported by studies on other species that require active conservation worldwide [38–40]. Research conducted by Liu et al. [39,41] on the critically endangered species *Ardisia gigantifolia* revealed lower F_v/F_m values in plants introduced into natural habitats than in individuals grown under controlled conditions. According to the authors, this reduction was attributed to the cumulative effects of multiple environmental stressors acting simultaneously under field conditions during the early phase of acclimatization. This was also confirmed by the results of studies conducted by Pogorzelec et al. [16] and Arciszewski et al. [42] on species of the genus *Salix* during the initial stage of plant growth in the natural environment. These studies indicated a significant intensification of physiological processes related to plant growth during acclimatization in substitute habitats, as evidenced by an improvement in the measured chlorophyll fluorescence parameter. In contrast, experiments conducted under laboratory conditions, involving the induction of stress using a single environmental factor (temperature, high salinity, or substrate eutrophication), revealed significantly smaller fluctuations in F_v/F_m values in *S. lapponum* [43,44] than in the case of the combined effects of stress factors in substitute habitats during studies conducted in 2024–2025.

The fluctuations in the content of photosynthetic pigments (chlorophyll *a* and *b*) in the plant material of *S. lapponum* observed during the field experiment indicate adaptation of the photosynthetic apparatus to changing habitat conditions. At the reference site, the introduced plants exhibited a relatively constant level of pigment concentration in the examined leaf tissues during the entire period of the study. At the other experimental sites, changes in these values were observed, which may indicate unstable conditions and a potential, constant influence of stress factors of varying intensity in the substitute habitats.

During the analysis of photosynthetic pigment content in the tissues of introduced plants throughout the study period, particular attention was given to the Chl *a/b* ratio, which reflects structural and functional changes in the photosynthetic apparatus. A decrease in this parameter, especially in transformed and degraded habitats (D and E), indicated an increase in Chl *b* content, primarily associated with light-harvesting antenna complexes [45]. This phenomenon may be interpreted as an adaptive response to changing light conditions or as a result of photosynthetic apparatus reorganization in response to environmental stress [46,47].

Important insights into plant physiological status were also provided by the analysis of carotenoid content in plant tissues. These pigments play a key photoprotective role and are involved in the dissipation of excess excitation energy, thereby protecting the photosynthetic apparatus against oxidative stress [48,49]. An increase in the content of these pigments in plants at transformed habitats and in a peatland undergoing enhanced ecological succession may indicate the activation of protective mechanisms in response to less favorable environmental conditions. Similar relationships were also reported in conservation translocation studies conducted on other endangered species, including *Primulina* spp. and *Euryodendron excelsum*. These studies showed that individuals introduced to sites outside their natural range or translocated to microhabitats that did not fully meet the species' specific ecological requirements were characterized by reduced photosynthetic effi-

ciency, accompanied by lower photosynthetic pigment content [40,50]. These observations confirm the usefulness of studying changes in the levels of photosynthetic pigments as sensitive indicators of physiological stress and the effectiveness of acclimatization in plants introduced into new habitats.

Disruptions in photosynthesis may also result from disturbances in cellular water balance associated with a decline in relative water content (RWC). This parameter is considered one of the key indicators of plant water stress, reflecting the balance between water uptake and loss, as well as the hydration status of tissues [51]. The results obtained during the field experiment indicate a clear seasonal variation in RWC in the plant material of *S. lapponum* individuals at substitute sites, confirming the high sensitivity of this indicator to changes in habitat hydrological conditions. The greatest fluctuations in RWC in *S. lapponum* tissues were recorded during the summer months of 2024, particularly in degraded peatlands and raised bogs, whose functioning depends primarily on rainfall. This interpretation is supported by meteorological data from the nearest monitoring station in Włodawa (eastern Poland), which recorded exceptionally low precipitation in May 2024 (11.7 mm), followed by a gradual increase in June and July (unpublished data). Additional field observations also indicated temporary water deficits at the study sites, as physicochemical measurements of habitat water could not be performed at site D (drained peatland) in July 2024 and at sites B (raised bog) and D in August 2024, due to critically low groundwater levels (unpublished data). It should also be noted that the relative water content in tissues may depend on other habitat-related stress factors, such as excessive salinity or high nitrogen levels in the water available to plants [44].

Anthocyanins, classified as secondary plant metabolites, accumulate in tissues in response to various stress factors, such as low ambient temperature, water deficit, or excessive radiation [52,53]. Their content in the tissues of *S. lapponum* during the field experiment exhibited pronounced seasonal variability. However, no statistically significant differences in anthocyanin concentration were found among plants from different substitute sites. It can therefore be concluded that the plant response manifested, as anthocyanin accumulation is associated with environmental stress resulting from a combination of many abiotic factors acting seasonally at all substitute sites. The anthocyanin content in the fresh leaf biomass of *S. lapponum* at the experimental sites remained at a level similar to the values obtained in laboratory experiments, where acclimatized plants were subjected to stress associated with salinity and increased nitrogen supply [44]. Similar results were reported in studies on *Rhododendron minus*, where an increased accumulation of anthocyanins was observed in plants exposed to high levels of radiation. The authors suggest that this phenomenon was linked to photoprotective mechanisms that limit oxidative stress [54]. According to Naing and Kim [55], reactive oxygen species induced by abiotic stress may stimulate anthocyanin biosynthesis, which in turn may help to increase stress resistance by scavenging excess reactive oxygen species. Summing up, this indicator can be used to supplement the monitoring of the acclimatization of introduced plants. Its values should be interpreted in relation to other indicators, such as RWC or antioxidant enzyme activity.

Guaiacol peroxidase activity and the results of histochemical ROS detection indicate the response of plants to environmental stresses under various conditions in alternative habitats. In contrast to previous studies on *S. lapponum* conducted under controlled laboratory conditions, where plants were exposed to single stress factors such as salinity, eutrophication, or temperature extremes [43,44], the present field experiment revealed markedly higher guaiacol peroxidase activity, particularly in plants growing in transformed habitats such as drained and degraded peatlands. This suggests that under field conditions, stronger defense mechanisms are activated in response to multifactorial stress [56]. An increase in guaiacol peroxidase activity under environmental stress is a widely observed

phenomenon, as demonstrated in studies on drought and salinity stress, where GPOX activity increased with the intensity of stressors, reflecting the activation of plant defense mechanisms associated with the neutralization of reactive oxygen species [57,58].

Under controlled laboratory conditions, histochemical detection of ROS in *S. lapponum* tissues exposed to single stress factors (low and high temperature, varying salinity levels, and increased nitrogen supply) [43,44] resulted in lower staining intensity than that observed in field experiments conducted in 2024–2025. These differences may result not only from the nature of the applied stress factors, but also from differences in the age and morphological traits of the studied plants. In the laboratory experiments, young individuals derived from ex situ cultivation were used, characterized, among other features, by a less developed indumentum layer on the leaf surface [43,44]. In contrast, plants introduced into natural sites exhibited well-developed, dense indumentum on thicker leaf blades. It should also be emphasized that the staining method is time-consuming and less standardized, so it can be used to confirm the presence of ROS detected by another, more sensitive method. As demonstrated by Grellet Bournonville and Díaz-Ricci [59], reliable quantitative determination of superoxides requires additional analytical steps, including extraction of the formazan and its spectrophotometric quantification.

The results of the study, together with an evaluation of the utility of individual biomarkers, enabled the identification of a set of indicators suitable for monitoring the outcomes of active conservation of the endangered relict species *S. lapponum*. These indicators are characterized by high sensitivity, functional relevance, and non-invasiveness, and their determination does not require advanced technical expertise. The values of these three indicators: maximum photochemical efficiency of photosystem II (F_v/F_m), relative water content (RWC), and guaiacol peroxidase activity, can support an objective assessment of the effectiveness of active protection measures based on the physiological responses of plants under natural conditions. However, it should be emphasized that the initial step in evaluating acclimatization success and identifying individuals of reduced vitality should be an expert-based assessment of plant morphological traits, subsequently complemented by more sensitive physiological indicators [60].

4. Materials and Methods

4.1. Plant Material and Experimental Sites

The plant material used in the experiment consisted of one-year-old *Salix lapponum* seedlings, introduced into five sites differing in terms of habitat conditions. The plants had previously been obtained through micropropagation from vegetative parts of parent plants [61] and acclimatized both in the laboratory and at the field plant acclimatization station located in close proximity to the planned introduction sites, where they grew for 2 months (according to the procedure described by Pogorzelec et al. [16]). After this period, 40 *S. lapponum* individuals were introduced to each experimental site in autumn 2023. The plants were manually planted with intact root balls into peat mats or directly into the soil, depending on habitat type. The seedlings were randomly distributed over an area of approximately 25 m² at each site.

The sites where the plants were introduced (5 substitute habitats) were located in eastern Poland within the wetland complex of the Łęczna-Włodawa Lake District. This is an area where, as late as the 1950s, the presence of several numerous populations of the studied species was recorded [62]. Due to habitat transformation and fragmentation resulting from anthropogenic pressure, only a few remaining populations of *S. lapponum* were confirmed in this area after the year 2000 [15].

The substitute sites represented habitats intended to reflect potential transformations of peatland ecosystems under the influence of global warming (climate change) in the

study area. A habitat exhibiting characteristics typical of transitional mires (site A) was designated as the reference site, as the largest currently existing population of *S. lapponum* in eastern Poland occurs under such conditions [15,63]. Additional introduction sites were identified during a field survey in the study area, in habitats that are both natural and unaffected by direct human activity (site B—a continental-type raised bog, whose proper functioning depends on rainfall; site C—a transitional mire undergoing accelerated ecological succession—as an example of a habitat whose abiotic environment is being modified by quantitative and qualitative changes in plant cover), as well as those that have been altered or degraded and can represent the effect of natural habitat transformation under the influence of climate warming (site D—a mesic meadow on peat soil, resulting from the drainage of peatland habitats—simulating the drainage of natural wetlands; site E—a degraded, desiccated transitional mire adjacent to a lake, resulting from the covering of the peatland surface with waste rock, representing a habitat permanently transformed by long-term fluctuations in groundwater levels and increased temperature).

4.2. Assessment of Physiological Parameters

The physiological parameters of the plants introduced into each substitute site were measured at monthly intervals during the growing season in the first year after planting (from May to September 2024) and at the beginning of the growing season in the second year (May and June 2025). At each sampling date, leaf material was collected from randomly selected individuals to ensure independence of observations.

4.2.1. Selected Chlorophyll Fluorescence Parameters

Chlorophyll *a* fluorescence parameters, including minimal fluorescence (F_0), maximal fluorescence (F_m) and maximum quantum efficiency of photosystem II (F_v/F_m), were measured in situ on leaves located in the middle part of shoots of 10 randomly selected plants from each experimental population. Measurements were conducted using a field fluorometer (Handy PEA fluorometer, Hanstech Instruments, UK) following 15 min of dark adaptation.

4.2.2. Photosynthetic Pigment Content

The content of chlorophyll *a*, chlorophyll *b*, and carotenoids was determined in leaves collected from the middle parts of the plant shoots. The plant material was homogenized in a 96% ethanol solution and then incubated in a water bath at 70 °C for 5 min to ensure complete extraction of the pigments. The samples were centrifuged (10,000 rpm, 10 min) to separate the solid phase from the liquid. The absorbance of the extracts was measured using a spectrophotometer (SPECORD 40, Analytik Jena, GmbH, Jena, Germany) at wavelengths of 470, 649, and 665 nm. Each sample was analyzed in three technical replicates. Pigment concentration was calculated using a modified method of Lichtenthaler and Welburn [64] as follows:

$$\text{Chl } a = (13.95 \times A_{665}) - (6.88 \times A_{649}) \quad (1)$$

$$\text{Chl } b = (24.96 \times A_{649}) - (8.12 \times A_{665}) \quad (2)$$

$$\text{Car} = ((1000 \times A_{470}) - (2.05 \times \text{Chl } a) - (114.8 \times \text{Chl } b))/245 \quad (3)$$

4.2.3. Anthocyanin Content

The anthocyanin content was determined according to the method described by Martinez and Favret [65]. Leaf blade fragments (100 mg, composite sample; each composite sample consisted of 3 plants) were extracted in methanol acidified with HCl (99:1, *v/v*) for 24 h at 4 °C. The samples were centrifuged (10,000 rpm, 10 min), after which the absorbance

of the supernatant was measured spectrophotometrically at wavelengths of 527 and 652 nm, with each biological sample measured in three technical replicates.

4.2.4. Relative Water Content

Relative water content (RWC) was determined according to the method of González and González-Vilar [66]. Five leaves collected from randomly selected individual plants were used for the analysis, and their fresh weight (FW) was determined. The samples were then placed in distilled water for 24 h to achieve full turgor and were weighed again (TW). In the final stage, the leaf blades were dried at 60 °C for 24 h in a drying oven (SML 32/250, Zalmed, Warsaw, Poland), and the dry weight (DW) was determined. The RWC index was calculated using the formula:

$$\text{RWC} = (\text{FW} - \text{DW}) / (\text{TW} - \text{DW}) \times 100\%. \quad (4)$$

4.2.5. Guaiacol Peroxidase Activity

To determine guaiacol peroxidase activity (GPOX, EC 1.11.1.7), 100 mg of leaf material (composite sample; each composite sample consisted of three plants) was used and homogenized in 1 mL of 0.05 M phosphate buffer (pH 7.0). The obtained homogenates were centrifuged for 10 min (10,000 rpm) at 4 °C. Enzyme activity was determined spectrophotometrically (SPECORD 40 spectrophotometer, Analytik Jena GmbH, Jena, Germany). GPOX activity was assayed according to the method of Małoplesza and Urbanek [67]. Changes in absorbance were recorded at 480 nm for 4 min, with readings taken at 1-min intervals. Each biological sample was analyzed in three technical replicates.

4.2.6. Histochemical Detection of Reactive Oxygen Species (ROS)

Qualitative analyses of hydrogen peroxide (H₂O₂) and superoxide anion (O₂^{•−}) accumulation in leaf tissues were conducted in spring and summer 2024, during the first year following translocation. For each experimental site, four leaves collected from randomly selected plants were used for histochemical staining. The analyses followed the method described by Kumar et al. [68]. Reactive oxygen species were detected using 3,3'-diaminobenzidine (DAB) for H₂O₂ and nitro blue tetrazolium chloride (NBT) for O₂^{•−}. Leaves were incubated for 12 h in darkness at room temperature in previously prepared solutions of DAB (1 mg mL^{−1}) and 0.2% NBT in 50 mM phosphate buffer (pH 7.5). After incubation, the leaves were immersed in hot water and subsequently decolorized in hot ethanol to remove chlorophyll. As a result of the reaction, the presence of H₂O₂ led to the formation of a brown DAB polymerization product, whereas the reduction of NBT resulted in the formation of a blue–purple diformazan, indicating the presence of O₂^{•−}.

4.3. Statistical Analysis

The normality of data distribution was tested using the Shapiro–Wilk test. Since most of the analyzed variables did not meet the assumptions of normal distribution, further analyses were performed using non-parametric statistics. Differences in physiological parameters among the experimental sites within individual sampling terms were assessed using the Kruskal–Wallis test (significance level $p \leq 0.05$). The same test was also applied to evaluate temporal changes within individual sites across consecutive sampling periods. All statistical analyses and data visualizations were performed using the R statistical environment.

5. Conclusions

This study constitutes the first attempt to identify physiological indicators that may enhance monitoring the condition of *Salix lapponum* plants following translocation into

substitute habitats representing different stages of habitat transformation. Analysis of the results suggests that the most effective approach is the combined use of parameters reflecting photosynthetic performance, plant water status, and the level of antioxidant response to environmental stress. Among the parameters evaluated in this study, the most informative biomarkers for post-translocation monitoring of *S. lapponum* were maximum photochemical efficiency of photosystem II (F_v/F_m), relative water content (RWC), and guaiacol peroxidase activity. At the same time, the analysis of physiological parameters should be conducted in parallel with observations of plant morphological traits.

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Abbreviations

The following abbreviations are used in this manuscript:

ACNs	Anthocyanins
GPOX	Guaiacol Peroxidase
ROS	Reactive Oxygen Species
RWC	Relative Water Content
PSII	Photosystem II

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9. Oświadczenia doktoranta oraz współautorów

Lublin, 29.09.2026 r.

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Nauki Biologiczne

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Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracach:

(P1) Arciszewski M., Pogorzelec M., Hawrylak-Nowak B., Parzymies M., & Piejak M. (2024). Towards successful reintroduction of *Salix myrtilloides*: the importance of monitoring plant physiological indicators during acclimatization. *Dendrobiology*, 92.

Mój wkład w publikację obejmował: opracowanie koncepcji i założeń metodologicznych badań, udział w prowadzeniu badań i analizie wyników, zebranie oraz uporządkowanie danych, przygotowanie pierwszej wersji manuskryptu i materiałów graficznych.

(P2) Arciszewski M., Pogorzelec M., Parzymies M., Bronowicka-Mielniczuk U., & Mieczan T. (2025). Do Endangered Glacial Relicts Have a Chance for Effective Conservation in the Age of Global Warming? A Case Study: *Salix lapponum* in Eastern Poland. *Biology*, 14(1), 19.

Mój wkład w publikację obejmował: opracowanie koncepcji i założeń metodologicznych badań, udział w prowadzeniu badań i analizie danych, zebranie i uporządkowanie danych, przygotowanie materiałów graficznych, opracowanie manuskryptu.

(P3) Arciszewski M., Pogorzelec M., Bronowicka-Mielniczuk U., & Parzymies M. (2025). Eutrophication and Salinization as Risk Factors in the Translocation Process of an Endangered Glacial Relict Species. *Water*, 17(16), 2451.

Mój wkład w publikację obejmował: opracowanie koncepcji i założeń metodologicznych badań, udział w prowadzeniu badań oraz analizie danych, zapewnienie materiałów niezbędnych do badań, zebranie i uporządkowanie danych, przygotowanie materiałów graficznych i manuskryptu.

(P4) Arciszewski M., Pogorzelec M., Bronowicka-Mielniczuk U., & Piejak M. (2026). Could species translocation be an effective conservation method for *Salix lapponum* populations in wetlands affected by anthropogenic changes?. *Journal for Nature Conservation*, 127403.

Mój wkład w publikację obejmował: opracowanie koncepcji i założeń metodologicznych badań, prowadzenie badań, analizę wyników, zebranie i uporządkowanie danych oraz przygotowanie pierwszej wersji manuskryptu i materiałów graficznych.

(P5) Arciszewski M, Pogorzelec M. (2026). Physiological Indicators for Post-Translocation Monitoring of *Salix lapponum* in Natural vs. Degraded Peatlands. *Plants*, 15(10):1557.

Mój wkład w publikację obejmował: opracowanie koncepcji i założeń metodologicznych badań, przeprowadzenie badań oraz analizę wyników, zebranie i uporządkowanie danych, a także przygotowanie pierwszej wersji manuskryptu i materiałów graficznych.

Michał Arciszewski

Czytelny podpis

Lublin, 29.09.2026 r.

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Nauki Biologiczne

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Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracach:

(P1) Arciszewski M., Pogorzelec M., Hawrylak-Nowak B., Parzymies M., & Piejak M. (2024). Towards successful reintroduction of *Salix myrtilloides*: the importance of monitoring plant physiological indicators during acclimatization. *Dendrobiology*, 92.

Mój wkład w publikację obejmował: współpracowanie koncepcji i założeń metodologicznych badań, udział w prowadzeniu badań oraz redagowanie manuskryptu.

(P2) Arciszewski M., Pogorzelec M., Parzymies M., Bronowicka-Mielniczuk U., & Mieczan T. (2025). Do Endangered Glacial Relicts Have a Chance for Effective Conservation in the Age of Global Warming? A Case Study: *Salix lapponum* in Eastern Poland. *Biology*, 14(1), 19.

Mój wkład w publikację obejmował: współpracowanie koncepcji i założeń metodologicznych badań, udział w prowadzeniu badań i analizie danych oraz redagowanie i edycję manuskryptu.

(P3) Arciszewski M., Pogorzelec M., Bronowicka-Mielniczuk U., & Parzymies M. (2025). Eutrophication and Salinization as Risk Factors in the Translocation Process of an Endangered Glacial Relict Species. *Water*, 17(16), 2451.

Mój wkład w publikację obejmował: współpracowanie koncepcji i założeń metodologicznych badań, udział w prowadzeniu badań, redagowanie i edycję manuskryptu oraz nadzór merytoryczny nad pracą.

(P4) Arciszewski M., Pogorzelec M., Bronowicka-Mielniczuk U., & Piejak M. (2026). Could species translocation be an effective conservation method for *Salix lapponum* populations in wetlands affected by anthropogenic changes?. *Journal for Nature Conservation*, 127403.

Mój wkład w publikację obejmował: współpracowanie koncepcji i założeń metodologicznych badań, udział w prowadzeniu badań, nadzór merytoryczny nad pracą oraz redagowanie i edycję manuskryptu.

(P5) Arciszewski M., Pogorzelec M. (2026). Physiological Indicators for Post-Translocation Monitoring of *Salix lapponum* in Natural vs. Degraded Peatlands. *Plants*, 15(10):1557.

Mój wkład w publikację obejmował: współpracowanie koncepcji i założeń metodologicznych badań, weryfikację metod i wyników, nadzór merytoryczny nad pracą oraz redagowanie i edycję manuskryptu.


Czytelny podpis

Lublin, 29.09.2026 r.

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Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracach:

(P1) Arciszewski M., Pogorzelec M., Hawrylak-Nowak B., Parzymies M., & Piejak M. (2024). Towards successful reintroduction of *Salix myrtilloides*: the importance of monitoring plant physiological indicators during acclimatization. *Dendrobiology*, 92.

Mój wkład w publikację obejmował: weryfikację poprawności zastosowanych metod i uzyskanych wyników, udział w prowadzeniu badań oraz redagowanie i edycję manuskryptu.

(P2) Arciszewski M., Pogorzelec M., Parzymies M., Bronowicka-Mielniczuk U., & Mieczan T. (2025). Do Endangered Glacial Relicts Have a Chance for Effective Conservation in the Age of Global Warming? A Case Study: *Salix lapponum* in Eastern Poland. *Biology*, 14(1), 19.

Mój wkład w publikację obejmował: współpracowanie założeń metodologicznych badań, zapewnienie materiałów niezbędnych do badań oraz analizę i redagowanie i edycję manuskryptu.

(P3) Arciszewski M., Pogorzelec M., Bronowicka-Mielniczuk U., & Parzymies M. (2025). Eutrophication and Salinization as Risk Factors in the Translocation Process of an Endangered Glacial Relict Species. *Water*, 17(16), 2451.

Mój wkład w publikację obejmował: współpracowanie założeń metodologicznych badań, redagowanie i edycję manuskryptu oraz nadzór merytoryczny nad pracą.



Czytelny podpis

Lublin, 29.09.2026 r.

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Niniejszym oświadczam, że w pracach:

(P2) Arciszewski M., Pogorzelec M., Parzymies M., Bronowicka-Mielniczuk U., & Mieczan T. (2025). Do Endangered Glacial Relicts Have a Chance for Effective Conservation in the Age of Global Warming? A Case Study: *Salix lapponum* in Eastern Poland. *Biology*, 14(1), 19.

Mój wkład w publikację obejmował: przeprowadzenie analiz statystycznych, wizualizację danych, udział w redagowaniu i korekcie manuskryptu.

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U. Bronowicka-Mielniczuk

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Lublin, 29.09.2026 r.

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Lublin, 29.09.2026 r.

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Mój wkład w publikację obejmował: weryfikację metod i wyników oraz redagowanie i edycję manuskryptu.


Czytelny podpis

Lublin, 29.09.2026 r.

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Nauki Biologiczne

Uniwersytetu Przyrodniczego w Lublinie

Oświadczenie o współautorstwie

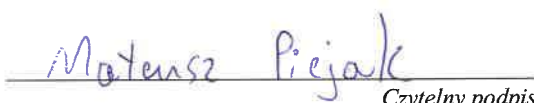
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(P1) Arciszewski M., Pogorzelec M., Hawrylak-Nowak B., Parzymies M., & Piejak M. (2024). Towards successful reintroduction of *Salix myrtilloides*: the importance of monitoring plant physiological indicators during acclimatization. *Dendrobiology*, 92.

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Mój wkład w publikację obejmował: udział w prowadzeniu badań, przygotowanie materiałów graficznych oraz redagowanie manuskryptu.


Czytelny podpis

Aneks – życiorys naukowy

Wykształcenie

- **2022-2026 Szkoła Doktorska Uniwersytetu Przyrodniczego w Lublinie**
Kształcenie w dyscyplinie: nauki biologiczne
Tytuł rozprawy: Uwarunkowania czynnej ochrony zagrożonych gatunków roślin z rodzaju *Salix* metodami translokacji w perspektywie globalnych zmian klimatycznych.
Promotor: dr hab. Magdalena Pogorzelec, prof. uczelni
Promotor pomocniczy: dr hab. Marzena Parzymies, prof. uczelni
- **2025-2026 Studia podyplomowe**
Wydział Leśny, Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie
Kierunek: Siedliskoznawstwo
- **2021-2022 Studia II stopnia**
Wydział Biologii Środowiskowej, Uniwersytet Przyrodniczy w Lublinie
Kierunek: Ochrona środowiska
Tytuł pracy: Wpływ wybranych czynników siedliskowych na przebieg i powodzenie procesu reintrodukcji zagrożonych gatunków z rodzaju *Salix*
Promotor: dr hab. Magdalena Pogorzelec, prof. uczelni
- **2018-2022 Studia I stopnia**
Wydział Biologii Środowiskowej, Uniwersytet Przyrodniczy w Lublinie
Kierunek: Ochrona środowiska
Tytuł pracy: Wpływ zawartości przyswajalnych form azotu w siedlisku *Aldrovanda vesiculosa* (aldrowandy pęcherzykowatej) na zróżnicowanie cech morfofizjologicznych roślin
Promotor: dr hab. Magdalena Pogorzelec, prof. uczelni
- **2017-2021 Studia I stopnia**
Wydział Agrobioinżynierii, Uniwersytet Przyrodniczy w Lublinie
Kierunek: Leśnictwo, specjalność: Ochrona zasobów leśnych
Tytuł pracy: Wybrane aspekty ekologii lelka *Caprimulgus europaeus* L. w Nadleśnictwie Sobibór
Promotor: dr hab. Grzegorz Grzywaczewski, prof. uczelni

Stáže naukowe i mobilności zagraniczne

- **03-25.02.2022 mobilność zagraniczna w ramach programu Erasmus+**
“Multi-Spectral Imaging and System Analysis tools for smart water management Sentinel-2 MSI for water quality monitoring”. Polytechnic University of Beja, Portugal
- **30.10.2023-08.12.2023 zagraniczny staż naukowy**
Department of Plant Physiology and Metabolomics, Agricultural Institute, Centre for Agricultural Research, HUN-REN, Martonvásár, Węgry
- **24.06.2024-28.06.2024 mobilność zagraniczna w ramach programu Erasmus+**
Uniwersytet Mendla w Lednicach: “Sustainable Horticulture of the 21st century - From theory to practice”, Czechy
- **22.07-26.07.2024- mobilność zagraniczna w ramach programu Erasmus+**
Samsun Ondokuz Mayıs University: “Blended Intensive Programme for Sustainable Agriculture”, Samsun, Turcja
- **10.03.2025-11.04.2025 zagraniczny staż naukowy**
CCB - Biodiversity Conservation Centre, Department of Life and Environmental Sciences, University of Cagliari, Włochy
- **13.04.2026-17.04.2026 zagraniczny staż naukowy**
CCB - Biodiversity Conservation Centre, Department of Life and Environmental Sciences, University of Cagliari, Włochy

Aktywność publikacyjna

1. Serafin, A., Pogorzelec, M., Banach-Albińska, B., Zalewska, E., Bronowicka-Mielniczuk, U., & **Arciszewski, M.** (2022). The importance of groundwater quality and other habitat parameters for effective active protection of an endangered plant species in Eastern Poland. *Water*, 14(8), 1270.
2. Pogorzelec, M., Parzymies, M., Pawlik-Skowrońska, B., **Arciszewski, M.**, & Mielniczuk, J. (2022). Searching for optimal substitute habitats for plants by biological experiments—A case study of the endangered species *Aldrovanda vesiculosa* L.(*Droseraceae*). *International Journal of Environmental Research and Public Health*, 19(17), 10743.
3. **Arciszewski, M.**, Pogorzelec, M., Bronowicka-Mielniczuk, U., Niedźwiecki, M., Parzymies, M., & Serafin, A. (2023). The search for suitable habitats for endangered

- species at their historical sites—conditions for the success of *Salix lapponum* and *Salix myrtilloides* reintroduction. *International Journal of Environmental Research and Public Health*, 20(2), 1133.
4. **Arciszewski, M.**, Pogorzelec, M., Hawrylak Nowak, B., Parzymies, M., & Piejak, M. (2024). Towards successful reintroduction of *Salix myrtilloides*: the importance of monitoring plant physiological indicators during acclimatization. *Dendrobiology*, 92.
 5. **Arciszewski, M.**, Pogorzelec, M., Parzymies, M., Bronowicka-Mielniczuk, U., & Mieczan, T. (2024). Do Endangered Glacial Relicts Have a Chance for Effective Conservation in the Age of Global Warming? A Case Study: *Salix lapponum* in Eastern Poland. *Biology*, 14(1), 19.
 6. Parzymies, M., Głębocka, K., Pogorzelec, M., Banach-Albińska, B., Świstowska, A., & **Arciszewski, M.** (2025). Phenotypic and genetic stability of the *Aldrovanda vesiculosa* L. plants regenerated in tissue culture. *Genes*, 16(9), 1003.
 7. **Arciszewski, M.**, Pogorzelec, M., Bronowicka-Mielniczuk, U., & Parzymies, M. (2025). Eutrophication and Salinization as Risk Factors in the Translocation Process of an Endangered Glacial Relict Species. *Water*, 17(16), 2451.
 8. Piejak, M., Pogorzelec, M., & **Arciszewski, M.** (2026). UAV-Based Detection of *Molinia caerulea* in Wet Grasslands Using a Lightweight Deep Learning Model. *Global Ecology and Conservation*, e04329.
 9. **Arciszewski, M.**, & Pogorzelec, M. (2026). Physiological Indicators for Post-Translocation Monitoring of *Salix lapponum* in Natural vs. Degraded Peatlands. *Plants*, 15(10), 1557.
 10. **Arciszewski, M.**, Pogorzelec, M., Bronowicka-Mielniczuk, U., & Piejak, M. (2026). Could species translocation be an effective conservation method for *Salix lapponum* populations in wetlands affected by anthropogenic changes?. *Journal for Nature Conservation*, 127403.

Sumaryczny IF - 27,200

Sumaryczna liczba punktów MNiSW - 930

Udział w konferencjach naukowych

Referaty wygłoszone na konferencjach międzynarodowych:

1. **Arciszewski M., 2021.** Preferencje siedliskowe lelka *Caprimulgus europaeus* Linnaeus, 1758 w lasach Sobiborskich. II Międzynarodowe Sympozjum Studenckich Kół Naukowych "Środowisko- Roślina-Zwierzę-Produkt", 15.04.2021, Lublin.
2. **Arciszewski M., 2022.** Co zagraża drapieżnej osobliwości, czyli aldrawanda pęcherzykowata (*Aldrovanda vesiculosa*) w zmieniającym się środowisku. III Międzynarodowe Sympozjum Studenckich Kół Naukowych "Środowisko- Roślina-Zwierzę-Produkt", 21.04.2022, Lublin.
3. **M. Arciszewski, M. Pogorzelec, 2023.** Translocation – a tool for active conservation of endangered species. 2nd International PhD Student's Conference at the University of Life Sciences in Lublin, Poland: Environment – Plant – Animal – Product. 19- 20.04.2023, Lublin, Polska.
4. **M. Arciszewski, M. Pogorzelec, 2023.** Active protection of endangered species of willows in times of climate changes. XIX International Scientific Conference for Students and PhD Students: Youth and Progress of Biology, Lwów, Ukraina.
5. **M. Arciszewski, M. Pogorzelec, 2025.** Glacial relics in the age of global warming – threats and prospects for conservation. 4th International PhD Student's Conference at the University of Life Sciences in Lublin, Poland: Environment – Plant – Animal – Product. 09.04.2025, Lublin, Polska.

Referaty wygłoszone na konferencjach krajowych:

1. **Arciszewski M., Zielińska M., Klimczak A., Kołodziejczyk M. 2019.** Ochrona prawna wilka szarego *Canis lupus* vs. rzeczywiste zagrożenia. V Konferencja Naukowo-Techniczna „Nowe kierunki badań w inżynierii środowiska, energetyce, geodezji i gospodarce przestrzennej”, 22-24.05.2019, Krynica Zdrój.
2. **Arciszewski M., 2019.** Edukacja ekologiczna w zakresie zmian klimatu w wybranych parkach narodowych w Polsce. 24. Konferencja z cyklu Współczesne Zagadnienia Edukacji Leśnej Społeczeństwa „Edukatorzy dla Klimatu”, 04.12.2019, Rogów.

Postery zaprezentowane na konferencjach międzynarodowych:

1. **M. Arciszewski**, M. Pogorzelec, **2024**. Acclimatisation of *Salix myrtilloides* L. Key physiological indicators on the path to successful reintroduction. 3rd International PhD Student's Conference at the University of Life Sciences in Lublin, Poland: Environment – Plant – Animal – Product. 24.04.2024, Lublin, Polska.
2. **Arciszewski M.**, Pogorzelec M., **2026**. Eutrophication as a risk factor in the active conservation of *Salix lapponum* in wetland habitats. 5th International PhD Students' Conference at the University of Life Sciences in Lublin, Poland: Environment – Plant – Animal – Product. 22.04.2026, Lublin, Polska.

Postery zaprezentowane na konferencjach krajowych:

1. **Arciszewski M.**, Korcz N., **2019**. Zmiany klimatu w oczach leśników. 24.Konferencja z cyklu Współczesne Zagadnienia Edukacji Leśnej Społeczeństwa „Edukatorzy dla Klimatu”, 04.12.2019, Rogów.
2. **Arciszewski M.**, Zielińska M., Klimczak A., Kołodziejczyk M., **2019**. Wykorzystanie markerów genetycznych w procesie rozpoznawania drewna pokradzieżowego. V Konferencja Naukowo-Techniczna „Nowe kierunki badań w inżynierii środowiska, energetyce, geodezji i gospodarce przestrzennej”, 22-24.05.2019, Krynica Zdrój.

Nagrody i wyróżnienia

- Dyplom za zajęcie III miejsca w sesji referatowej Sekcji Biologii Środowiskowej II Międzynarodowego Sympozjum Studenckich Kół Naukowych „Środowisko-Roślina-Zwierzę-Produkt” (Lublin), 2021.
- Dyplom Polskiego Towarzystwa Zoologów za zajęcie III miejsca w kategorii „referat” ogłoszonego na Międzynarodowym Sympozjum Studenckich Kół Naukowych „Środowisko-Roślina-Zwierzę-Produkt” (Lublin), 2021.
- Dyplom za zajęcie I miejsca w sesji referatowej Sekcji Biologii Środowiskowej III Międzynarodowego Sympozjum Studenckich Kół Naukowych „Środowisko-Roślina-Zwierzę-Produkt” (Lublin), 2022.
- Stypendium Studenckie Marszałka Województwa Lubelskiego w latach 2020, 2021, 2022.

- Dyplom oraz nagroda za szczególne zaangażowanie w organizację wydarzeń na rzecz Uniwersytetu Przyrodniczego w Lublinie przyznany przez Rektora oraz Prorektora ds. Studenckich i Dydaktyki Uniwersytetu Przyrodniczego w Lublinie.
- Wyróżnienie pracy dyplomowej (magisterskiej) „Wpływ wybranych czynników siedliskowych na przebieg i powodzenie procesu reintrodukcji zagrożonych gatunków z rodzaju *Salix*”.
- Dyplom Wyróżniającego się Absolwenta za wybitne osiągnięcia w nauce. (Studia I i II stopnia na kierunku ochrona środowiska).
- Wyróżnienie za działalność naukową przyznane przez Prorektora ds. Nauki i Współpracy z Zagranicą Uniwersytetu Przyrodniczego w Lublinie- 2023.
- III miejsce w konkursie „Drive Innovation – Rozwiązania dla zrównoważonego rozwoju” zorganizowanym przez BASF Polska – 2024.
- Wyróżnienie za prezentację w sekcji „Environment” podczas 4th International PhD Students’ Conference: Environment-Plant-Animal-Product (I-SDUPL) – 2025.
- Wyróżnienie za poster podczas 5th International PhD Students’ Conference at the University of Life Sciences in Lublin, Poland: Environment – Plant – Animal – Product, 2026.

Członkostwo w towarzystwach naukowych

- Członek Polskiego Towarzystwa Hydrobiologicznego (od 2025 r.)

Udział w projektach

- Wykonawca w projekcie „Opracowanie narzędzia do wykrywania i mapowania rozmieszczenia nawłoci (*Solidago* spp.) z wykorzystaniem danych obrazowych BSP i modeli uczenia głębokiego oraz identyfikacja cech diagnostycznych populacji na potrzeby monitoringu terenowego” finansowanym z Funduszu Grantowego na badania i działania na rzecz ochrony środowiska dla naukowców z województwa lubelskiego (UMCS-Bogdanka); Konkurs Grantowy „Środowisko i Nauka”.
- Wykonawca w projekcie „Ochrona czynna i ograniczanie zagrożeń dla różnorodności biologicznej cennych przyrodniczo terenów mokradłowych województwa lubelskiego” (FELU.03.09-IZ.00-0001/25) – Fundusze Europejskie dla Lubelskiego 2021–2027 / Europejski Fundusz Rozwoju Regionalnego.
- Wolontariusz w projekcie „Ochrona czynna aldrowandy pęcherzykowatej (*Aldrovanda vesiculosa*) na terenie Lubelszczyzny”. Projekt nr POIS.02.04.00-00-

0034/18, współfinansowany ramach Programu Operacyjnego Infrastruktura i Środowisko 2014-2020.

- Wolontariusz w projekcie „Ochrona czynna szczególnie zagrożonych gatunków roślin reliktowych z rodziny Salicaceae w siedliskach torfowiskowych”. Projekt nr POIS.02.04.00-00-0008/17, współfinansowany w ramach Programu Operacyjnego Infrastruktura i Środowisko 2014-2020.

Działalność organizacyjna i popularyzatorska:

- Członek Wydziałowej Komisji ds. jakości kształcenia Wydziału Biologii Środowiskowej (2019-2022)
- Przewodniczący Wydziałowej Rady Samorządu Studentów Wydziału Biologii Środowiskowej Uniwersytetu Przyrodniczego w Lublinie (2019-2022)
- Członek Senatu Uniwersytetu Przyrodniczego w Lublinie (2019-2022)
- Aktywny członek Rady Uczelnianej Samorządu Studentów Uniwersytetu Przyrodniczego w Lublinie, w tym: Koordynator ds. Kół naukowych (2019-2021) oraz Wiceprzewodniczący ds. kultury i wymiany międzynarodowej (2021-2022)
- Członek Komitetu Organizacyjnego Konferencji „Nowe kierunki badań w inżynierii środowiska, energetyce, geodezji i gospodarce przestrzennej” (2020)
- Członek Kolegium Wydziału Biologii Środowiskowej (2021-2022)
- Członek Rady Programowej Kierunku Ochrona Środowiska (2021-2022)
- Członek Komitetu Organizacyjnego III Międzynarodowego Sympozjum Studenckich Kół Naukowych „Środowisko-Roślina-Zwierzę-Produkt” (2022)
- Członek Odwoławczej Komisji Dyscyplinarnej dla Studentów Uczelni Uniwersytetu Przyrodniczego w Lublinie (2022)
- Członek Rady Studentów Lublina (2022)
- Aktywny członek Samorządu Doktorantów Uniwersytetu Przyrodniczego w Lublinie (2022-2023)
- Członek Komitetu Naukowego II Międzynarodowej Konferencji Doktorantów „2nd International PhD Student’s Conference Environment-Plant-Animal-Product (ICDSUPL)” (2023)

- Członek Komitetu Organizacyjnego III Międzynarodowej Konferencji Doktorantów „3rd International PhD Student’s Conference Environment-Plant-Animal-Product (ICDSUPL)” (2024)
- Członek Komisji ds. weryfikacji zapisów Regulaminu Szkoły Doktorskiej Uniwersytetu Przyrodniczego w Lublinie (2024)
- Współorganizator Obozu Naukowego Wydziału Biologii Środowiskowej (2024, 2025, 2026)
- Członek Komitetu Organizacyjnego IV Międzynarodowej Konferencji Doktorantów „4th International PhD Students’ Conference: Environment-Plant-Animal-Product (I-SDUPL)” (2025)
- Współorganizator wydarzenia „Próbna Matura z Biologii na Uniwersytecie Przyrodniczym w Lublinie” (2025, 2026)
- Członek Komitetu Organizacyjnego V Międzynarodowej Konferencji Doktorantów “5th International PhD Students’ Conference at the University of Life Sciences in Lublin, Poland: Environment – Plant – Animal – Product” (2026)
- Organizator warsztatów w ramach wydarzenia „Noc Biologów” (2026)
- Organizator warsztatów naukowych podczas 29 Pikniku Naukowego im. Prof. Łukasza Turskiego pn. „Z życia wzięte” w Warszawie, realizowanego przez Polskie Radio i Centrum Nauki Kopernik (2026)