UNIVERSITÉ DU QUÉBEC EN OUTAOUAIS

CARACTÉRISATION DES RÉPONSES PHYSIOLOGIQUES ET MOLÉCULAIRES À LA SÉCHERESSE DE L'ÉRABLE À SUCRE (*Acer saccharum*) ET DE L'ÉPINETTE BLANCHE (*Picea glauca*) : APPROCHES ÉCOPHYSIOLOGIQUE ET TRANSCRIPTOMIQUE

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Crédit photo : Zoé Ribeyre.

DÉDICACE

À mon père, ma mère et mon frère,

“La vraie forme de l’intelligence est de comprendre qu’on n’a pas encore compris et de faire le nécessaire pour comprendre quand même.”

Albert Jacquard

AVANT PROPOS

Cette étude a été réalisée sur les territoires traditionnels non cédés de la nation Anishinabeg Omàmiwininiwak (nation algonquine).

Mon projet de doctorat a été mené sous la direction de Christian Messier, professeur à l'Université du Québec en Outaouais (UQO) et à l'Université du Québec à Montréal (UQAM), et sous la co-direction de Philippe Nolet, professeur à l'UQO. Le financement de cette thèse de doctorat a été assuré par la chaire du Canada sur la résilience des forêts face aux changements globaux de Christian Messier.

Cette thèse contient un premier chapitre sous la forme d'une revue scientifique qui fait l'état des lieux des connaissances actuelles sur les mécanismes épigénétiques des arbres forestiers, intitulé "*Advances and promises of epigenetics for forest trees*". Cette revue a été coécrite avec deux autres étudiants au doctorat dans le cadre d'une collaboration internationale et a été publiée dans le journal *Forests* en septembre 2020. Puis, la thèse est également constituée de trois autres chapitres rédigés sous la forme d'articles scientifiques originaux pour caractériser et mieux appréhender les mécanismes physiologiques et moléculaires qui influencent le potentiel d'acclimatation à la sécheresse de l'érable à sucre (*Acer saccharum* [Marsh]) et de l'épinette blanche (*Picea glauca* [Moench] Voss). Le deuxième chapitre s'intitule : "*No stress memory pattern was detected in sugar maple and white spruce seedlings subjected to experimental droughts*" et a été publié dans la revue *Ecosphere* en décembre 2022. Le troisième chapitre dont le titre est : "*De novo transcriptome assembly and discovery of drought-responsive genes in white spruce (Picea glauca)*" a été publié dans le journal *PlosOne* en janvier 2025. Enfin, le quatrième chapitre porte le titre de : "*Insights into drought responses: comparative transcriptomics of sugar maple and white spruce in short- and long-term perspectives*", et sera soumis pour publication dans le journal *Tree Physiology*.

Comme tout projet de doctorat, mon parcours fut long et semé d'embûches. Après une pandémie et le besoin de repenser mon projet à mi-parcours, je pense avoir mieux cerné le concept de résilience (autant d'un point de vue écologique que personnel). Un des points clés de mon parcours a été ma capacité à aller chercher des personnes-ressources et à établir des collaborations avec plusieurs équipes de recherches, sans quoi ma thèse de doctorat n'aurait pas pu aboutir. Je tire donc de mon expérience doctorale, en plus d'un enrichissement personnel et d'une formation scientifique, la création d'un réseau professionnel multidisciplinaire. L'ensemble de mon parcours universitaire en biologie et écologie m'a permis de rencontrer et de côtoyer des personnes averties aux enjeux écologiques et sociétaux associés aux changements climatiques. Mais, il m'a aussi permis de comprendre qu'il est essentiel et urgent de sortir de notre bulle académique et de repenser notre rôle et nos responsabilités en tant que chercheur et chercheuse, afin d'assurer et de promouvoir une vraie transition écologique. Les actions pour l'environnement et les programmes de gestion et de conservation ne peuvent pas être séparés d'une prise de conscience et d'une modification profonde du fonctionnement de nos sociétés. Les efforts placés dans l'élitisme à outrance et la course à la publication devraient être davantage redirigés vers l'éducation et la transmission des connaissances à la population pour contribuer à l'éveil général des consciences, sans quoi aucune action et mesure politique concrète n'est possible.

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LISTE DES ABRÉVIATIONS

ABC: *ATP-binding cassette*, transporteur à cassette liant l'ATP

ABA: Acide abscissique

ADN: Acide désoxyribonucléique (DNA : *Deoxyribonucleic acid*)

AQP: Aquaporine

ATP: Adénosine triphosphate

ARN: Acide ribonucléique (RNA : *Ribonucleic acid*)

ARNpm: Acide ribonucléique pré-messager

AS: *Acer saccharum*

BP: *Biological process,* processus biologique

CTAB: *Cetrimonium bromide*, bromure de cétrimonium

CYT: Cytochrome

DB: *Database,* base de données

DBH: *Diameter at breast height,* diamètre à hauteur de poitrine

DEG: *Differentially expressed gene,* gène différentiellement exprimé

DET: *Differentially expressed transcript,* transcrit différentiellement exprimé

DM: *Dry matter,* matière sèche

ENA: *European Nucleotide Archive*

FAO: *Food and Agriculture Organization*

FDR: *False discovery rate*, taux de fausses découvertes

FM: *Fresh mass*, masse fraîche

GO: *Gene ontology*, ontologie génique

GST: Glutathione-S-transferase

GTC: *Guanidine thiocyanate*, thiocyanate de guanidine

H: *Height*, hauteur

H_2O_2: Peroxyde d'hydrogène

HW: *Heat wave*, vague de chaleur

HSP: *Heat shock protein*, protéine de stress

IDENT: *International Diversity Experiment Network with Trees*

IPCC: *Intergovernmental Panel on Climate Change*

LFC: *Log fold change*

LEA: *Late embryogenesis abundant protein*

LRT: *Likelihood ratio test*, taux de vraisemblance

MATE: *Multidrug and toxic compound extrusion*

MF: *Molecular function*, fonction moléculaire

MFFP: Ministère des Forêts de la Faune et des Parcs

MSAP: *Methylation sensitive amplification polymorphism*

MSF: *Major facilitator superfamily*

MscS: *Mechanosensitive ion channel*

NADPH: Nicotinamide adénine dinucléotide phosphate

NCS: *Non-structural carbohydrate*, glucides non structuraux

NGS: *Next generation sequencing*, séquençage de future génération

NCBI: *National Center for Biotechnology Information*

NPK: Nitrogen – phosphorus - potassium, azote – phosphore - potassium

NS: *Not significant*, non significatif

OPT: *Oligopeptide transporter*

ORF: *Open reading frame*, cadre de lecture ouvert

OTL: *Osmotin/thaumatin-like*

PG: *Picea glauca*

PIP: *Plasma membrane intrinsic protein*

PSI: *Photosystem I*, photosystème I

PSII: *Photosystem II*, photosystème II

QTL: *Quantitative trait loci*, locus de caractères quantitatifs

RNA-seq: RNA-sequencing

ROS: *Reactive oxygen species*, espèces réactives de l'oxygène

RT-qPCR: *Reverse transcription-quantitative real-time polymerase chain reaction*, PCR quantitative en temps réel

RWC: *Relative water content*, teneur relative en eau

SAM: *Shoot apical meristem*, méristème apicale de la tige

S: *Species*, espèce

SLA: *Specific leaf area*, surface foliaire spécifique

SNP: *Single nucleotide polymorphism*, polymorphisme nucléotidique

T: *Treatment*, traitement

TE: *Transposable element*, élément transposable

TF: *Transcription factor*, facteur de transcription

WS: *Water stress*, stress hydrique

WW: *Well-watered*, bien irrigué

XTH: *Encoding xyloglucan endotransglucosylase/hydrolase*

Y: *Year*, année

LISTE DES SYMBOLES ET DES UNITÉS

°C : Degrés Celsius

h : Heure

°N : Degrés de latitude Nord

°W : Degrés de longitude Ouest

% : Pourcentage

Ppm : Partie par million

µm : Micromètre

mm : Millimètre

cm : Centimètre

cm^2 : Centimètre carré

$m^2.kg^{-1}$: Mètre carré par kilogramme

$\mu mol.m^{-2}.s^{-1}$: Micromole par mètre carré par seconde

m a.s.l. : Mètre au-dessus du niveau de la mer

nM : Nanomolaire

mM : Millimolaire

ng : Nanogramme

Gb : Gigabase

Pb : paire de base (bp : *base paire*)

RÉSUMÉ

Les forêts jouent un rôle fondamental dans l'équilibre des écosystèmes en offrant de nombreux services écosystémiques qui régulent le climat et bénéficient aux sociétés humaines. La dégradation des forêts et de leurs réseaux écologiques suscite donc de vives préoccupations. La sécheresse est une perturbation majeure pour les forêts. Elle se manifeste entre autres en impactant le taux de croissance et la vigueur des arbres et en accentuant leur vulnérabilité aux autres perturbations et leur taux de mortalité. Dans le contexte des changements globaux, il est attendu que la fréquence et l'intensité des évènements de sécheresse s'accentuent d'ici la fin du siècle. L'altération et le déplacement des niches écologiques déjà en cours pourraient donc s'accentuer et dépasser la capacité d'adaptation et de migration des arbres. Intervenant à l'échelle individuelle, le processus d'acclimatation permet aux arbres de s'ajuster aux modifications environnementales auxquelles ils sont soumis tout au long de leur cycle de vie. La capacité d'acclimatation d'une espèce reste toutefois limitée et les mécanismes impliqués dans le potentiel d'acclimatation des arbres face à la sécheresse sont encore peu décrits. L'épigénétique est un domaine en extension depuis les années 1990 et offre une approche très prometteuse pour compléter notre compréhension de l'impact de l'environnement sur les plantes. Cependant, les mécanismes épigénétiques sont encore très peu étudiés chez les arbres, car de nombreux défis doivent encore être relevés pour utiliser pleinement cette approche à l'étude d'organismes complexes et possédant un large génome. Les angiospermes et les gymnospermes présentent de nombreuses spécificités moléculaires et physiologiques qui découlent de leur grande distance évolutive datant de plus de 300 millions d'années. La comparaison de ces deux lignées évolutives en contexte de sécheresse offre donc un cadre avantageux pour identifier les différentes réponses et stratégies adoptées par les arbres face à ce stress. Les espèces cibles de cette étude sont l'érable à sucre (*Acer saccharum* [Marsh]) et l'épinette blanche (*Picea glauca* [Moench] Voss), deux espèces ligneuses avec une large aire de répartition en Amérique du Nord et avec une forte influence écologique et économique au Canada.

L'objectif principal de cette étude était de caractériser les processus physiologiques et moléculaires induits par la sécheresse qui pourraient contribuer au potentiel d'acclimatation à la sécheresse de l'érable à sucre et de l'épinette blanche. Le premier chapitre de cette thèse est une revue de littérature qui synthétise les connaissances actuelles des mécanismes épigénétiques chez les arbres et qui pointe les avancées récentes dans ce domaine. Cette revue propose également des pistes de recherches pour combler les lacunes du domaine pour l'étude des arbres. La revue pointe notamment qu'une des prochaines étapes pour intégrer pleinement l'épigénétique à l'étude d'espèces ligneuses non modèles est d'améliorer la disponibilité de leurs ressources génomiques. Le deuxième chapitre s'est intéressé au concept de mémoire de stress environnementale, un processus sous le contrôle des mécanismes épigénétiques. Ce processus a été décrit chez de nombreuses espèces herbacées pour améliorer la perception et la tolérance au stress après plusieurs expositions. Cependant, les études dans ce domaine sur les arbres sont rares et se limitent majoritairement à des genres modèles comme *Populus* et *Picea* ou *Pinus*. Ce chapitre avait pour objectif de tester si des traitements de sécheresses successives (modérée, puis sévère) au cours d'une même saison de croissance, permettraient de déclencher et de mesurer une mémoire de stress, ou au contraire, une accumulation des effets délétères des

stress chez l'érable à sucre et l'épinette blanche. Les résultats obtenus n'ont pas permis de mettre en évidence un patron de mémoire de stress chez les deux espèces. L'érable à sucre a démontré une accumulation de stress des plants doublement stressés avec une réduction de la photosynthèse par rapport aux plants soumis à un seul stress. En revanche, bien qu'aucune des deux hypothèses n'ait pu être validée pour l'épinette blanche, celle-ci a démontré une tolérance inattendue aux différents traitements de sécheresse. Cette étude a révélé la complexité de déclencher et de mesurer un patron de mémoire de stress chez les arbres en milieu semi-contrôlé. Par ailleurs, la forte variabilité intraspécifique observée a souligné la nécessité de repenser l'approche sur plusieurs saisons de croissance. Dans les troisième et quatrième chapitres, un assemblage transcriptome *de novo* a été réalisé pour chacune des deux espèces pour servir de référence aux analyses transcriptomiques. L'objectif du troisième chapitre était d'établir une caractérisation temporelle du transcriptome des plants d'épinettes blanches soumis à une sécheresse sévère en serre de 22 jours. Les résultats ont montré que la régulation transcriptomique s'accentuait fortement en fonction du temps d'exposition au stress. Il a notamment été observé une régulation à la baisse des processus de croissance et de photosynthèse, et une régulation à la hausse des processus de défense antioxydante. Cette étude a également souligné le rôle potentiel, encore très peu décrit dans la littérature, du métabolisme des lipides dans la réponse à la sécheresse chez l'épinette blanche. De plus, plusieurs gènes potentiellement clés dans la réponse à la sécheresse chez cette espèce ont été identifiés. Enfin, le quatrième chapitre avait pour objectif d'identifier les gènes clés et de comparer les fonctions et les processus biologiques mobilisés par différentes conditions de sécheresse (courte et longue) chez l'érable à sucre et l'épinette blanche en utilisant une approche transcriptomique. Le premier objectif de ce dernier chapitre était de réaliser une caractérisation intraspécifique des effets d'une sécheresse longue et modérée par rapport à une sécheresse courte et sévère sur la régulation des gènes de l'érable à sucre et de l'épinette blanche. Puis, le second objectif était d'établir une comparaison interspécifique de la réponse à la sécheresse des deux espèces sur la base des fonctions et des processus induits ou inhibés en réponse aux deux types de sécheresse. Pour ce faire, des jeux de données issus d'une expérience d'exclusion hydrique à long terme (6 ans) dans un jardin expérimental, et des jeux de données provenant d'expériences de sécheresse à court terme (3 semaines) en serre ont été utilisés. Les résultats ont mis en avant une augmentation des processus de transport transmembranaire chez les deux espèces face à une sécheresse longue. Cependant, les espèces présentaient globalement des réponses distinctes face aux deux types de stress, où l'érable à sucre s'est distingué avec une régulation transcriptomique plus accentuée par rapport à l'épinette blanche face à la sécheresse longue. De plus, l'érable à sucre présentait une plus forte similitude de réponse entre les deux conditions que l'épinette blanche avec cependant une forte différence dans les patrons de régulation génique. Ces analyses ont également permis d'identifier plusieurs gènes qui constituent de potentiels bons candidats pour de futures études fonctionnelles chez les deux espèces.

L'ensemble de cette thèse de doctorat apporte une meilleure compréhension des processus physiologiques et moléculaires engagés dans la réponse à différentes modalités de sécheresse de l'érable à sucre et de l'épinette blanche. Cette étude a également participé à améliorer les ressources transcriptomiques des deux espèces et à identifier plusieurs gènes potentiellement impliqués dans l'acclimatation à la sécheresse des deux espèces cibles.

Mots-clés : Érable à sucre, épinette blanche, forêts, sécheresse, transcriptomique, mémoire de stress, épigénétique, acclimatation

ABSTRACT

Forests are crucial for ecosystem balance, delivering many ecosystem services which regulate our climate and support human societies. The degradation of forests and forest ecology networks is alarming. Drought is an important disturbance of forests, primarily through its effects on tree growth rates and vigor and by increasing their vulnerability to other disturbances and their mortality rate. Without taking mitigation measures, climate change projections indicate that the frequency and intensity of drought events will increase through the end of the century. The shift and displacement of ecological niches, which we are already witnessing, may thus be dramatic and push tree adaptive and migratory limits. At the individual level, acclimation is the process by which trees adapt to the environmental changes they experience over their lifetime. But the acclimation capacity of a species is limited, and the mechanisms governing the acclimation potential of trees to drought are poorly understood. Since the 90s, epigenetics has emerged as a growing field and a novel way to study how the environment affects plants. However, epigenetic mechanisms remain largely unexplored in trees, as many challenges still need to be addressed before fully applying this approach to the study of complex organisms with large genomes. Angiosperms and gymnosperms have sharply diverged from each other for more than 300 million years, and they possess many molecular and physiological specificities. This enables a useful framework for understanding the variable responses and strategies of trees to drought by comparing the evolutionary lineages of two lineages. This study focuses on sugar maple (Acer saccharum [Marsh]) and white spruce (Picea glauca [Moench] Voss), two tree species that are distributed widely across North America and have high ecological and economic value in Canada. The main objective of this study was to characterize the physiological and molecular responses induced by drought that could contribute to the acclimation potential of sugar maple and white spruce. The first chapter of this thesis is a literature review that synthesizes current knowledge on epigenetic mechanisms in trees and highlights recent advances in the field. This review also proposes research directions to address existing gaps in tree studies. Notably, one of the next steps to fully integrate epigenetics into the study of non-model woody species is to improve the availability of their genomic resources. The second chapter focuses on the concept of environmental stress memory, a process controlled by epigenetic mechanisms. Such a process has been reported for various species in herbaceous systems to improve stress perception and tolerance to recurrent stresses. Nonetheless, research on this issue in trees is still limited, mostly having focused on model genera such as Populus, Picea, or Pinus. This chapter sought to investigate if consecutive drought treatments (moderate, then severe) within one growing season would induce and reveal a stress memory, or rather, lead to cumulative detrimental effects in sugar maple and white spruce. Neither species showed strong evidence for a stress memory pattern in the results. Sugar maple showed accumulation of stress in seedlings exposed to double stress, with a reduction in photosynthesis compared to plants exposed to a single stress event. Alternatively, white spruce was not supported by either hypothesis but displayed unexpected drought tolerance in treatments. This study highlighted the complexity of triggering and measuring a stress memory pattern in trees under semi-controlled conditions. Furthermore, the strong intraspecific variability observed underscored the need to reconsider the approach over multiple growing seasons.

Chapter 3 and 4 are based on de novo transcriptome assembly for both species to be used as a reference for transcriptomic analyses. The third chapter aimed to establish a temporal characterization of the transcriptome of white spruce seedlings subjected to severe drought over 22 days in a greenhouse. The results showed that transcriptomic regulation significantly increased with the duration of stress exposure. Notably, downregulation of growth and photosynthesis processes, and upregulation of antioxidant defense mechanisms, were observed. This study also pointed out the potential role for lipid metabolism in white spruce response to drought, which is still poorly described in the literature. Additionally, several genes potentially crucial for drought response in this species were identified. Lastly, chapter four focused on identifying important genes, and comparing what biological functions and processes activated under different drought conditions (short and severe versus long and moderate) in sugar maple and white spruce using a transcriptomic approach. The first objective of this chapter was to characterize the intraspecific effects of prolonged moderate drought compared to short severe drought on gene regulation in both species. The second objective was to establish an interspecific comparison of drought responses based on the biological functions and processes activated or inhibited under both drought conditions. To do this, we relied on data sets from a long-term (six-year) drought exclusion experiment within an experimental garden as well as from short-term (three-week) drought experiments in a greenhouse. The results highlighted an increase in transmembrane transport processes in both species in response to prolonged drought. Overall, the species differed in their response to the two stress types, with sugar maple demonstrating stronger transcriptomic regulation than white spruce under prolonged drought. However, with different patterns of gene regulation, sugar maple was also more similar in its response to the two conditions than white spruce. These analyses also pinpointed several genes as promising candidates for future functional studies in each species.

This thesis provides new insights on the physiological and molecular responses of sugar maple and white spruce responding to varying drought conditions. This study also aided in the enhancement of transcriptomic resources for both species, and the identification of several genes possibly implicated in their acclimation to drought.

Keywords: Sugar maple, white spruce, forests, drought, transcriptomics, stress memory, epigenetics, acclimation

INTRODUCTION

0.1 Contexte de l'étude

0.1.1 Les fluctuations environnementales à l'échelle des temps géologiques : une source de diversité

Les écosystèmes qui peuplent notre planète sont des systèmes complexes et dynamiques dont la structure et la composition résultent de processus évolutifs en réponse aux fluctuations environnementales (Lytle, 2001). Depuis 540 millions d'années, la Terre a connu cinq extinctions massives dont la plus grande s'est produite au Permien-Trias (il y a 250 millions d'années) causant l'extinction de plus de 90% des espèces animales et végétales (Barnosky et al., 2011). Les cataclysmes environnementaux associés à ces extinctions (p. ex. éruptions volcaniques massives, modifications climatiques extrêmes, impact de météorites) ont réduit la surface habitable terrestre (Benton, 2018). Bien que ces extinctions aient entraîné la disparition de nombreuses espèces très rapidement à l'échelle des temps géologiques (75% des espèces en moins de 2 millions d'années) (Barnosky et al., 2011), elles ont également ouvert la voie à de nouvelles opportunités évolutives, permettant l'émergence de nouvelles espèces (Rull, 2022). Si depuis toujours la Terre est soumise à des bouleversements climatiques, et que l'extinction d'espèces est un processus naturel nécessaire à la diversification de la vie, pourquoi les changements globaux actuels sont-ils si alarmants ?

0.1.2 Quelles sont les différences avec les changements globaux actuels ?

Les changements globaux en cours se distinguent des événements qui ont eu lieu dans l'Histoire de notre planète au niveau de leur rapidité (IPCC, 2023; Shaw & Etterson, 2012), mais surtout de leur origine (Rull, 2022). Les activités humaines génèrent une multitude de perturbations sur les forêts (p. ex. déforestation, dissémination d'espèces invasives, pollution des sols et des eaux souterraines) (Curtis et al., 2018; Scanes, 2018) qui réduisent considérablement leur complexité, leur connectivité et leur résilience (Grantham et al., 2020; Moore & Schindler, 2022). Ainsi, contrairement aux précédentes crises imputées à des forces environnementales, la

plupart des études tendent à pointer les activités anthropiques de ces deux derniers siècles comme la source principale de ces changements (Rull, 2022).

0.1.3 La sécheresse : une menace sans précédent pour les forêts

Les forêts jouent un rôle important dans l'équilibre planétaire et offrent de nombreux services écosystémiques. Elles ont notamment un rôle prépondérant dans les cycles de la matière (p. ex. les cycles de l'eau, du carbone, des nutriments) (Anderegg, Hicke, et al., 2015; Cardinale et al., 2012) et participent à la stabilisation du climat en agissant comme une source et un puits de carbone (Grassi et al., 2017; Mori et al., 2017). Les forêts constituent donc un patrimoine naturel et culturel inestimable dont la dégradation et la destruction sont des tragédies, non seulement pour les communautés animales et végétales qui y vivent, mais également pour les sociétés humaines (Watson et al., 2018).

Une des fortes inquiétudes de la communauté scientifique de ces dernières décennies est l'accentuation de la fréquence et de l'intensité des événements de sécheresse et de leurs impacts sur la santé des forêts (Hammond et al., 2022; Q. Liu et al., 2023). Une sécheresse est le résultat d'une diminution de la réserve en eau du sol notamment induit par une réduction des précipitations, une hausse des températures et de l'évapotranspiration du couvert végétal, une faible densité du couvert neigeux et la rapidité de sa fonte (Barnett et al., 2005). Le Canada et le Québec sont des territoires où l'eau est abondante, cependant des projections climatiques récentes prévoient une diminution de l'eau dans les sols en saison estivale de l'ordre de 20 à 40% d'ici 2099 (Houle et al., 2012). Ainsi, au courant de ce siècle, les forêts tempérées de ces territoires seront soumises à des saisons de croissance plus longues et plus chaudes et à un risque de sécheresse accru (Cholet et al., 2022). Une modification profonde de la composition des forêts est donc à prévoir avec notamment une migration des populations pour suivre leur niche écologique et une augmentation des évènements de dépérissements (Allen, 2009; Aubin et al., 2018; Clark et al., 2016). Dans le contexte de modifications environnementales rapides, l'évaluation du potentiel d'acclimatation des arbres apparaît comme un bon outil pour déterminer, au sein d'une génération, leur capacité à faire face à l'augmentation des perturbations et à se maintenir dans leur environnement (Demmig-Adams et al., 2008; Gunderson et al., 2010; Kramer et al., 2020). Cependant le potentiel d'acclimatation à la sécheresse est encore peu documenté pour la plupart des arbres.

0.2 Le potentiel d'acclimatation des arbres face à la sécheresse

0.2.1 Le potentiel d'acclimatation, une question d'échelle temporelle

Les arbres doivent faire face à d'importants changements des conditions environnementales au cours de leur longue durée de vie. Pour s'ajuster à ces changements et maintenir leur *fitness* (la capacité de survivre et de se reproduire), les arbres disposent de mécanismes d'acclimatation et d'adaptation qui opèrent à des échelles temporelles et spatiales distinctes. L'adaptation est une réponse évolutive qui implique l'acquisition ou la recombinaison de caractéristiques génétiques à travers plusieurs générations sous l'effet de la pression de sélection (Demmig-Adams et al., 2008; Lambers & Oliveira, 2019). L'adaptation se mesure donc mieux à l'échelle des populations. En revanche, l'acclimatation est un ajustement des traits physiologiques et morphologiques qui améliore la performance et la *fitness* à l'échelle individuelle face à un stress. Ces ajustements ne nécessitent pas de modifications génétiques et peuvent se mettre en place très rapidement après le début du stress, de l'ordre de quelques heures à quelques semaines (Demmig-Adams et al., 2008). Dans le contexte des changements globaux qui démontrent une évolution rapide (Shaw & Etterson, 2012), il est donc primordial de mieux caractériser les réponses physiologiques et moléculaires mises en place à l'échelle individuelle par les arbres face à une sécheresse pour mieux appréhender leur potentiel d'acclimatation.

0.2.2 Les facteurs qui influencent le potentiel d'acclimatation des arbres

L'acclimatation est un processus qui améliore la survie à l'échelle de l'individu en ajustant le fonctionnement de l'organisme à l'environnement sous l'action de modifications moléculaires, physiologiques et anatomiques (Harb et al., 2010). Ces ajustements peuvent être temporaires et réversibles, comme ceux qui concernent la régulation osmotique ou photosynthétique, alors que d'autres sont irréversibles, car ils engagent des modifications anatomiques, comme la synthèse de nouveaux tissus (Demmig-Adams et al., 2008; Lambers & Oliveira, 2019). La diversité génétique intraspécifique et le niveau de plasticité (la capacité d'un génotype à présenter différents phénotypes en réponse à des conditions environnementales variées) sont deux facteurs cruciaux pour le potentiel d'acclimatation d'une espèce (Alberto et al., 2013; Nicotra et al., 2010). En effet, une grande diversité génétique permet à une espèce de résister à une plus large gamme de stress. De même, une plasticité élevée confère à une espèce la capacité d'ajuster

rapidement ses caractéristiques phénotypiques en réponse à des changements environnementaux.

0.2.3 Les mécanismes épigénétiques ont-ils un rôle dans l'acclimatation des arbres ?

Ces dernières décennies, les mécanismes épigénétiques ont suscité un vif intérêt en raison de leur implication dans l'acclimatation des plantes aux variations environnementales (Gallusci et al., 2017; C. L. Richards et al., 2017). Ils sont définis comme des mécanismes stables, mais potentiellement réversibles, n'affectant pas la séquence de l'ADN et qui peuvent se transmettre par mitose et/ou par méiose (Baulcombe & Dean, 2014; Bossdorf et al., 2007). Ces mécanismes peuvent donc transcender l'échelle individuelle et impacter la génération suivante. Les marques épigénétiques modifient l'accès des gènes à la machinerie de transcription et influencent leur niveau d'expression en jouant sur le niveau de compaction de la chromatine (Jaskiewicz et al., 2011; Sow, Allona, et al., 2018). Ces mécanismes permettent d'augmenter la diversité des profils d'expression génique et modulent, par ce fait, la plasticité phénotypique (Moore et al., 2013). La mémoire épigénétique, ou mémoire de stress environnementale, est un processus qui conserve les informations environnementales par le biais de modifications épigénétiques (p. ex. la méthylation de l'ADN, la modification de protéines histones) (Lämke & Bäurle, 2017). Cette mémoire moléculaire permet à la plante de mobiliser son expérience passée pour améliorer sa tolérance aux stress futurs (Conrath et al., 2006, Figure 0.1). Cette amélioration peut, par exemple, s'illustrer par une meilleure perception du stress, une mobilisation accrue des processus de défense, ainsi que le maintien de variables physiologiques, telles que le rendement photosynthétique ou la croissance (Lämke & Bäurle, 2017; Murata et al., 2012; Tanou et al., 2009). Bien étudiée chez les plantes modèles, l'évaluation du rôle des mécanismes épigénétiques et de leur importance pour le potentiel d'acclimatation des espèces ligneuses est plus récente (Sow, Allona, et al., 2018), principalement en raison du manque de ressources génétiques et du coût élevé des analyses.

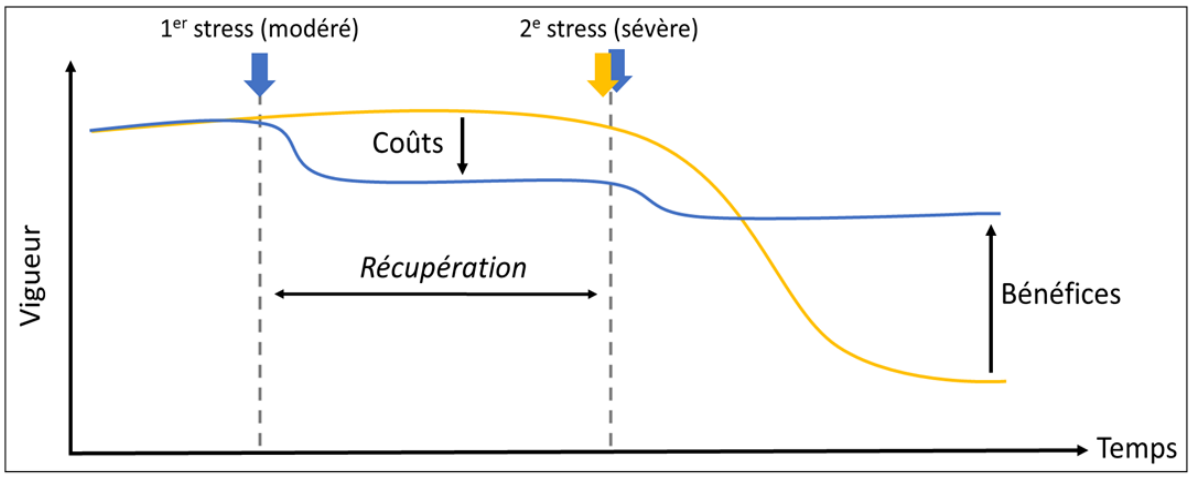


Figure 0.1. Schéma du concept de mémoire de stress. La courbe bleue représente la vigueur au cours du temps d'une plante qui a déclenché une mémoire de stress après avoir été soumise à un stress modéré, puis à un second stress plus sévère. La courbe jaune représente une plante uniquement soumise au second stress sévère et qui n'a pas déclenché de mémoire de stress (Figure modifiée de Hilker et al., 2015).

0.3 Modifications physiologiques et moléculaires induites par la sécheresse : caractéristiques des angiospermes et des gymnospermes

0.3.1 Comparer les angiospermes et les gymnospermes

Les gymnospermes sont considérés comme un clade ancien par rapport aux angiospermes. Malgré leur domination pendant la majeure partie du Mésozoïque, les gymnospermes ont été fortement impactés par des évènements climatiques extrêmes, ce qui aurait causé l'extinction de la plupart des lignées anciennes (M. D. Crisp & Cook, 2011; De La Torre et al., 2017). Les espèces actuelles de gymnospermes et d'angiospermes auraient donc le même âge évolutif (M. D. Crisp & Cook, 2011; De La Torre et al., 2017), mais leurs trajectoires évolutives distinctes leur confèrent de nombreuses différences anatomiques, physiologiques et écologiques (Carnicer et al., 2013; De La Torre et al., 2020; Díaz-Sala et al., 2013). Les angiospermes possèdent des caractéristiques physiologiques innovantes (p. ex. une croissance rapide, un système de pollinisation par les animaux, une diversification des systèmes de défenses et de tolérance aux stress) qui les ont rendus extrêmement compétitifs et leur ont permis de coloniser

la plupart des écosystèmes terrestres (90% des espèces végétales sont des angiospermes) (Condamine et al., 2020). Bien que la diversité des gymnospermes soit moindre (environ 600 espèces actuelles de conifères), celles-ci dominent de nombreux écosystèmes forestiers de l'hémisphère Nord et démontrent une bonne capacité d'adaptation aux conditions extrêmes (McLoughlin, 2021). Comparer les fonctions et les processus biologiques mobilisés en contexte de sécheresse d'espèces évolutivement très distantes permet d'approfondir notre compréhension de la diversité des réponses des arbres face aux changements globaux et d'identifier les stratégies communes et distinctes entre les deux taxons (De La Torre et al., 2020; X. Li et al., 2020).

0.3.2 Cavitation et défaillance hydraulique au cœur de la réponse à la sécheresse

La sécheresse impacte lourdement les arbres à plusieurs niveaux, mais la défaillance hydraulique est une des principales causes qui affecte leur vigueur et leur survie pendant une sécheresse (H. D. Adams et al., 2017; Choat et al., 2018). L'eau se déplace à travers le continuum sol-arbre-atmosphère grâce à la force de tension-cohésion (Steudle, 2001), dont le moteur est l'évaporation des feuilles. En période de sécheresse, le potentiel hydrique du sol diminue, augmentant ainsi la tension exercée sur la colonne d'eau. Une tension excessive induit la rupture des liaisons hydrogènes des molécules d'eau de la sève, entrainant sa vaporisation (passage de l'état liquide à gazeux) et la cavitation des vaisseaux (Cochard, 2006). Si elle n'est pas contenue, la cavitation peut causer un embolisme généralisé du système vasculaire, c'est-à-dire une propagation d'air dans les vaisseaux qui empêche la circulation de la sève dans l'arbre. Un haut taux d'embolisme conduit progressivement à la mort des zones affectées, voir à la mort progressive de l'individu. Le seuil d'embolie conduisant à des dommages irréversibles est en général plus élevé chez les angiospermes que les gymnospermes (estimé autour de 88% pour les angiospermes, et 50% pour les gymnospermes) (Choat et al., 2012; Urli et al., 2013). En parallèle de la défaillance hydraulique, la sécheresse impacte l'équilibre carboné des arbres. Sous sécheresse, la réduction de la croissance (un des principaux puits de carbone) et la réduction de la photosynthèse (la source de carbone) entrainent une modification de l'allocation des sucres au sein de l'organisme (Hartmann & Trumbore, 2016; Piper et al., 2017). À terme, si le stress est trop sévère et long, le maintien de la respiration cellulaire et l'arrêt ou la diminution de la photosynthèse conduisent à l'épuisement progressif des réserves carbonées (Sala et al., 2012; Skelton et al., 2017) et accentuent la vulnérabilité des arbres. L'augmentation

du stress oxydatif contribue aussi à affaiblir les arbres en causant une perte de l'homéostasie et une dérégulation des processus biologiques et des fonctions moléculaires essentielles à leur survie (Lei et al., 2022; Wujeska et al., 2013; Zlobin et al., 2019).

0.3.3 Les impacts physiologiques et moléculaires de la sécheresse

Pour s'acclimater à la sécheresse, les arbres mobilisent diverses stratégies basées sur des compromis physiologiques, anatomiques et d'allocation de ressources. Ainsi, l'étude de la variation des traits phénotypiques, physiologiques et de la régulation des gènes sont des approches qui permettent de mieux appréhender la capacité des arbres à faire face à une sécheresse.

0.3.3.1. Entre la production photosynthétique et l'équilibre hydrique

La photosynthèse est un processus partagé par l'ensemble des plantes chlorophylliennes qui convertit l'énergie lumineuse inorganique en énergie chimique organique et qui est donc la source de la production autotrophe. L'évaluation de production photosynthétique permet d'appréhender l'état physiologique de l'organisme face à une sécheresse (Demmig-Adams et al., 2017; Drake et al., 2017a). Pendant la journée, les photons du soleil sont captés par les photosystèmes II et I (PSII et PSI), des complexes constitués de protéines et de pigments situés dans les chloroplastes (Rochaix, 2014). Le transfert d'électrons et la force proton motrice conduisent à la production de NADPH, un agent réducteur, et d'ATP, une source d'énergie cellulaire. Durant la nuit, le cycle de Calvin (ou cycle de Calvin-Benson-Bassham), utilise les produits de la phase lumineuse pour catalyser la fixation et la réduction du CO_2 atmosphérique et générer des molécules de glucides (Rochaix, 2014). La limitation de la photosynthèse pendant une sécheresse peut être causée par la fermeture des stomates et par des processus non stomatiques comme des défaillances métaboliques causées par le stress oxydatif (Drake et al., 2017a).

Les stomates répondent aux variations de la disponibilité en eau par l'intermédiaire de la signalisation hormonale, dont l'acide abscissique (ABA) est un acteur majeur (Bauer et al., 2013; Brunner et al., 2015). La fermeture des stomates limite la perte en eau, mais réduit également l'incorporation du CO_2 atmosphérique, entraînant une réduction de la production autotrophe en carbone (McDowell et al., 2008). Les arbres sous sécheresse se retrouvent alors

face à un dilemme, qui se résume sommairement par un compromis entre une optimisation de leur gestion hydraulique ou de leur production photosynthétique. Les angiospermes sont généralement caractérisés comme des espèces capables de tolérer de plus fortes amplitudes de potentiel hydrique que les gymnospermes. En revanche, ces derniers montrent une gestion hydrique plus conservatrice avec une haute marge de sécurité hydraulique (Carnicer et al., 2013; Choat et al., 2012; D. M. Johnson et al., 2012). Cependant, ces tendances ne sont pas un classement strict et les stratégies adoptées par les arbres se distribuent le long d'un continuum de réponses. Ces réponses sont fortement dépendantes de l'espèce et de ses caractéristiques vasculaires (p. ex. des angiospermes avec des vaisseaux conducteurs à pores diffus ou poreux) (Bryant et al., 2022; Kannenberg et al., 2019), mais dépendent aussi de la sévérité de la sécheresse (Hochberg et al., 2018).

0.3.3.2. Croissance et anatomie vasculaire

La croissance et la production de biomasse sont des variables très étudiées, car elles constituent de bons indices pour estimer la vigueur des arbres et aussi parce qu'elles intéressent fortement le secteur agroforestier (Grulke et al., 2020). La diminution de la croissance pendant une sécheresse est une des premières réponses communément observées chez les angiospermes et les gymnospermes (Carnicer et al., 2013; L. Chen et al., 2017; Goldblum & Rigg, 2011; Moreau et al., 2020). Elle est induite par une réduction de la division et de l'extension cellulaire, liée entre autres par une baisse de la force de turgescence et d'énergie allouée à ces processus (Mitchell et al., 2014). Les caractéristiques anatomiques des vaisseaux conducteurs des arbres, comme leur longueur et leur diamètre et leur épaisseur, influencent le transport de l'eau (l'efficacité hydraulique) et la sécurité hydraulique (Hacke et al., 2017). Les angiospermes ont un système conducteur composé de vaisseaux longs et larges (atteignant parfois plusieurs mètres de long et jusqu'à 500 µm de diamètre) (Brodribb et al., 2012). Ces caractéristiques favorisent une bonne conductance hydraulique et un fort taux de croissance, mais les rendent plus sujets à la cavitation (Carnicer et al., 2013; D. M. Johnson et al., 2012). Comparativement, le système vasculaire des gymnospermes est constitué de trachéides de faible diamètre (entre 5 et 80µm de diamètre) avec un torus (système de valve qui isole les trachéides embolisées) (Brodribb et al., 2012; Díaz-Sala et al., 2013). Ce système vasculaire offre une plus faible conductance hydraulique, mais est moins enclin à la cavitation (Hacke et al., 2015; D. M. Johnson et al., 2012; Sala et al., 2012).

0.3.3.3. Le rôle des carbohydrates non structuraux face à la sécheresse

Les carbohydrates non structuraux (NSC) sous leur forme soluble (p. ex. le saccharose) constituent une source majeure de carbone utilisée par le métabolisme des arbres. Ils jouent un rôle important en contexte de sécheresse, car ils participent au maintien du transport de l'eau et à la régulation osmotique (Hartmann & Trumbore, 2016; W. He et al., 2020). Les NSC sont également impliqués dans la récupération et la résilience à la cavitation (Brodersen & McElrone, 2013; Klein et al., 2018). Les différences anatomiques et physiologiques soulevées entre les angiospermes et les gymnospermes se reflètent sur leur métabolisme carboné et leur capacité de stockage. Par exemple, les angiospermes disposent d'une plus grande fraction de cellules de stockage (les cellules parenchymateuses du bois) et une plus grande teneur xylémienne en NSC que les gymnospermes, ce qui pourrait expliquer leur meilleure capacité de récupération à la cavitation (D. M. Johnson et al., 2012; Trifilò et al., 2019; Urli et al., 2013). Le recouvrement après une embolie a aussi été observé chez des gymnospermes (Klein et al., 2018). Cependant, cette capacité de recharge du xylème après une sécheresse chez les arbres fait encore débat auprès des spécialistes.

Par ailleurs, les lipides constituent une autre source importante d'énergie et sont principalement utilisés comme un substrat pour la respiration, la communication cellulaire et les mécanismes de défense (Hartmann & Trumbore, 2016), mais peu d'études sur les arbres ont caractérisé leurs rôles en contexte de sécheresse.

0.3.3.4. Les mécanismes de défense et les molécules protectrices

La sécheresse induit une augmentation du stress oxydatif caractérisé par l'accumulation de molécules oxydantes de haute énergie appelées ROS (*Reactive oxygen species*) (Regier et al., 2009; Wujeska et al., 2013). En faible quantité, les ROS occupent une fonction de signalisation cellulaire. Cependant, en condition de stress, la perturbation des voies métaboliques induit une accumulation excessive des ROS (Mukarram et al., 2021). En excès, ils peuvent notamment induire la dégradation des photosystèmes, la perte d'intégrité cellulaire et augmenter la protéolyse (Lei et al., 2022; Zlobin et al., 2019). Pour atténuer les dommages causés par le stress oxydatif, les arbres disposent de mécanismes antioxydants enzymatiques (p. ex. la glutathione S-transférase, la catalase, la superoxyde dismutase), et non enzymatiques (p. ex. les caroténoïdes, l'acide ascorbique, le glutathion) dont le rôle est de protéger les cellules en

dissipant l'excès d'énergie des ROS (Du et al., 2018; H. Fox et al., 2018; Wujeska et al., 2013). En plus des processus antioxydants, les arbres synthétisent des molécules protectrices dont l'action est relativement bien conservée entre les angiospermes et les gymnospermes, bien qu'il existe des spécificités (Baldi & La Porta, 2022). Ces molécules protectrices sont issues du métabolisme secondaire comme les terpènes ou les flavonoïdes (Almeida et al., 2020; Y. Zhang et al., 2023), ou encore des protéines chaperonnes, comme les *Heat Shock Protein* (HSP), dont le rôle est de limiter la dénaturation des protéines (Zhang et al., 2021), ou des déhydrines (*Late embryogenesis abundant protein*, LEA), qui assurent la stabilisation de l'homéostasie cellulaire pour limiter leur déshydratation (Karas et al., 2024; Stival Sena et al., 2018). L'homéostasie cellulaire est aussi maintenue par la biosynthèse de composés osmoprotectants (p. ex. des amino-acides comme la proline) qui agissent en protégeant l'intégrité des cellules et qui augmentant la capacité des arbres à extraire l'eau du sol par un ajustement du potentiel hydrique (Aranda et al., 2021; Sancho-Knapik et al., 2017).

Les différences anatomiques et physiologiques entre les angiospermes et les gymnospermes peuvent induire des stratégies de réponse à la sécheresse distinctes et influent notamment sur leur niveau de résistance. L'ensemble des réponses induites par une sécheresse répondent à une régulation fine de l'expression des gènes. Les approches génomiques, et notamment les analyses transcriptomiques, permettent donc d'identifier les acteurs clés induits par le stress.

0.4 La transcriptomique des arbres forestiers

0.4.1 Le développement de la génomique en écologie forestière

La génomique dans le contexte de l'écologie forestière a été dans un premier temps utilisée pour améliorer la maîtrise de la domestication et de la sélection des variétés les plus productrices (Harfouche et al., 2012). Mais plus récemment, l'accélération des changements globaux a orienté les innovations génomiques vers une amélioration de la gestion et de la conservation des forêts (Laverdière et al., 2022; Parent et al., 2015). Une des plus grandes avancées dans le domaine est la démocratisation des méthodes de séquençage à haut débit (*Next Generation Sequencing*, NGS). Les NGS, comparativement aux méthodes de première génération, sont plus rapides et moins coûteuses. Les NGS ont donc rendu plus accessibles les recherches sur les espèces non modèles dont les références génomiques sont inexistantes ou très fragmentées,

comme c'est le cas pour la majorité des arbres forestiers (Parent et al., 2015; Prunier et al., 2015). Les espèces les plus étudiées chez les arbres appartiennent à la famille des *Pinaceae* pour les gymnospermes et des *Salicaceae*, *Rosaceae*, *Fabaceae* et *Fagaceae* pour les angiospermes (Lopez de Heredia & Vázquez-Poletti, 2016). La transcriptomique est un outil fondamental en écologie, car elle permet de comparer l'expression génique d'individus ou de tissus issus de différentes conditions et/ou à différents stades de développement. De plus, cette technique permet également de classer les transcrits, de déterminer les structures transcriptionnelles des gènes et de réaliser des annotations fonctionnelles (Neale & Wheeler, 2019).

0.4.2 La transcriptomique

0.4.2.1 L'ARN messager : de la transcription à la protéine

La transcription est la première étape du processus à l'origine de la biosynthèse des protéines. Celle-ci se déroule dans le noyau des cellules et consiste à copier par complémentarité de séquence une portion d'un gène porté par l'ADN en ARN pré-messager (ARNpm). Chez les eucaryotes, les gènes sont constitués de parties codantes (les exons) et non codantes (les introns). Une fois synthétisé, l'ARNpm est maturé en ARN messager (ARNm) par un épissage constitutif qui consiste en l'excision des introns. Dans certains cas, l'ARNpm est maturé par un épissage alternatif qui entraîne la rétention de certains introns et/ou l'excision de certains exons (Chen et al., 2020). En plus de permettre la synthèse de différentes protéines à partir d'un même gène, l'épissage alternatif jouerait également un rôle important dans la régulation de l'expression de 60% à 85% des gènes des végétaux et influencerait notamment le développement et la réponse aux stress des plantes (Chen et al., 2020; Zhu et al., 2017). Une fois matures, les ARNm sortent du noyau cellulaire et sont traduits en protéines dans le cytoplasme par les ribosomes.

Les eucaryotes disposent d'ARN codants (ARNm), dont la finalité est de coder pour une protéine, et d'ARN non codants qui jouent de multiples rôles dans le fonctionnement et la régulation cellulaire (ANR ribosomique, ARN de transfert, microARN) (Chen, 2009; Giegé et al., 2012; Sáez-Vásquez & Delseny, 2019). Dans cette thèse de doctorat, seuls les ARNm ont été utilisés. L'essentiel à retenir est donc que l'ARNm est une molécule simple brin qui constitue l'intermédiaire entre l'ADN et la protéine et qu'un même gène peut coder pour

différents ARNm. L'ensemble des ARNm constituent le transcriptome et peuvent être utilisés pour évaluer le niveau d'expression des gènes d'un organisme soumis à différentes conditions environnementales à un moment donné (Neale & Wheeler, 2019; Parent et al., 2015).

0.4.2.2 Le séquençage de l'ARN

Le séquençage de l'ARN ou *RNA-seq*, est une méthode très populaire en transcriptomique pour l'étude des organismes non modèles. Cette méthode ne nécessite pas obligatoirement de référence génomique et est relativement abordable et facile d'accès. De plus, elle présente un faible taux d'erreur comparativement aux anciennes techniques (p. ex. les puces à ADN) (Raghavan et al., 2022; Tyagi et al., 2022). Après extraction, les échantillons d'ARN sont convertis en ADN complémentaire (ADNc) par rétrotranscription, puis séquencés. Il existe différentes plateformes de séquençage, dont la plus courante est la technologie Illumina qui génère des séquences (*reads*) courtes de 100 à 300 paires de bases (pb). D'autres plateformes de séquençage existent comme les technologies ION Torrent PGM ou AB SOLID, mais leur utilisation reste très marginale pour les espèces d'arbres forestiers (Lopez de Heredia & Vázquez-Poletti, 2016; Metzker, 2010). Les séquences obtenues sont ensuite filtrées et nettoyées, puis assemblées en de plus longues séquences pour pouvoir être étudiées.

0.4.3 L'assemblage de novo du transcriptome : construire sa référence transcriptomique

Les séquences obtenues après le séquençage peuvent être assemblées en un transcriptome en les alignant sur un génome de référence de l'organisme étudié, ou sur celui d'une espèce apparentée. Dans le cas où aucune référence de qualité n'est disponible (p. ex. des références très fragmentées ou incomplètes), l'assemblage des séquences peut être réalisé par une méthode *de novo* (du latin "du début") (Martin & Wang, 2011; Raghavan et al., 2022). L'objectif de l'assemblage *de novo* est d'aligner les fragments de séquences les uns aux autres par des méthodes sans à priori afin de reconstruire les séquences d'origine du transcriptome de l'espèce. Un fois assemblé, le transcriptome *de novo* peut servir de référence pour réaliser des analyses transcriptomiques de l'espèce concernée (Figure 0.2). Concernant les arbres forestiers, l'approche par assemblage *de novo* est souvent privilégiée, car peu d'espèces disposent d'un génome suffisamment complet et annoté (Lopez de Heredia & Vázquez-Poletti, 2016), comme c'est le cas pour l'érable à sucre et l'épinette blanche, les deux espèces cibles de cette étude. Une fois que l'assemblage *de novo* du transcriptome est effectué, une annotation fonctionnelle

des séquences contenues dans l'assemblage peut être réalisée par homologie de séquences avec des bases de données géniques et/ou protéiques. Cette annotation fonctionnelle permet d'identifier des gènes potentiels homologues aux séquences, mais aussi d'obtenir des informations sur leurs fonctions.

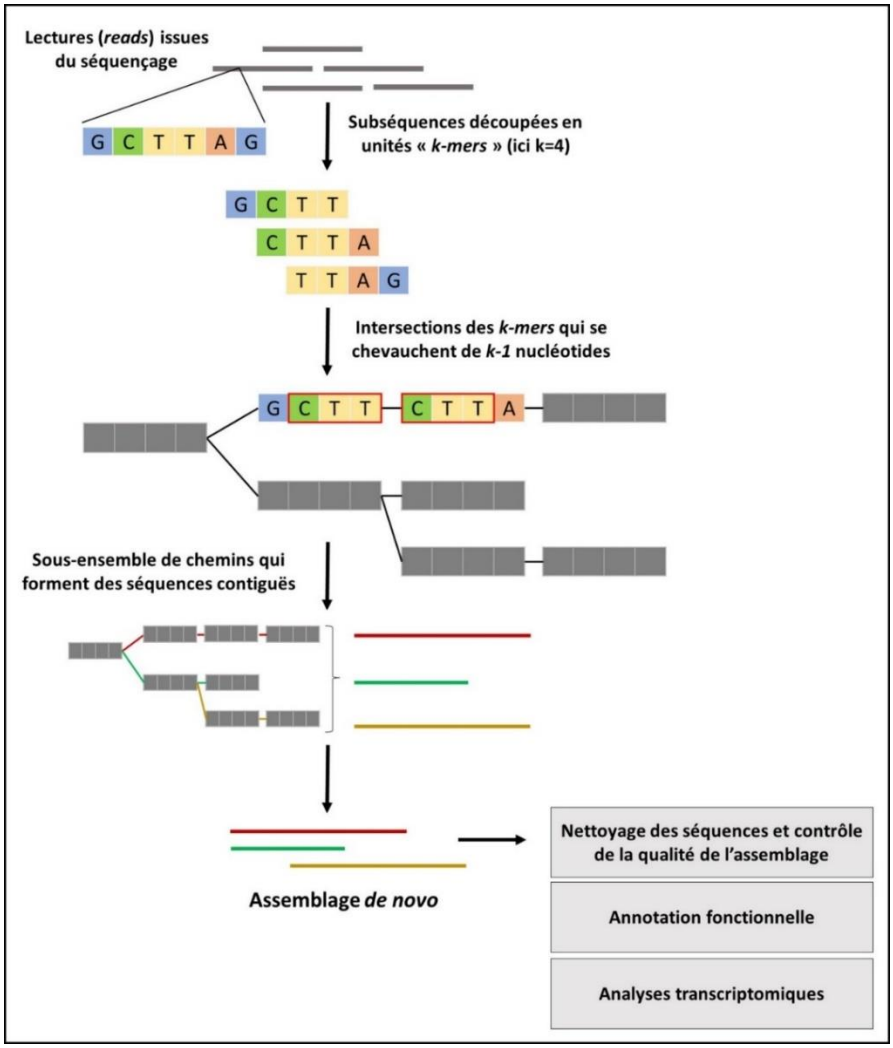


Figure 0.2. Approche simplifiée des différentes étapes à la réalisation d'un assemblage *de novo* d'un transcriptome. Cette procédure d'assemblage consiste à établir un catalogue de subséquences issues des lectures obtenues par le séquençage de l'ARN et de créer des graphiques constitués de chaînes de séquences qui se chevauchent d'au moins k-1 nucléotides. Les graphiques contiennent différents chemins qui correspondent aux potentiels transcrits d'origine. Après l'assemblage, diverses étapes de nettoyage et

de contrôle de la qualité sont nécessaires. Une fois assemblé et annoté par homologie de séquences avec diverses bases de données, l'assemblage *de novo* peut servir de référence pour des analyses transcriptomiques. Figure inspirée et modifiée de Raghavan et al. (2022).

0.5 Les espèces cibles de l'étude

Les deux espèces cibles de l'étude sont l'érable à sucre (*Acer saccharum* [Marsh]) qui est un angiosperme et l'épinette blanche (*Picea glauca* [Moench] Voss) qui est un gymnosperme. Ces deux arbres sont des espèces de fin de succession et dominantes dans les forêts canadiennes. Bien qu'elles démontrent une écologie distincte et des spécificités propres, elles ont toutes deux été caractérisées comme étant susceptibles d'être fortement impactées par l'augmentation des évènements de sécheresse d'ici 2100 (Aubin et al., 2018). De plus, ce sont deux espèces généralement décrites pour avoir une gestion hydraulique conservatrice (comportement plutôt isohydrique, c'est-à-dire qui ferme rapidement leurs stomates sous sécheresse pour limiter la perte en eau) (Roman et al., 2015; Sullivan et al., 2021; Yi et al., 2017).

0.5.1 L'érable à sucre

0.5.1.1. L'écologie de l'espèce

L'érable à sucre est une espèce monoïque à croissance lente de fin de succession qui arrive à maturité vers l'âge de 40 ans et qui a une longévité pouvant atteindre les 400 ans. L'érable à sucre présente une large aire de répartition en Amérique du Nord, où elle est notamment très abondante dans les forêts du nord-est et de l'ouest des États-Unis, ainsi que dans l'est du Canada (Horsley et al., 2002, Figure 0.3). L'érable à sucre est une espèce tolérante à l'ombre qui affectionne les environnements frais et mésiques avec des sols alcalins riches en nutriments (Godman et al., 1990). L'érable à sucre est une espèce emblématique au Canada avec une forte valeur patrimoniale dont la production du sirop d'érable constitue un savoir-faire traditionnel qui contribue à l'identité du pays (Hinrichs, 1998). Par ailleurs, en plus d'être également très apprécié pour la qualité de son bois et ses belles couleurs automnales, l'érable à sucre est

également une espèce à haute valeur écologique (Long et al., 2009; Lucash et al., 2012). Ces dernières décennies, une diminution des populations d'érable à sucre a été observée, avec une augmentation des signes de dépérissement et une diminution du taux de régénération (Bal et al., 2015; Nolet & Kneeshaw, 2018; Oswald et al., 2018). L'acidification des sols forestiers, résultant des dépôts acides d'origines anthropiques a été identifiée comme l'un des principaux facteurs contribuant à ce déclin (Bal et al., 2015; Long et al., 2019; Moore & Ouimet, 2021).

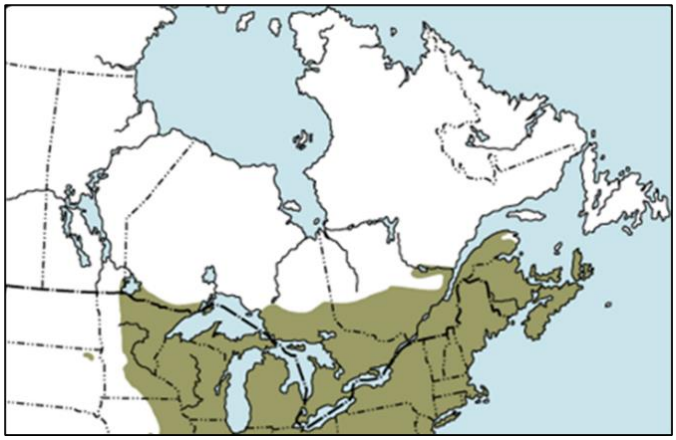


Figure 0.3. Répartition géographique en Amérique du Nord de l'érable à sucre (Ressources Naturelles Canada).

Mais des travaux s'accordent sur le fait que d'autres facteurs, en lien avec les changements climatiques, et notamment la sécheresse, accentuent la problématique (Moreau et al., 2020; Muhr et al., 2016; Nolet & Kneeshaw, 2018). En effet, 49% de la distribution actuelle des populations d'érables à sucre d'ici 2100 pourrait se retrouver en dehors de sa niche hydrique (Aubin et al., 2018). L'érable à sucre est une espèce avec un bois dense et un système vasculaire à pore diffus, une caractéristique qui améliore la résistance à la cavitation (Bryant et al., 2022). Décrite comme une espèce qui maintient un potentiel hydrique stable et qui ferme rapidement

ses stomates (comportement isohydrique) pendant une sécheresse (Roman et al., 2015; Yi et al., 2017), elle pourrait toutefois modifier sa gestion hydrique selon l'intensité de la sécheresse en maintenant ses échanges gazeux si elle dispose d'un accès à l'eau suffisant (par exemple en profondeur) (Guillén et al., 2022). Malgré les vives inquiétudes concernant cette espèce, des travaux ont mis en évidence qu'elle dispose d'une grande plasticité phénotypique et d'un bon potentiel d'adaptation locale (X. Guo et al., 2020, 2023; Solarik et al., 2016).

0.5.1.2. Les ressources génomiques

L'érable à sucre, en tant qu'espèce non modèle, présente une disponibilité limitée en ressources génomiques et très peu d'études menées à l'échelle moléculaire en contexte de sécheresse ont été réalisées (Mulozi et al., 2023). Récemment, des efforts de recherche ont été entrepris pour combler cette lacune, notamment par le développement de marqueurs de microsatellites polynucléaires (cet outil permet l'étude des flux génétiques et de la variation génétique intraspécifique) (Graignic et al., 2013; Harmon et al., 2017; Khodwekar et al., 2015), par l'assemblage du premier génome chromosomique de l'espèce (McEvoy et al., 2022), mais également par l'assemblage *de novo* d'un transcriptome et d'une étude transcriptomique en contexte de stress hydrique (Mulozi et al., 2023). Une partie des données brutes de Mulozi et al. (2023) a d'ailleurs été utilisée dans notre étude. Toutefois, la caractérisation des processus biologiques et des fonctions moléculaires induits par la sécheresse chez cette espèce n'en est qu'à ses débuts et cette thèse contribue à son amélioration.

0.5.2 L'épinette blanche

0.5.2.1 L'écologie de l'espèce

L'épinette blanche est un gymnosperme à croissance lente et de fin de succession très présente dans les forêts tempérées et boréales canadiennes. Cette espèce monoïque et anémophile commence à produire des cônes dès l'âge de quatre ans, mais atteint sa pleine production autour de 30 ans. Elle dispose d'une très large distribution transcontinentale qui s'étend du nord du Canada jusqu'au nord-est des États-Unis (Nienstaedt & Zasada, 1990, Figure 0.4). Décrite comme une espèce plastique, elle présente également une grande variabilité génétique intraspécifique (De Lafontaine et al., 2010; Depardieu et al., 2020) lui permettant de prospérer dans une vaste gamme de conditions climatiques. Elle est retrouvée sur des sites très variés avec

des paramètres du sol assez larges (p. ex. plusieurs gammes de pH, de fertilité ou de niveaux d'humidité), malgré une préférence pour les sols bien drainés (Abrahamson, 2015; Nienstaedt & Zasada, 1990). L'épinette blanche est une espèce économiquement très importante au Canada et représente environ un quart de l'inventaire forestier canadien (Canadian Forest Service, 2015). Son bois, léger et peu dense, est principalement utilisé dans la fabrication de contreplaqué, de pâte à papier, et comme bois de construction (Middleton & Zhang, 2009). L'épinette blanche est une espèce décrite comme relativement sensible à la sécheresse et avec une stratégie hydraulique assez conservatrice (comportement isohydrique), comme la plupart des espèces du genre *Picea* (Brodribb et al., 2014; Sullivan et al., 2021). Les conditions de sécheresse prolongée impactent fortement sa croissance radiale (Chen et al., 2017; Hogg et al., 2017) et augmentent les risques de mortalité (C. Peng et al., 2011). Cette espèce semble également plus sensible à la chaleur que d'autres espèces de conifères (D'Orangeville et al., 2018; McGuire et al., 2010).

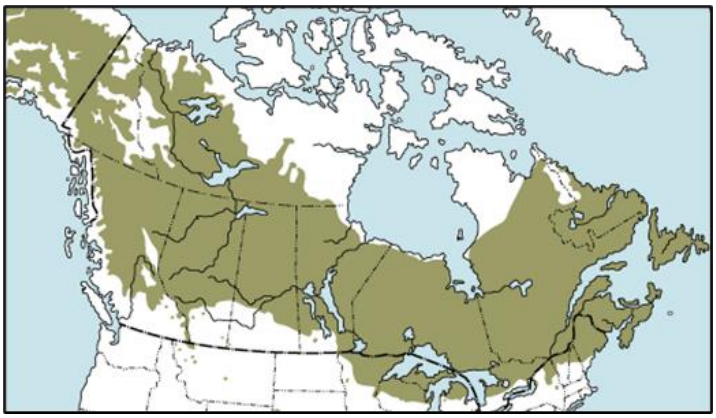


Figure 0.4. Répartition géographique en Amérique du Nord de l'épinette blanche (Ressources Naturelles Canada).

0.5.2.2 Les ressources génomiques

L'épinette blanche disposent de plusieurs ressources génomiques bien que la plupart soient fragmentées et avec une annotation incomplète. Les ressources disponibles sont principalement issues d'analyses à l'échelle d'un ou plusieurs gènes cibles, plutôt que sur l'ensemble du génome et/ou du transcriptome (Depardieu et al., 2021; Hornoy et al., 2015). Ces ressources comprennent notamment des génomes nucléaires (Birol et al., 2013; Warren, Keeling, et al., 2015), des génomes mitochondriaux et chloroplastiques (Jackman et al., 2016), un catalogue de gènes annotés (Rigault et al., 2011), des données de génotypages basés sur les SNP (*single nucleotide polymorphism*) (Pavy et al., 2008, 2013, 2016) et des analyses QTL (*quantitative traits loci*) (Laoué et al., 2021; Pavy et al., 2017; Pelgas et al., 2011). L'épinette blanche fait l'objet de programmes d'amélioration génétique et de sélection génomique, dont les principaux paramètres concernent principalement des traits reliés à la croissance et à la productivité sylvicole (p. ex. le taux de survie après la plantation, la croissance primaire et radiale, la qualité du bois) (Beaulieu et al., 2014; Mullin et al., 2011). Plus récemment, ces programmes ont élargi leurs objectifs de sélection pour intégrer des critères visant à améliorer la résilience aux facteurs environnementaux, dont la sécheresse (Beaulieu et al., 2020; Laverdière et al., 2022; Lenz et al., 2020).

0.6 Objectifs de l'étude

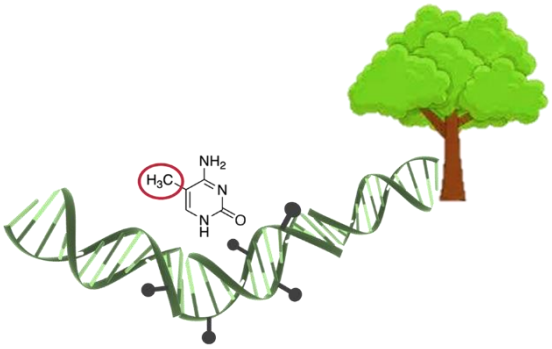
L'objectif principal de cette thèse de doctorat était d'améliorer nos connaissances sur les mécanismes physiologiques et moléculaires qui contribuent au potentiel d'acclimatation à la sécheresse de l'érable à sucre et de l'épinette blanche. Ces réponses ont été étudiées par des approches écophysiologiques et transcriptomiques dans divers contextes de sécheresse : des sécheresses répétées plus ou moins longues sur une même période de croissance, une sécheresse à court terme et sévère et une sécheresse à long terme plus modérée. Cette thèse est composée d'un premier chapitre écrit sous la forme d'un article de revue de littérature qui présente les avancées et les connaissances actuelles des mécanismes épigénétiques chez les arbres en contexte de changements globaux. Puis, les trois autres chapitres sont rédigés sous la forme d'articles de recherche. Chaque chapitre est relié à des objectifs spécifiques décrits ci-dessous.

L’objectif spécifique du chapitre 1 était de faire l’état de nos connaissances sur les mécanismes épigénétiques mobilisés par les arbres en contexte de changements globaux afin de pointer les lacunes actuelles et de guider les futures recherches dans le domaine.

L’objectif spécifique du chapitre 2 était d’explorer la capacité de l’érable à sucre et de l’épinette blanche à déclencher une mémoire de stress après avoir été soumis à des sécheresses consécutives d’intensité croissante. La mémoire de stress est un processus sous le contrôle de mécanismes épigénétiques qui pourrait contribuer à améliorer l’acclimatation des arbres à la sécheresse. La première hypothèse était que deux sécheresses consécutives, une modérée (courte), puis une plus sévère (plus longue), entrecoupées par une période de réhydratation, déclenchent la mise en place d’une mémoire de stress chez les arbres. Il était donc attendu que la mise en place d’un patron de mémoire de stress se caractérise par une amélioration ou un maintien de la vigueur des plants d’érables à sucre et d’épinettes blanches soumis aux deux sécheresses consécutives par rapport aux plants soumis uniquement à la deuxième sécheresse. En opposition, la seconde hypothèse était que deux sécheresses consécutives induisent une accumulation des effets délétères des deux stress. Dans ce cas, il était attendu qu'un patron d’accumulation de stress se caractérise par un déclin de la vigueur plus marqué des arbres doublement stressés par rapport à ceux soumis à une seule sécheresse.

L’objectif spécifique du chapitre 3 était de faire une caractérisation temporelle fine des réponses du transcriptome chez l’épinette blanche lors d’une sécheresse sévère à court terme (3 semaines). Cette caractérisation temporelle avait pour but d’identifier les gènes clés impliqués dans la réponse à la sécheresse chez cette espèce et de mettre en évidence les principales fonctions de ces gènes. Dans ce chapitre, un assemblage *de novo* du transcriptome de l'épinette blanche a été réalisé pour servir de référence aux analyses transcriptomiques. L’épinette blanche dispose d’une grande marge de sécurité hydraulique et est décrite pour réduire drastiquement ses échanges gazeux pendant une sécheresse. L’hypothèse était donc que face à une sécheresse sévère et courte, cette espèce favorise une régulation rapide des processus visant à améliorer la gestion hydrique au détriment des processus liés à la croissance. Il était donc attendu d'observer une régulation à la hausse des gènes impliqués dans les processus d’osmorégulation et de transport, et une régulation à la baisse des gènes associés aux processus de photosynthèse et de croissance.

Le chapitre 4 comportait deux objectifs spécifiques. Dans ce chapitre, un jeu de données d'une expérience hydrique à long terme (6 ans) issu d'un jardin expérimental, et un jeu de données d'une expérience à court terme (3 semaines) en serre ont été utilisés pour chacune des deux espèces (les données brutes à court terme sont issues de l'étude de Mulozi et al. (2023) pour l'érable à sucre et du chapitre 3 pour l'épinette blanche). Afin d'avoir des références robustes pour les analyses transcriptomiques, un assemblage *de novo* de l'érable à sucre a été réalisé dans ce chapitre et l'assemblage transcriptomique *de novo* de l'épinette blanche issu du chapitre 3 a été utilisé de nouveau. **Le premier objectif spécifique** était d'établir une comparaison intraspécifique de l'effet d'une sécheresse longue et modérée par rapport à une sécheresse courte et sévère sur la régulation des gènes de l'érable à sucre et de l'épinette blanche. L'hypothèse était que les gènes clés de la réponse à la sécheresse au sein d'une même espèce sont régulés en réponse à différentes modalités de stress hydrique. Il était donc attendu que les gènes régulés à la hausse en réponse aux deux types de sécheresses soient des gènes clés de réponse à la sécheresse. **Le deuxième objectif spécifique** de ce chapitre était d'établir une comparaison interspécifique des fonctions moléculaires et des processus biologiques observés chez les deux espèces de l'étude en réponse aux deux types de sécheresse. Ces deux espèces ne font pas partie du même groupe (angiosperme *versus* gymnosperme), mais sont toutes deux caractérisées pour présenter une gestion hydraulique assez conservatrice en réponse à une sécheresse. L'hypothèse était que les voies de régulation à la sécheresse sont conservées chez les arbres, même entre deux espèces séparées par une grande distance évolutive. Il était donc attendu d'observer une similitude de fonctions entre les deux espèces soumises à des conditions hydriques similaires.



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1) CHAPITRE 1

ADVANCES AND PROMISES OF EPIGENETICS FOR FOREST TREES

Cet article de revue a été publié dans *Forests* en septembre 2020.

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Résumé

La variabilité génétique des arbres joue un rôle important dans la capacité des forêts à répondre et à s'adapter aux changements environnementaux. Elle est également une variable cruciale à intégrer pour les questions de gestion et de conservation forestière. En plus de la génétique, des études récentes ont mis en évidence “l’épigénétique” comme un domaine de recherche prometteur pour améliorer notre compréhension sur les mécanismes sous-jacents à la plasticité phénotypique et aux réponses adaptatives des arbres. Dans cet article, nous passons en revue les avancées dans ce domaine et leurs applications potentielles pour les chercheurs, ainsi que pour les gestionnaires forestiers. Tout d'abord, nous présentons les bases de l'épigénétique chez les plantes avant de discuter de son potentiel pour l’étude des arbres. Nous proposons ensuite une vue d'ensemble des recherches en épigénétique chez les arbres, en mettant notamment en avant une description du processus de mémoire de stress et de *priming*. Enfin, nous soulignons le potentiel de l’utilisation d’approches épigénétiques pour la recherche et la gestion forestière, ainsi que les lacunes actuelles et les défis futurs. La recherche en épigénétique offre de nombreuses perspectives, notamment par le biais d’approches sylvicoles innovantes pour aider les forêts à s'adapter aux changements globaux.

Mots-clés : Adaptation, sélection, changements climatiques, méthylation de l’ADN, épigénomique, mémoire de stress, *priming*, stress, arbres

Abstract

The importance of tree genetic variability in the ability of forests to respond and adapt to environmental changes is crucial in forest management and conservation. Along with genetics, recent advances have highlighted “epigenetics” as an emerging and promising field of research for the understanding of tree phenotypic plasticity and adaptive responses. In this paper, we review recent advances in this emerging field and their potential applications for tree researchers and breeders, as well as for forest managers. First, we present the basics of epigenetics in plants before discussing its potential for trees. We then propose a bibliometric and overview of the literature on epigenetics in trees, including recent advances on tree priming. Lastly, we outline the promises of epigenetics for forest research and management, along with current gaps and future challenges. Research in epigenetics could use highly diverse paths to help forests adapt to global change by eliciting different innovative silvicultural approaches for natural- and artificial-based forest management.

Keywords: Adaptation, breeding, climate change, DNA methylation, epigenomics, memory, priming, stress, trees

1.1 Forests and epigenetics in a time of global change

Forests represent a prime resource covering around 30% of the world's land surface, or 4 billion hectares (Food and Agriculture Organization (FAO), 2018). These are dynamic ecosystems with a variety of functions that provide numerous social, economic, and environmental benefits. Forest productivity and health are currently threatened by global change, especially due to increasing drought and/or episodes of high temperature, as well as invasive insects and pests (H. Adams et al., 2010; Allen et al., 2010; Anderegg et al., 2016; Messier et al., 2019). As the effects caused by abiotic and biotic stresses can last for long periods, there is a high probability of interaction with other stresses or disturbances to produce more stressful combined events (Denny et al., 2009; Millar & Stephenson, 2015; Nolet & Kneeshaw, 2018). The United Nations (https://www.un.org/esa/forests/wp-content/ uploads/2016/12/UNSPF_AdvUnedited.pdf) and the European Forest-Based Sector (https://www.forestplatform.org/wp-content/uploads/2020/05/SIRA_2030.pdf) recognize the need for a collaborative global response to achieve more adapted and resilient forests under a climate change scenario, such as the initiative from the International Union Of Forest Research Organizations (https://www.iufro.org/ fileadmin/material/publications/spotlights/spotlight75-iufro-task-forces.pdf). Forest trees are sessile, perennial, and modular organisms with complex life cycles that are often challenged by environmental variations during their long lifespan. Surviving tree populations can respond to these environmental changes through complex and interacting mechanisms (Bruce et al., 2007; Feeley et al., 2012): Migration, adaptation, and phenotypic plasticity. At the individual scale, organisms can acclimate through phenotypic plasticity, i.e., the ability to produce different phenotypes in response to different environments (Fox et al., 2019). Such plasticity is particularly important for non-mobile organisms, such as trees. Across generations, populations can shift their distribution (i.e., migrate) to remain at equilibrium with the climate (Parmesan, 2006), or they can remain in their present geographic location by improving their fitness and adapting to new climate conditions (Davis & Shaw, 2001; Herrel et al., 2020; Marin et al., 2020; Yona et al., 2015). Such local adaptation is crucial for many tree species that will not be able to keep pace with climate change, such as sugar maple in North America (Solarik et al., 2020). The real challenge for ecologists, conservation biologists, and forest managers is to predict which of these two options, favor migration or adaptation, will be best for tree species (Heilmeier, 2019; Rey et al., 2020). The role of forest genetic material in responding to environmental changes is a key player in forest management and conservation

(Alfaro et al., 2014). As many species are unlikely to migrate fast enough to keep up with the rapidly changing climate, breeding programs should be rethought to incorporate climate change-related traits, including plasticity and adaptation, as selection criteria when exploring the high genetic variation in forest trees. Information from trials established to focus on growth/productivity traits should now be reinterpreted from the perspective of these new large-scale environmental challenges (Alberto et al., 2013; Laitinen & Nikoloski, 2018) and new trials should be established addressing these explicit responses. Functional traits are morphological, physiological, and phenological attributes that determine an organism's performance to a given environmental filter by impacting growth, reproduction, and survival (Heilmeier, 2019; Violle et al., 2007). It is crucial to identify relevant traits and thresholds for both a better selection of genetic material and species combinations to maximize sustainable ecosystem functions and resilience to climate change. Special attention should be given to traits not often previously considered in breeding depending on bioclimatic zones but likely to be fostered in the context of global change: Pest and disease resistance, drought resistance, fire resistance/tolerance, flood resistance, cyclone resistance, or salt tolerance (Alfaro et al., 2014; Messier et al., 2019). However, resistance and/or tolerance to stress are often difficult to measure and depend on a complex network of functional traits at multiple scales. For instance, 'drought resistance' can rely on diverse processes from those promoting growth maintenance under moderate water deficit to those promoting survival and recovery under severe and/or long-lasting water deficit (Bartlett et al., 2016; Volaire, 2018). These include traits related to the control of water loss (Buckley, 2005; Klein, 2014), the maintenance of cell turgor (Bartlett et al., 2012, 2014; Kozlowski & Pallardy, 2002), shifts in sink-source relationships and adjustments between transpiring vs. conducting or absorbing surface areas (Bogeat-Triboulot et al., 2007; Poorter et al., 2012; F. Wu et al., 2008) or the maintenance of xylem hydraulic continuity (Bartlett et al., 2016; Brodribb, 2017). Another example, 'insect defoliation resistance', is influenced by leaf strength, palatability, and phenolic content (Cornelissen et al., 2003; Gillison, 2019). Hence, a holistic view of trees' adaptation to stress and the identification of sets of key functional traits are imperative. Due to their wide ecological distributions and long-lifespans, trees have evolved numerous adaptations. Considerable effort has been paid to understanding the genetic basis of the traits underlying adaptive responses (Lind et al., 2018). In the context of global change, such information will be especially useful for breeding, conservation, and resilient forest-based industry. Recent advances have, however, highlighted "epigenetics" as an emerging and promising field of research, along with genetics, for the

understanding of tree phenotypic plasticity and adaptive responses (Bräutigam et al., 2013; Carbó et al., 2019; Plomion et al., 2016; Sow, Segura, et al., 2018). Here, we argue that trees as long living organisms may particularly use epigenetics to facilitate phenotypic modifications in response to environmental changes. Research in epigenetics could use highly diverse paths to help forests adapt to global change by eliciting different innovative silvicultural approaches for natural and artificial-based forest management. In this paper, we review recent advances in this emerging field and their potential for tree researchers and breeders, as well as the whole forest management community. We begin by presenting the basics of epigenetics in plants before discussing its potential for trees. We propose a bibliometric and overview of the literature on epigenetics in trees, including recent advances on tree priming, an adaptive strategy that improves their defensive capacity and resulting from a prior experience (Mauch-Mani et al., 2017). Finally, we outline the promise of epigenetics for forest research and management, along with current gaps and future challenges.

1.2 Epigenetics in plants: the basics

Epigenetics has been initially defined as "the branch of biology which studies the causal interactions between genes and their products, which bring the phenotype into being" (Waddington, 1959). The author also proposed the existence of a conceptual picture of this new discipline with the "epigenetic landscape", which represents the interactions between the environment and the genes leading to the development of a certain phenotype (Waddington, 1940). Here, we define it as the study of all the processes affecting the expression of genes and/or the activity of transposable elements (TEs) without altering the DNA sequence that may be heritable by mitosis (during development) and/or meiosis (across generations) (Bossdorf et al., 2007; Ledón-Rettig, 2013; Richards, 2011) (Figure 1.1). Epigenetics has been proposed as being the missing part of heritability since the information encoded into the DNA nucleotide sequence by itself is often insufficient to explain biological variation in all of its complexity (Danchin, 2013; Maher, 2008). Accordingly, epigenomic studies chart the locations and understand the functions of all chemical tags that mark the genome (named epigenome). Epigenomics has quickly become a big data science, posing tremendous challenges of its translation into knowledge (Xiao et al., 2014). The main molecular support for epigenetic mechanisms in eukaryotes is chromatin structure (compaction and 3D positioning in the nucleus).

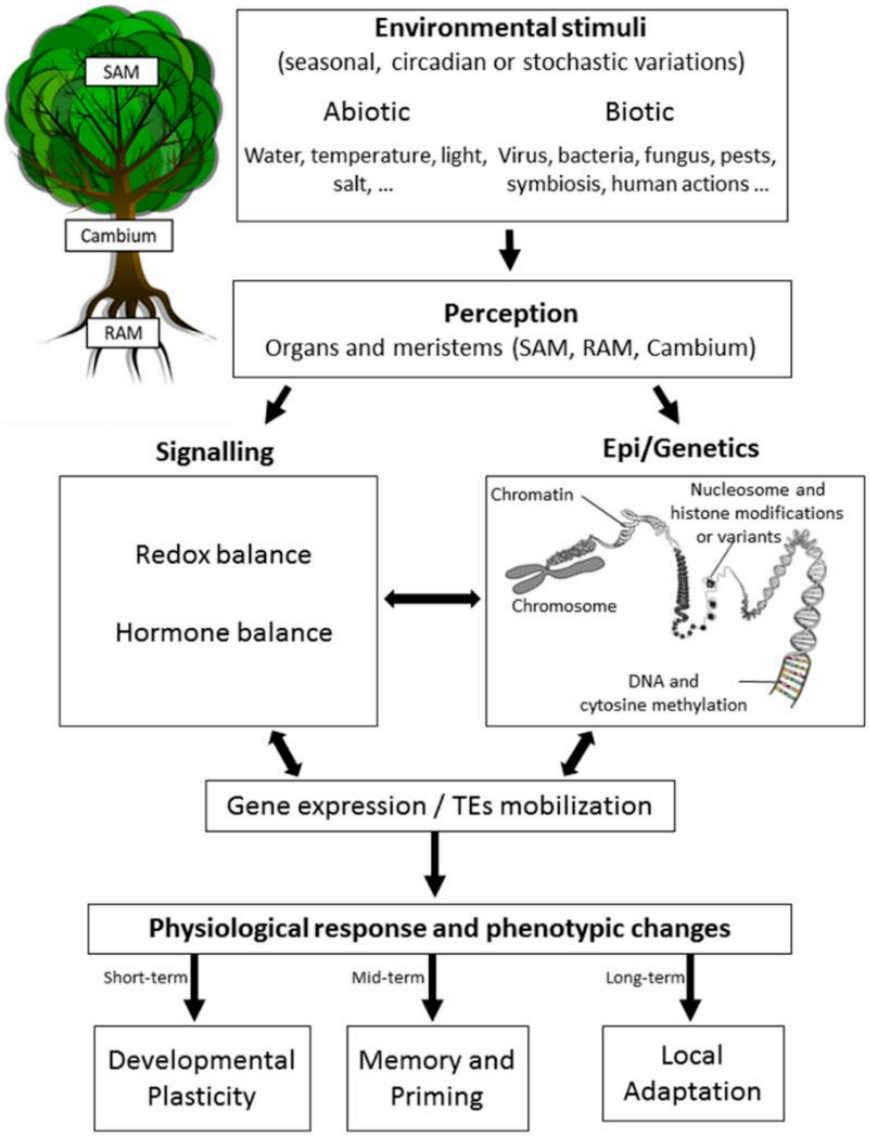

Figure 1.1. Epigenetic response to environmental changes in trees. Trees are known to recognize various abiotic and/or biotic stimuli occurring rhythmically (circadian or seasonal) or stochastically. These changes are perceived at different tissue levels, with most studies focusing on the leaves, roots, and meristems (SAM, shoot apical meristem; Cambium; or RAM, root apical meristem). Perception is then followed by signaling mechanisms that may include changes in the redox or hormonal balance, which have been shown to be related to epigenetic changes (chromatin remodeling, DNA methylation, non-coding RNA mechanisms (not shown), and histone modifications and variants). This complex crosstalk between signaling processes, epigenetics, and genetics results in an altered gene expression status and/or the mobilization of transposable elements (TEs). A physiological response is then observed together with phenotypic changes that allow trees to acclimate to the environmental changes initially sensed depending on the time-scale considered and the heritable transmission of epigenetic changes.

Chromatin is formed through the association of DNA wrapped around nucleosomes (histones octamer). Its compaction is characterized either by a decondensed state (open chromatin or euchromatin) enriched in genes and permissive to transcription, or by a condensed state (closed chromatin or heterochromatin) enriched in repetitive sequences and silenced sequences. These states are controlled by marks defining chromatin domains (Bannister & Kouzarides, 2011; Roudier et al., 2011) and allowing chromatin remodeler activity. The latter are protein complexes modifying the chromatin structure that then allow or prevent the transcription of genes to their destinations on the DNA (Becker & Hörz, 2002; Harikumar & Meshorer, 2015). The chromatin marks or "epigenetic marks" (when transmitted by cell division) are mainly DNA methylation, non-coding RNAs (ncRNAs), and histone modifications and variants (Hussey et al., 2015; Jiang & Berger, 2017; Kawashima et al., 2015; Matzke & Mosher, 2014; H. Zhang et al., 2018; H. Zhao et al., 2019). Indeed, the incorporation of histone variants into nucleosomes and the addition of post-translational modifications to histones can alter the properties of nucleosomes and chromatin. The major histones are deposited during DNA replication, and all eukaryotes have variant types of H2A and H3, such as H2A.Z and H3.3 that are also incorporated into chromatin. In addition, histone proteins have shown that they can be extensively modified post-translationally at their N-terminal tails through the addition of acetyl, methyl, phosphoryl, and ADP-ribose groups, as well as peptides, such as SUMO and ubiquitin. The key players associated with these modifications are: (a) Writers–enzymes that modify the nucleotide bases and specific amino acid residues on histones, (b) erasers–enzymes that remove these marks, and (c) readers–proteins with specific domains that bind or interact with specific epigenetic marks of a locus (e.g., transcription factors) (Biswas & Rao, 2018; Zhu et al., 2016). Epigenetic marks and their machinery may change in response to environmental variations occurring throughout the life cycle of an organism, and in some cases, may be inherited in the progeny (Figure 1.1). DNA methylation is the most studied epigenetic mark because of its occurrence in both plants and mammals (Zemach et al., 2010),its stability (mitosis and/or meiosis), its role in gene regulation (Bewick & Schmitz, 2017; Niederhuth & Schmitz, 2017; Zhang et al., 2018; Zhu et al., 2016), in the silencing of TEs (Mirouze et al., 2009; Slotkin et al., 2009; H. Zhang et al., 2018), or in chromosome interaction (Feng et al., 2014; Zhang et al., 2018), and its facility to be studied at the genome-scale (Kurdyukov & Bullock, 2016; Sow, Segura, et al., 2018; Yong et al., 2016). DNA methylation consists of the addition of a methyl group on the carbon in position 5' of a cytosine residue. Resulting in various patterns at the whole genome level (methylome), the dynamics of DNA methylation (establishment,

maintenance, and active removal) are considered of great importance for plant development and response to environmental variations (Richards et al., 2017; Zhang et al., 2018; Zhang et al., 2006).

In plants, methylation-induced modifications may either be reversible or retained during cell division (mitosis and intra-generational transmission) for a memory process as exemplified by vernalization (Baulcombe & Dean, 2014; Y. He & Li, 2018) or passed on to the next generations (meiosis) as highlighted by the identification of natural epivariants (Cubas et al., 1999; Lisch, 2013, 2013; Manning et al., 2006; Miura et al., 2009) or artificially-induced epivariants and epigenetic recombinant inbred lines (EpiRILs) (Johannes et al., 2009; Reinders et al., 2009). Studies with EpiRILs, where the recombinant offspring is created by crossing two parents with similar DNA sequences, but highly contrasting DNA methylation profiles have shown that part of the DNA methylation variation is inherited in a Mendelian manner (Colomé-Tatché et al., 2012). Moreover, these EpiRILs show significant phenotypic variation in morphology, growth rate, and responses to biotic and abiotic stress (Johannes et al., 2009; Kooke et al., 2015; Latzel et al., 2012; Roux et al., 2011; Y.-Y. Zhang et al., 2013). Epigenetic diversity has thus emerged as a new source of broadened phenotypic variations for improving the adaptation of wild species to changing environments and ensuring the yield and quality of domesticated species whose genetic diversity has been eroded by intense breeding (Gallusci et al., 2017; Richards et al., 2017; Springer & Schmitz, 2017). Epigenetic phenomena can affect plant phenotype and fitness, be stably inherited across multiple generations, and vary across populations and individuals, thus contributing to the ability of plants to colonize, adapt or evolve in variable environments (Herrel et al., 2020; Kawakatsu et al., 2016; Marin et al., 2020; Richards et al., 2017; Schmid et al., 2018; Skinner, 2015; Thiebaut et al., 2019). Yona et al. (2015) suggested that “organisms often adapt by progressing the adaptation spectrum, starting with rapidly attained physiological and epigenetic adaptations and culminating with slower long-lasting genetic ones”. This suggests (i) direct interactions between epigenetics and genetics that can occur through the DNA methylation control of TEs transposition inducing mutations, but also (ii) an interplay between physiological responses and epigenetics (Figure 1.1) (Alonso et al., 2019; Chang et al., 2020). This last point has been recently proposed to occur through chromatin and hormones crosstalk (Maury et al., 2019; Ojolo et al., 2018; Yamamuro et al., 2016) (Figure 1.1).

1.3 Advances in forest tree epigenetics: memory and priming

Our knowledge of forest tree epigenetics has not evolved at the same pace as knowledge about other plant model organisms and crops. The studies on trees represent only a small proportion of those carried out in the field of Plant Sciences (Figure 1.2) and are limited to only a few species (see Table S1.1). Nevertheless, research in forest trees is now well-positioned to enter a productive phase due to the higher availability of genomic resources, especially if the associated costs become more accessible. The sequencing of forest tree genomes such as *Populus trichocarpa* (Tuskan et al., 2006), *Picea abies* (Nystedt et al., 2013), or *Eucalyptus grandis* (Myburg et al., 2014) may have contributed to the increase in forest epigenetic studies over the last decade as such studies rely on high-quality genome sequence assemblies (Sow, Segura, et al., 2018). In addition to recent reviews on the methodologies for tree epigenomics, as well as features of forest tree breeding (Carbó et al., 2019; Plomion et al., 2016; Sow, Segura, et al., 2018), we highlight there after recent advances in this fast-evolving field of research with a special focus on stress epigenetic memory and priming in trees. Indeed, one example of memory-controlling plant response to pathogens, herbivore attacks, or abiotic stresses is defense priming (Bäurle & Trindade, 2020; Crisp et al., 2016; Lämke & Bäurle, 2017; Mauch-Mani et al., 2017). The priming event is followed by a period of stress memory, storing information about the priming stress through an epigenetic phenomenon and resulting in a modified response upon recurring stress exposure or a sustained response after the priming. This memory may last from several days to years for somatic stress memory, and in some cases, may even be extended to the offspring. Table S1.1 summarizes relevant studies that evidence the role of epigenetics in development, biotic and abiotic stress-responses, epigenetic memory, and stress priming, as well as initial and potential uses for tree breeding, conservation of genetic resources, or biotechnology.

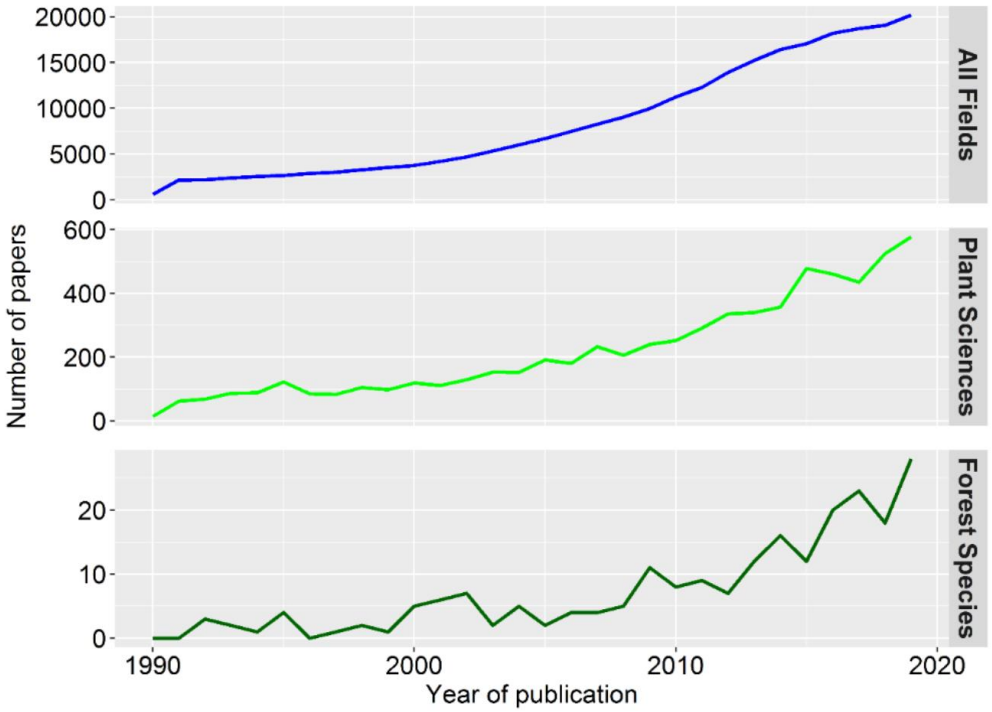

Figure 1.2. The number of papers on Epigenetics published per year from 1990 to 2019 (2020 being underway) in three Research Areas: all fields, plant sciences, and forest species. The number of studies was obtained from the Web of Science Core Collection (www.webofknowledge.com) with the topic search (TS) "epigenetics" and its variants (e.g. "epigenomics"). The main mechanisms behind epigenetics (DNA methylation, histone modification, chromatin remodeling, and non-coding RNA mechanisms) were also considered. An advanced search was therefore conducted as follows: TS = (epigen* OR DNA methyl* OR histone OR chromatin remodel* OR (epigen* AND RNA)). All documents written in English and part of the Science Citation Index Expanded were examined. Results were then refined to retain the papers from the "Plant Sciences" Web of Science Category (adding "AND WC = Plant Sciences" to the query). Data was further filtered for epigenetic research in forest tree species by adding TS fields of "forest" and of the main forest tree genera studied (Picea/spruce, Pinus/pine, Populus/poplar, Quercus/oak, and Eucalyptus/eucalypt) and avoiding results referring to the "random-forest" method (NOT TS = random forest).

Only a few studies have paid attention to histone modifications, probably due to the fact that histone marks are numerous, labile, and difficult to analyze (Li et al., 2017; Ma et al., 2016). DNA methylation is one of the well-understood epigenetic marks and is commonly used in

studies with forest trees. However, due to a lack of high-quality genome sequence assemblies for forest trees, most DNA methylation studies have focused on a global approach (High-Performance Liquid Chromatography) or a locus approach, such as MSAP (Methylation Sensitive Amplification Polymorphism), which uses methylation sensitive restriction enzymes to identify methylated DNA fragments (Klimaszewska et al., 2008; Rico et al., 2014; Sow, Segura, et al., 2018) (Table S1.1). Despite the potential of such techniques in the absence of an assembled genome, they do not offer high resolution making it difficult to identify the specific targets (features) of DNA methylation variations. Once reference genomes are established and available (Sow, Segura, et al., 2018), methods (such as Whole Genome Bisulfite Sequencing) can provide methylation at a single nucleotide resolution over the whole genome (Yong et al., 2016). Another epigenetic mark that is attracting considerable attention in forest trees is ncRNAs (Table S1.1). Expression of specific microRNAs (miRNAs) has been associated with temperature-dependent epigenetic memory-controlling bud set/burst in Norway spruce and with habitat memory in poplar under phosphate starvation (Schönberger et al., 2016; I. Yakovlev et al., 2012; I. A. Yakovlev et al., 2014, 2016).

One striking point in Table S1.1 is that both DNA methylation and ncRNAs raise the fundamental question of the priming effect in forest trees. This epigenetic memory could help trees respond to recurrent biotic and/or abiotic stresses (Avramova, 2019; D'Urso & Brickner, 2017; Hilker & Schmülling, 2019).

Studies in forest tree epigenetics in the context of biotic stresses remain limited. However, recent findings have highlighted variation in the epigenome of individuals infected by pathogens compared to healthy individuals. These variations have been observed at the methylome level for ash trees infected with the fungus *Hymenoscyphus fraxineus* (Sollars & Buggs, 2018). A study of *Paulownia fortunei* infected with *Paulownia* witches'-broom disease showed a differential level in two histone methylations (H3K4me3, H3K36me3) and one histone acetylation (H3K9ac). The modifications of these histones seemed to be involved in the regulation of genes related to secondary metabolism, such as phenylpropanoid metabolism and the phytohormonal pathway, and to a lesser extent, genes related to plant-pathogen interactions (Yan et al., 2019).

Regarding abiotic disturbances, variation in DNA methylation levels has been reported following drought (Correia et al., 2016; Gourcilleau et al., 2010; Lafon-Placette et al., 2018; A. L. Le Gac et al., 2019; A.-L. Le Gac et al., 2018; Neves et al., 2017; Plitta-Michalak et al., 2018; Raj et al., 2011; Sow, Segura, et al., 2018), salt stress (Liu et al., 2018; Liu et al., 2019; Murata et al., 2012), exposure to heavy metals (Cicatelli et al., 2014), or temperature extremes (Carón et al., 2015; Conde, Le Gac, et al., 2017; Conde, Moreno-Cortés, et al., 2017; Deng et al., 2018; Dewan et al., 2018; Schönberger et al., 2016; Yakovlev et al., 2011). Other investigations have reported changes in histone proteins related to water deficiency (Fox et al., 2018; Li et al., 2019) or chronic radiation exposure (Duarte et al., 2019), showing that these variations may occur at a genome-wide level, such as in *Populus* clones (Raj et al., 2011) or in mangrove trees (*Laguncularia racemosa*) (Lira-Medeiros et al., 2010). Integrative studies have attempted to establish correlations between phenotypic or molecular responses and epigenetic modifications to highlight how epigenetic mechanisms potentially controlled the response of trees to environmental disturbances. For example, a study showed that salt exposure in *Pyrus betulaefolia* (Pb) influences the expression of demethylases (DME), which highlights the important role of PbDME genes in response to salt stress (Liu et al., 2018). Moreover, a study on *Pinus radiata* suggested that PrELIP1 (*P. radiata* Early Induced Protein 1), which has a role in resistance to photo-oxidative stress, is partly regulated by an epigenetic pathway during UV-B exposure (Valledor et al., 2012).

For forest tree species, the best-known example of epigenetic memory is the environmental regulation of the performance of *Picea abies* progeny during seed production. Johnsen et al. (2005) first suggested that seed production temperature and photoperiod interact to develop a long-lasting memory mechanism regulating phenology and frost hardiness, as well as bud burst timing (Kvaalen & Johnsen, 2008) in *P. abies* progeny. This temperature-regulated epigenetic memory was associated with changes in gene expression either during its embryogenic development (Johnsen et al., 2005) or in the progenies (Carneros et al., 2017; Yakovlev et al., 2011). Besnard et al. (2008) further supported the existence of a memory mechanism in *P. abies* as maternal environmental conditions during reproductive steps only induced limited genotypic selection. This epigenetic memory could also explain the rapid acclimation in Norway of *P. abies* originating from Central Europe, which showed a bud set more similar to Norwegian seedlings than to seedlings from the same origin (Skrøppa et al., 2010). Evidence for the epigenetic basis of the memory formed in *P. abies* embryos influencing adult tree traits is well

documented. Changes in the transcriptome of genetically identical *P. abies* somatic embryos during morphogenesis at 18 and 30°C were putatively based on chromatin modifications (I. A. Yakovlev et al., 2014). Moreover, the expression of a significant number of epigenetic regulators changed according to different epitype-inducing conditions, supporting the key role of DNA and histone methylation and small RNAs (sRNAs) to establish an epigenetic memory in *P. abies* (Yakovlev et al., 2016). The putative role of miRNAs in epigenetic regulation was suggested by the differential expression of specific miRNAs in seedlings of a full-sib family showing distinct epigenetic differences in bud set originated from seeds developed in cold and warm environments (Yakovlev et al., 2010). An *in silico* analysis of sRNAs further supported the involvement of miRNAs in the formation of epigenetic memory in somatic embryos of *P. abies* (Yakovlev & Fossdal, 2017).

The influence of the maternal environment has also been shown to play an important role in the tolerance level of the offspring to various pathogens. Individuals from mother plants exposed to pathogens appear to be more tolerant to these same pathogens, and authors hypothesized that epigenetic mechanisms may be involved in this priming phenomenon (Camisón et al., 2019; Vivas et al., 2013). Recently, Lamelas et al. (2020) proposed the importance of epigenetic mechanisms for *P. radiata* heat stress tolerance and priming by evaluating the nuclear proteome and tissue DNA methylation dynamics of seedlings submitted to 45°C for 10 days, allowed to recover, and submitted to another stress cycle. Several proteins involved in epigenomic-driven gene regulation were identified. The authors state that a priming-induced epigenetic memory may develop novel approaches to improve pine survival under extreme heat stress in the context of climate change. Also, the application of the phytohormone methyl jasmonate on a stand of 48-year-old *P. abies* 35 days before exposure to the tree-killing bark beetle resulted in a primed state or immunological memory, which allowed these trees to resist insect attack (Mageroy et al., 2020). The authors argue that the establishment of this priming memory is likely related to epigenetic mechanisms, such as DNA methylation and histone modifications as in *Arabidopsis* (Wilkinson et al., 2019), but acknowledge that further studies are needed.

Recent studies have suggested that epigenetic mechanisms are also involved in drought-induced developmental plasticity through the regulation of the phytohormonal pathway in the shoot apical meristem (SAM) (Lafon-Placette et al., 2018; Le Gac et al., 2018; Maury et al., 2019). Developmental plasticity (Pigliucci, 1998) involves the pluripotency of stem cells located in

meristems through the fine-tuning control of cell identity genes by hormones (Gaillochet & Lohmann, 2015). Crosstalk between chromatin and phytohormones in the meristem is supported by (i) direct control of phytohormone signaling on chromatin modifiers or marks, and vice versa, and (ii) their effects on cell identity genes in the meristem, on the stabilization of gene expression beyond the initial hormonal signal, and on the integration of separate hormones by chromatin acting as a hub. This process could explain, at least in part, the epigenetic memory and priming of organs after recurrent stress. An environmentally-induced epigenetic memory that may facilitate tree acclimation has been suggested in winter-dormant SAMs of field-grown poplar trees (Le Gac et al., 2018). Trees grown under moderate drought conditions during the vegetative period exhibited differentially methylated regions on stress-responsive genes and hormonal pathways in winter-dormant SAMs six months later, suggesting an epigenetic memory mediated by DNA methylation (Le Gac et al., 2018). A significant decrease in global DNA methylation has also been detected in winter-dormant SAMs from natural populations of black poplar subjected to a summer-drought (Sow, Segura, et al., 2018). Furthermore, Le Gac et al. (2019) showed that the first leaf emerging from SAMs also exhibited changes in DNA methylation under variable water availability. This result suggests a possible mitotic transmission (memory) from the SAM to the emerging leaf formed during the stress period. Other studies have reported an epigenetic memory in trees in response to drought with poplar transcriptomics depending on clone history (Raj et al., 2011), or in response to phosphorus nutrition with site-dependent growth of clonal *Populus trichocarpa* (Schönberger et al., 2016).

Altogether, although it is well-established that global changes represent a higher risk for insect and disease outbreaks in forests worldwide, there is a lack of epigenetic studies focusing on the response of forest species to biotic stresses. Most of the work in forest tree epigenetics is related to the response of several species to abiotic stresses, mainly drought and extreme temperatures. Moreover, our understanding of what functional traits allow plants to thrive under stressful conditions and their relationships with epigenetic mechanisms is lacking. In poplar and Norway spruce, epigenetic memory has proven to provide trees with adaptive plasticity, based on previous environmental conditions that facilitate plant responses to new challenges (Table S1.1). In line with these findings, epigenetics has emerged as a potential tool to understand and improve priming strategies against both biotic and abiotic stresses.

1.4 Promises of epigenetics for tree improvement, breeding, conservation of genetic resources and forest management

The potential of epigenetics in plant breeding is already being considered in crops (Gallusci et al., 2017, 2018; C. L. Richards et al., 2017; Ryder et al., 2019; Springer & Schmitz, 2017). Only limited studies have been implemented with trees, and results coming from crops will need to be adapted to fit with the specific features of tree breeding and forest management (Sow, Segura, et al., 2018). Although most of the studies on forest trees have focused on the role of epigenetics in tree development, and response to environmental changes and priming (Table S1.1), recent studies have pointed out the potential relevance of epigenetics in tree improvement. Three main approaches using epigenetics may be foreseen: (i) Exploit natural or artificially-induced epigenetic diversity; (ii) use epigenetic marks in addition to classic genetic markers for trait improvement with trans-omics approach (Yugi et al., 2016), statistics and modeling, and (iii) use epigenome editing (Bewg et al., 2018). Epigenetics could be used to expand the material for tree breeders that may be used by sexual or asexual propagation methods.

The best-known example in trees for exploiting natural or induced epigenetic diversity is the existence of an epigenetic memory mechanism that operates during embryo development in Norway spruce (see above section). This process adjusts the timing of bud burst in the progeny, but also in genetically identical epitypes in a manner usually associated with ecotypes in accordance with the temperature conditions during embryogenesis (Carneros et al., 2017; Johnsen et al., 2005; Kvaalen & Johnsen, 2008; Skrøppa et al., 2010; Yakovlev et al., 2011). Kvaalen and Johnsen, (2008) demonstrated that environmentally induced epigenetic memory during somatic embryogenesis can give similar results for phenology as to those produced by provenance separation of 4–6◦ latitude. Environmentally induced epigenetic memory during somatic embryogenesis could potentially be used in tree breeding to prime trees regarding their phenology for their first years of plantation along a latitudinal gradient. This promising application opens up many possibilities for forest tree research considering the increasing literature on tree priming (Table S1.1), and unravels the importance of epigenetic natural diversity that should be taken into consideration for forest breeding, conservation of genetic resources and forest management and protection. Another example of exploiting natural epigenetic variation is emerging from the role of epigenetics in tree heterosis that has been

evaluated in *Populus deltoides* (Gao et al., 2014). Findings indicate that methylation patterns of the two *P. deltoides* parental lines are both partially and dynamically passed on to their intraspecific hybrids, resulting in a non-additive methylation pattern in F1 hybrids. However, studies on epigenetic and heterosis are still necessary for trees and will have to be evaluated by tree breeders.

Another perspective of forest tree epigenetics comes from studies conducted on *Arabidopsis* and crops concerning sRNAs and cross-kingdom RNA interference (RNAi) (Weiberg et al., 2015). Cai et al. (2018) have shown that *Arabidopsis* has adapted its exosome-mediated cross-kingdom RNAi as part of its immune responses against pathogens. Pathogens and pests can thus be controlled by sRNAs, targeting their essential or pathogenicity genes, raising the possibility of plants to be protected from diseases by a novel eco-friendly, durable, and highly specific RNA fungicide or pesticide (Cai et al., 2018; Muhammad et al., 2019). In addition, there is growing evidence to indicate that epigenetic mechanisms directly participate in plant immune responses. However, the evidence of transgenerational inheritance of pathogen-induced defense priming is still a matter of debate (Ramirez-Prado et al., 2018). Studies have shown that biotic stresses can also trigger an increase in the overall level of genomic methylation. Curiously, the methylation levels of some pathogen responses or resistance genes are reduced (Peng & Zhang, 2009). We still need to understand exactly how epigenetics controls trees' defenses against disease to then translate this knowledge into practical actions. By exploring natural and induced resistance, we may implement new breeding strategies for preventing disease while reducing the global reliance on harmful pesticides, a field of research still in its infancy for trees.

The application of epigenetic markers to tree breeding has been tested over the last few years, including studies highlighting the extent and variation of genome-wide DNA methylation in natural populations of trees. Ci et al. (2015) investigated the variation in DNA methylation and whether this variation correlates with important plant traits, including leaf shape and photosynthesis in *Populus simonii*, indicating that epigenetics bridges environmental and genetic factors in affecting plant growth and development. Sow et al. (2018) reported the first estimate of narrow-sense heritability (h2) and phenotypic differentiation (Pst) for global DNA methylation in trees by assessing global DNA methylation variations in *Populus nigra* clones from natural populations under varying soil water availability. Regardless of water regimes,

values of h2 and Pst were comparable to those found for shoot biomass production, a known heritable trait in poplar. Therefore, global DNA methylation, being genetically and environmentally determined in these populations, could be used as a potential marker for population differentiation, performance, and selection under stressful conditions. To explore the epigenetic diversity in breeding programs, we need to expand our knowledge regarding the link between DNA methylation and economic traits in forest trees. Ma et al. (2012) investigated 130 intraspecific hybrids of *Populus tomentosa* and concluded that the regions defined by the MSAP candidate markers are linked to genes that are essential for photosynthetic traits that respond to DNA methylation which affect growth traits.

The potential of epigenetic markers in quantitative breeding approaches has been recently suggested by Baison et al. (2019). The authors identified 52 QTLs (Quantitative Trait Loci) of wood properties in Norway spruce for marker-assisted breeding. However, these QTLs explain a small proportion of the genetic variation, in line with previous studies examining genetic variation in complex traits in coniferous species using forward genetic approaches. They suggest that this could be due to several factors, including epigenetic variation, highlighting the need for sophisticated epi/genotyping tools, as well as a combination of advanced statistical models, such as regional heritability mapping. Recently, Champigny et al. (2020) applied statistical learning experiments to genetically diverse populations of *Populus balsamifera* trees grown at two common garden sites and showed that traits in novel genotypes can be modelled using small numbers of methylated DNA predictors. Indeed, significant phenotypic variances in quantitative traits of the wood were explained by the natural variation of DNA methylation, such as total biomass (57.5%), wood density (40.9%), soluble lignin (25.3%), and carbohydrate content in the cell wall (mannose: 44.8%). The authors proposed that DNA methylation-based models can be used as a strategy to validate the identity, provenance, or quality of agroforestry products. While this approach presents innovative perspectives for forest trees, it should be evaluated in terms of cost, technical, and analytical efforts.

The last approach to use epigenetics for tree improvement is epigenome editing, i.e., a type of genetic engineering in which the epigenome is modified at specific sites using engineered molecules targeted to those sites. Genome editing in trees has been recently reviewed by Bewg et al. (2018), suggesting a great potential for stable CRISPR-induced mutations and associated phenotypes over multiple clonal generations. Authors suggest that this technology can be used

for the commercial production of elite trees that relies on vegetative propagation. The potential of epigenome editing to control recombination in plant breeding has been recently reviewed (Taagen et al., 2020). Manipulating the rate and positions of crossovers to increase the genetic variation accessible to breeders is of main interest. Epi/genome editing at desired sites of recombination, and manipulation of crossovers factors, are applicable approaches for achieving this goal, reducing the time and expense associated with traditional breeding, revealing inaccessible genetic diversity, and increasing control over the inheritance of preferred haplotypes (Taagen et al., 2020). However, there is no study on epigenome editing in trees, and the feasibility of such technological advances, depending on use regulations in different countries, will have to be evaluated by tree breeders in the future.

1.5 Gaps in knowledge and future challenges

Although the conceptual role of epigenetics in trees is established (see Figure 1.1) and supported by an increasing number of articles (Figure 1.2 and Table S1.1), there is still a long way to go before one will make use of this adaptive potential in forest management. Most studies in forest trees have focused on how developmental and/or environmental interactions affect epigenetic marks (particularly DNA methylation), mainly using descriptive or correlative approaches between phenotypic traits and epi/genomics data. Recently, Alonso et al. (2019) proposed suitable approaches to detect links between epigenetic variation and plant (*Arabidopsis* and crop) functional phenotypic traits for biotic stress. The strategy combines the use of epimutants, drugs (Jubierre et al., 2018), or wild populations across environmental gradients with direct phenotyping of fitness traits to study somatic or transgenerational relationships after stress exposure. The authors proposed (i) “concurrent analysis of epigenetic variation and phenotypic trait variation, including plant fitness between individuals exposed to contrasted biotic interactions” and (ii) “analysis of specific loci and physiological pathways to clarify the epigenetic contribution to the stabilization of environmentally induced phenotypes (priming) or across generations” to gain insights into functional relationships. In addition to these recommendations, we propose a complementary methodological plan for epi/genetics in trees by (i) using both forward and reverse (epi)genetic approaches and developing population epigenomics (Greally, 2017; Richards, 2008), (ii) assessing the effects of multiple, potentially interacting, stressful conditions (intensity, duration, frequency, interaction), (iii) favoring experimental designs with growing conditions in common garden to get as close as possible to

natural conditions, (iv) using kinetics approaches by sampling biological material along a developmental gradient for a better understanding of the molecular chain acting from short to long term during development × environment interactions (Pigliucci, 1998), (v) taking into account tree features (species, genotype, physiological and chronological ages, organs, tissues), but also the geographic origin, clone history, clonal propagation vs. sexual reproduction, and all features of breeding, management of genetic resources and forestry, and (vi) developing a trans-omics approach (Yugi et al., 2016) to overcome the lack of comprehensive understanding and the information gap regarding interaction across multiple -omic layers. Trans-omics is an approach for reconstructing molecular networks by connecting multiple -omic data. To reach that purpose, diverse statistical and modeling analyses must be tested to move from correlative to causal inference and predictions (Sow, Segura, et al., 2018), as developed in human brain research (for example, see Tasaki et al. (2018)).

In addition, this methodological plan for epi/genetics in trees must be adapted to natural- and artificial-based forest management. In artificial-based forest management, epigenetics could improve the response of planted trees to stress by promoting stress memory during the breeding process or at the very beginning of the seedling stage. While this idea is attractive, its implementation can face important challenges. First, because trees are long-lived organisms, they are highly susceptible to numerous stresses at various stages of their lifespan, and this is particularly true with global change. Hence, for stress memory to be useful, research in epigenetics should focus on the long-term effect of stress memory. Second, in the context of global change, trees are also susceptible to stresses that are variable in their nature, such as droughts, thaw-freeze events, early or late frosts, or insect defoliations. In natural-based forest management, it is impossible to have a direct effect on regeneration (i.e., choosing a specific seed to grow as a tree) through breeding programs. However, recent research shows that epigenetics could be, on the one hand, partly responsible for the large intraspecific plasticity observed in plants, and on the other hand, that stressful conditions can induce epigenetic responses. These findings appear promising because we may not yet have seen the extent of phenotypic plasticity for some species. Moreover, if further research can identify how mature tree species respond to various stresses “epigenetically”, this opens the door to new ideas in terms of silvicultural treatments to take advantage of this phenotypic plasticity. For example, if the progeny from trees that were under drought stress during seed formation show higher

resistance to drought, forest managers could develop strategies to favor their germination and the further development of this particular seedling cohort.

The advent of next-generation sequencing technologies pushed by the emergent need to explore epi/genetic diversity in response to climate challenges (Balao et al., 2018 ; Neale & Kremer, 2011; Plomion et al., 2016; Sollars & Buggs, 2018) contributed to the initiation and progress of research of tree epigenomics (Sow, Segura, et al., 2018). Genomics will play a major role not only in unveiling the epigenetic mechanisms underlying adaptation, but also in developing and implementing innovative management actions to preserve forest adaptability and identify traits for selection in breeding programs. To achieve these goals, efforts are needed to establish forest tree reference (epi)genome sequences, to determine how to apply sequencing technologies to understand adaptation, and to develop resources for storing, accessing, and sharing forestry data (Neale et al., 2013; Plomion et al., 2016; Sow, Segura, et al., 2018). As forests and trees have shown an incredible ability to survive through the occurrence of various stresses for centuries, there is a need to understand to what extent epigenetics has played a role in this resistance and resilience of forests to past stressors, such as droughts, extreme heat events, insect defoliation, and dieback episodes. This question is hard to answer as epigenetic analyses require “fresh” material, but it might be possible to identify growth patterns in dendrochronological studies that support this hypothesis. No matter if it is with dendrochronology or by other means, epigenetic researchers would benefit from engaging collaborations with other disciplines to confirm the importance of epigenetics in forest resistance and resilience to past stresses. This is one of the concerns being addressed within the research project EPITREE (Evolutionary and functional impact of epigenetic variation in forest TREEs; ANR-17-CE32, https://www6.inrae.fr/epitree-project/), that should encourage translational research in the field of forest research. The recent EPI-CATCH Cost Action (CA19125; https://www.cost.eu/actions/CA19125/) has created an international network where researchers and stakeholders share and discuss information to improve laboratory epigenetic methodologies, in silico data analysis, and the integration of epigenomic data with other -omic data (genomic, transcriptomic, proteomic, phenomic, etc.) in crops, including relevant forest species. The knowledge and methodologies will be discussed with a focus on the investigation of epigenetic mechanisms modulating plant adaptation to environmental stresses driven by climate change.

Finally, priming seems to be rising as an interesting new research line in trees with the contribution from scientists worldwide, as it is evidenced by the "Priming in Trees Consortium" (https://lunadiezlab. com/priming-in-trees-consortium/). This alliance aims to understand the priming mechanism itself, its durability, and implementation in forests as a promising solution for current and future threats. Epigenetics has been identified as a key contributor to the priming mechanism, and thus, deeper studies that focus on unveiling the epigenetic mechanisms involved in priming in forest tree species are required. Besides identifying the epigenetic mechanisms involved in stress-response, it is important to apply this knowledge to the conservation of forest genetic resources, breeding, and management.

Supplementary Materials: The following are available online at http://www.mdpi.com/1999-4907/11/9/976/s1. Table S1.1: Current state of epigenetics in trees for development, abiotic stress or priming, biotic stress or priming, and markers, breeding and biotechnology topics.

Author Contributions: S.M. suggested and designed the article. J.A., Z.R. and J.V. designed the Figures and table and wrote a first draft of the manuscript and revised version with the help of M.D.S., R.F., C.M., G.P., P.N. All authors have read and agreed to the published version of the manuscript.

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Crédit photo : Zoé Ribeyre

2) CHAPITRE 2

NO STRESS MEMORY PATTERN WAS DETECTED IN SUGAR MAPLE AND WHITE SPRUCE SEEDLINGS SUBJECTED TO EXPERIMENTAL DROUGHTS

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Résumé

L'augmentation de la fréquence et de l'intensité des sécheresses menace les forêts, ainsi que les bénéfices économiques, écologiques et sociaux qu'elles fournissent. Il est bien établi que les arbres soumis à une succession de stress peuvent accumuler des lésions qui entraînent une diminution de leur vigueur et peuvent amener à leur mort. Cependant, des études récentes ont montré qu'un stress non létal pourrait également initier une mémoire de stress, déclenchant une réponse défensive des plantes plus rapide et plus forte lors de stress ultérieurs. Bien que ce mécanisme soit bien étudié chez de nombreuses plantes herbacées, une meilleure compréhension chez les arbres est nécessaire. L'objectif de notre étude était d'explorer la capacité de deux espèces d'arbres forestiers à initier une mémoire de stress. Une expérience en serre a été menée pour évaluer la vigueur des jeunes plants d'arbres après un ou deux épisodes consécutifs de sécheresse séparés par une période de réhydratation au cours de la même saison de croissance. Aucun patron de mémoire de stress n'a été observé pour les deux espèces d'arbres, en revanche, un patron d'accumulation de stress a été constaté chez l'érable à sucre. Il reste possible que certains individus de notre étude aient développé une mémoire de stress, mais que nous n'ayons pas été en mesure de la détecter. D’après nos résultats, l'affinement des paramètres expérimentaux et la réalisation d'études longitudinales sembleraient plus appropriés pour détecter la capacité individuelle à déclencher une mémoire de stress.

Mots-clés: Acclimatation, changements climatiques, sécheresse, arbres forestiers, accumulation de stress, mémoire de stress

Abstract

An increase in the frequency and magnitude of drought events threatens the health of forests and the economic, ecological, and societal services they provide. It has been widely demonstrated that trees undergoing a succession of stresses may accumulate lesions that in turn lead to a decrease in their vigor and eventually to death. However, recent studies have shown that a nonlethal stress should also initiate a stress memory, which triggers a faster and stronger plant defensive response when a new stress occurs. Although this mechanism is well understood in many herbaceous plants, a better understanding in trees is needed. The aim of our study was to explore the capacity of two forest tree species to develop a stress memory. A greenhouse experiment was conducted to evaluate the tree seedlings' vigor after one or two consecutive droughts separate from a rehydration period during the same growing season. No stress memory pattern was observed for the two tree species as, on the contrary, we even observed a stress accumulation pattern in sugar maple. It remains possible that some individuals in our study developed stress memory, but that we were not able to detect it. The fine-tuning of experimental parameters and the conducting of longitudinal studies would be helpful to detect individual capacity in stress memory activation.

Keywords: Acclimation, climate change, drought, forest tree, stress accumulation, stress memory

2.1 Introduction

Significant global habitat changes related to increasing temperatures and episodes of drought are expected in the coming decades (Hoegh-Guldberg et al., 2018). Such environmental changes are a major risk to the health and productivity of forests (Millar & Stephenson, 2015), with many cases of forest dieback already being linked to drought and extreme heat events worldwide (Allen et al., 2010). To survive, trees must migrate or adapt to the new environmental conditions. However, given the geographical barriers that limit the rate of migration (Aitken et al., 2008) and the rate of adaptation to the environment, it is expected that trees will have difficulty coping with global changes (Sittaro et al., 2017). Under such a context, forest survival depends on the ability of trees to cope with new local environmental conditions. This includes the capacity of the system to resist, recover, and return to a stable state after a disturbance (Lloret et al., 2011). Forest resilience has been observed after drought and seems to be strongly dependent on species (Vitasse et al., 2019), competition, and stress severity (Gazol et al., 2018). Population resilience is hard to predict and could be underestimated because the slow recovery of trees after a disturbance is often interpreted as a system imbalance but could instead be a sign of acclimation (Gessler et al., 2020). Past legacies can influence tree response to stresses like drought or heat events. Stress recurrence before the system is restored could increase any deleterious effects and increase the recovery time of the system (Mitchell et al., 2016), resulting in an accumulation of stress leading to a greater vulnerability or an increase in the mortality rate (Nolet & Kneeshaw, 2018; Peltier & Ogle, 2019). On the other hand, recent studies have highlighted a very different tendency where an acclimation process could be triggered after two or more perturbations. This stress memory (Lämke & Bäurle, 2017) or epigenetic memory (Sow, Allona, et al., 2018) exploits phenotypic plasticity, the ability of a genotype to express different phenotypes depending on environmental conditions (Nicotra et al., 2010), to improve the stress tolerance of the individual. Stress memory takes place after exposure to an initial low or moderate stress, referred to as a priming stress (Conrath et al., 2015), and could increase the speed of perception of a second stress, initiating a faster and stronger defensive response (Amaral et al., 2020). A greater stress tolerance is evidenced by the maintenance or improvement of physiological processes such as growth (Goswami et al., 2013) and photosynthetic yield (Murata et al., 2012; Neves et al., 2017) and leads to a better survival rate. Stress memory is attributed to a molecular imprint triggered by epigenetic mechanisms (Bruce et al., 2007; Hilker et al., 2016). These mechanisms operate at the individual level and modulate

gene expression in response to the environment without modifying the DNA sequence, making them reversible and highly reactive (Bräutigam et al., 2013). The study of stress memory in trees is still poorly documented. However, recent studies have revealed that several processes influenced by climatic conditions such as biomass production (A.-L. Le Gac et al., 2018), bud phenology (Carneros et al., 2017), vernalization (Ø. Johnsen et al., 2009; I. Yakovlev et al., 2011), or some defense pathways involving phytohormones (Lafon-Placette et al., 2018) are subject to epigenetic controls. Although these results suggest an important role for epigenetic mechanisms in response to the environment, many efforts are still needed to better understand their functioning and assess the extent of their influence on tree acclimation. This study was carried out on sugar maple (*Acer saccharum* [Marsh]) and white spruce (*Picea glauca* [Moench] Voss), two emblematic species in Canada that are very important, both economically and ecologically (Bishop et al., 2015; Hassegawa et al., 2020). Numerous studies have already revealed the vulnerability of these species to climate changes (Aubin et al., 2018; Oswald et al., 2018), particularly to drought (Pitel & Yanai, 2014; Sang et al., 2019). However, very little is known about their capacity for short-term acclimation. Moreover, their different ecological characteristics and their contrasting water management strategies (deciduous vs. coniferous) make these two species very interesting for exploring stress memory activation. An unexpected heat wave (HW) event occurred before the beginning of our experiment and deeply injured the white spruce seedlings but not the sugar maple ones. Following this event, the white spruce seedlings were all replaced, and the sugar maple seedlings were kept for the experiment (see Methods). The first objective of this study is to evaluate the possibility of triggering a stress memory pattern in tree seedlings via a succession of stresses during the same growing season: an initial moderate drought followed by a second more severe drought for white spruce and a HW followed by the same two drought treatments for sugar maple. Then, the second objective is to discuss the potential impact of the HW stress on sugar maple drought response. The first hypothesis is that the succession of stresses will promote the establishment of a stress memory pattern. In this instance, we expect to observe an improvement in drought tolerance during the second drought. Conversely, the second hypothesis is that the experimental conditions will trigger a stress accumulation pattern. In this case, we expect to observe evidence of an accumulation of the deleterious effects of multiple-stressed seedlings.

2.2 Materials and Methods

2.2.1 Plant material and growth conditions

Two-year-old sugar maple (*A. saccharum*) and white spruce (*P. glauca*) seedlings from the Ministère des Forêts de la Faune et des Parcs (MFFP, Berthier, Quebec, Canada) tree nursery were planted in 7-L plastic pots. The pots were filled with a 2:1 substrate (black soil:sand) and placed in a greenhouse. To avoid nutrient deficiencies, nitrogen, phosphorus, and potassium (NPK) fertilizer was applied during the season before each of the two drought treatments. Samples from the soil substrate were weighed prior to and after oven-drying at 50°C for 72 h to determine the dry mass (DM) of each pot. Soil substrate DM consistency was checked before each DM measurement. The target's mass of each pot was then calculated to maintain a field capacity of 65% for the stressed seedlings and 100% for the control and rehydrated seedlings. The pots were weighed daily, and their target mass was maintained by adding water. Temperature and humidity in the greenhouse were measured using three probes placed 20 cm above the seedlings. The experiment was separated into four periods: an acclimatization period before the first drought (Before drought), an initial moderate drought of 12 days (Drought 1), a rewatering period of 20 days (Rewatering), and a second more severe drought of 30 days (Drought 2). The experiment had four treatments: control (Ctl), seedlings stressed only by the first drought (D_{1+0}), seedlings stressed only by the second drought (D_{0+2}) and seedlings stressed by both droughts (D_{1+2}) (see Figure 2.1).

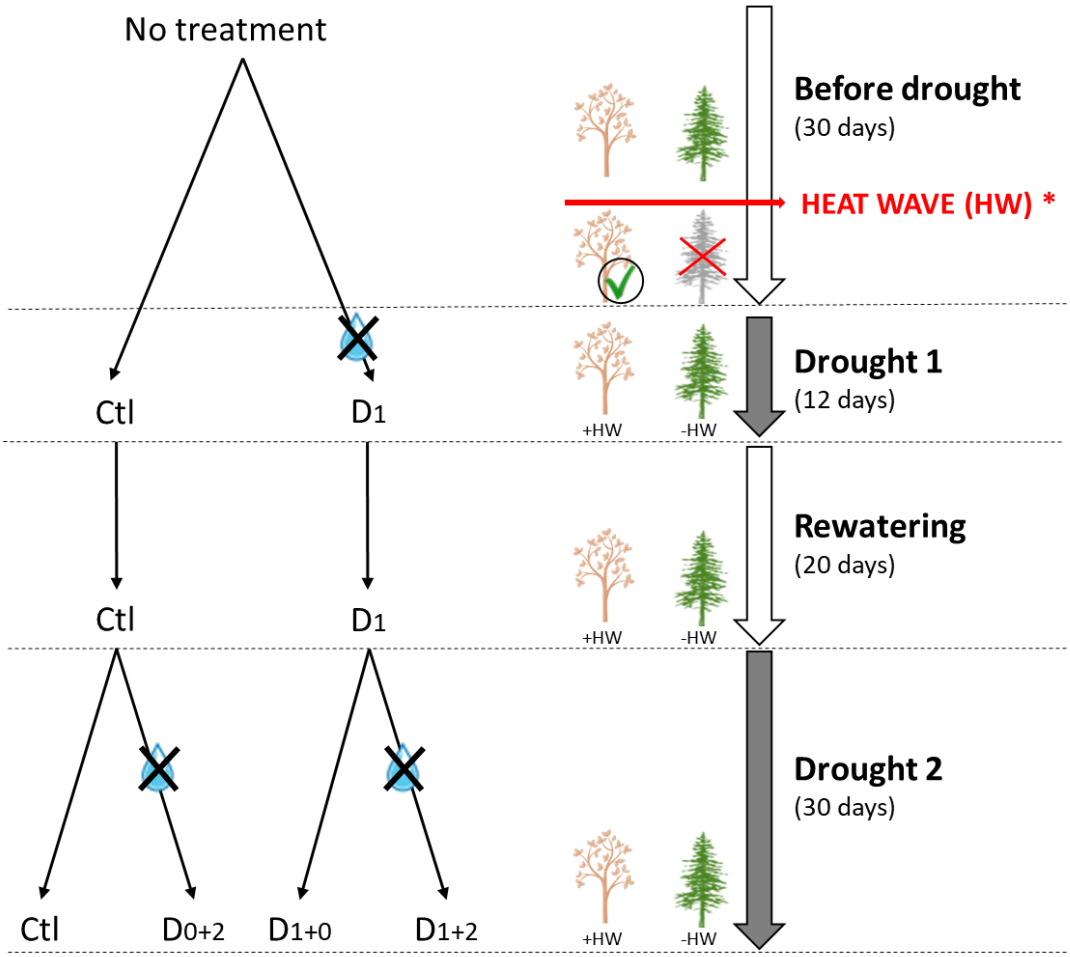

Figure 2.1. Overview of the experiment. The experience includes four periods: Before drought, Drought 1, Rewatering, and Drought 2. At the end of the experiment, there were four different treatments: Control (Ctl): seedlings well irrigated, D_1: seedlings submitted to Drought 1 before being separated in D_{1+0} or D_{1+2}, D_{1+0}: seedlings submitted only to Drought 1, D_{0+2}: seedlings submitted only to Drought 2, and D_{1+2}: seedlings submitted to Drought 1 and Drought 2. The heat wave (HW) occurred during the Before drought period. The asterisk marks the replacement of all white spruce seedlings after the HW, but only injured sugar maple seedlings were removed, and the healthy ones were kept. +HW: sugar maple seedlings were subjected to HW, –HW: replaced white spruce seedlings were not subjected to HW. n = 155 seedlings per species at the beginning of the experiment, then, n = 155 for the new planted white spruce seedlings and n = 135 for sugar maple seedlings.

2.2.2 Influence of HW

A HW occurred in the first week of July 2020 before the first drought (Drought 1) (Figure 2.1). The average daily temperature (from 8:00 am to 7:00 pm) recorded in the greenhouse during the HW was 32.8°C (Figure 2.2), with temperature peaks reaching 39°C. This HW had very damaging effects on white spruce seedlings. Indeed, more than 50% of the seedlings (78 of 155) suffered from severe dehydration (dry branches and needles). Following this sudden dehydration, the 78 plants died. To continue the experiment, all the white spruce seedlings were discarded, and new seedlings were planted. In contrast, sugar maple seedlings showed very few symptoms in response to the HW. Indeed, only 13% of the seedlings (20 of 155) showed some necrotic spots on leaves, while the remaining 135 seedlings appeared healthy and were kept until the end of the experiment. In summary, the results present the phenotypic responses of the newly planted white spruce seedlings (not subject to the heat wave, HW) and the 135 healthy sugar maple plants (subject to the heat wave, +HW). The impact of this event on sugar maple seedlings will be considered in the Discussion.

2.2.3 Vigor indicators' measurements

2.2.3.1 Destructive measures

Whole plant samples of both species were taken on the last day of each period (Before drought: n = 5 per treatment, Drought 1: n = 7 per treatment, Rewatering: n = 5 per treatment, Drought 2: n = 10–12 per treatment) to measure the dry biomass of root and shoot and the relative water content (RWC). Root and shoot samples were weighed after oven-drying at 48°C for 72 h to determine their DM. Seven hallmark leaves of 0.5 cm in diameter per sampled sugar maple and three sections of branch 4 cm in length per sampled white spruce were used to determine the RWC. Leaf and branch samples were taken from healthy leaves and branches between the second and fourth rows from the top of the seedlings. These samples were weighed immediately after sampling to determine their fresh mass (FM), after rehydration at 4°C for 24 h to determine their saturated fresh mass (at full turgidity) (FMsat), and after oven-drying at 48°C for 72 h to determine their DM. The verification of the DM constancy was randomly performed on 10 samples before each DM measurement.

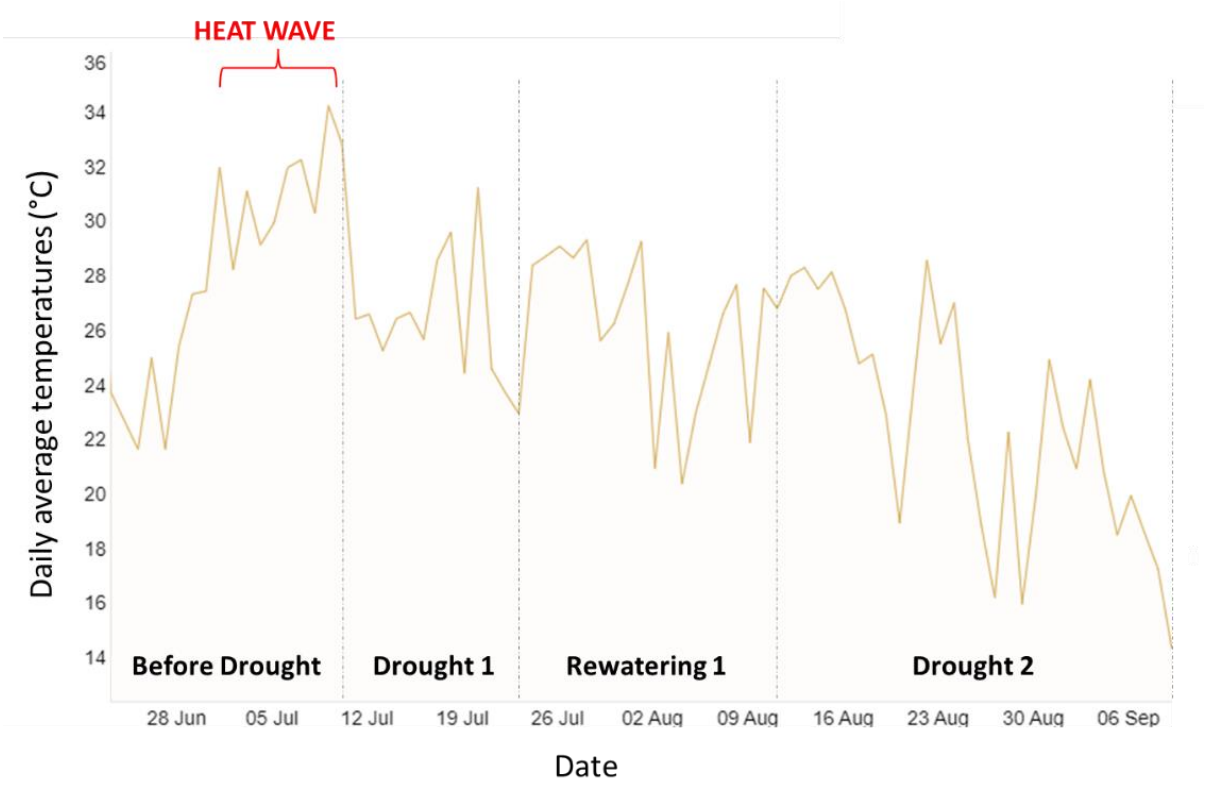

Figure 2.2. Average daily temperature recorded in the greenhouse as a function of periods of experiment. The temperature was recorded with three probes placed inside the greenhouse 20 cm above the seedlings. In red, the heat wave that occurred at the end of the Before drought period.

2.2.3.2 Nondestructive measures

Percentage of necrosis and healthy leaves

The percentage of leaf necrosis on sugar maple seedlings was measured on all plants before the first drought (Before drought, n = 40 per treatment), after the first drought (Drought 1, n = 35 per treatment), and after the second drought (Drought 2, n = 23 per treatment). For all seedlings, the total number of leaves per plant was counted and each leaf was classified into one of the following four categories: healthy leaf with necrosis on less than 10% of its area (Category 1), necrosis on 10%–50% of leaf area (Category 2) necrosis on 50%–90% of leaf area (Category 3), and necrosis on more than 90% of leaf area (Category 4). This measurement was not

performed on white spruce seedlings because there was no measurable difference before drought or after drought treatments.

Net photosynthesis

Net photosynthesis (A) was measured at 400 μmol.m^{-2}.s^{-1} with a LiCOR6400 between 6 am. and 11 am. on the last morning of each period of the experiment (n = 9–12 per treatment for both species). The system humidity was maintained between 70% and 80% and the CO_2 rate was maintained at an atmospheric rate of 400 ppm during measurements. The measured leaves were all healthy leaves taken from the second to fourth row from the top of each plant. Because the LiCOR chamber is not suitable for conifers, the white spruce net photosynthesis measurements for scanned needles were corrected using WinSEEDLE software.

2.2.4 Data analyses

An average RWC value was calculated for each sampled seedling for the seven hallmark leaves of sugar maple and the three branch sections of white spruce according to Equation (1). The average RWC for treatments and periods was then calculated from the average RWC per plant.

$$RWC = (FM - DM) / FMsat - DM) \quad (1)$$

The percentage of healthy leaves was first calculated for each sugar maple seedling by dividing the number of healthy leaves (Category 1) by the total number of leaves. From these individual percentages, an average per treatment and per period was then calculated. Finally, shoot and root DM were standardized by calculating the ratio of DM to initial live plant mass.

Statistical analyses were performed using the 4.0.5 version of R software. The RWC, the standardized dry root and shoot biomass variables for both species, and the net photosynthesis for white spruce only do not have a Gaussian distribution. A data normalization of RWC was not possible, so the effects of treatment on this variable were analyzed with a nonparametric Kruskal–Wallis rank sum test and a pairwise Wilcoxon rank sum test. On the other hand, the variables of standardized dry root and shoot biomass and the net photosynthesis of white spruce

were log-transformed. Thus, a single-factor ANOVA and a post hoc Tukey's multiple comparison test were used to test the effects of treatment on the standardized dry root and shoot biomass and on the net photosynthesis. In addition, as the percentage of healthy leaves per plant presented a binomial distribution, the effect of treatment on this variable was tested with a single-factor logistic regression.

2.3 Results

2.3.1 HW: Contrasted responses of species

The HW that occurred before the first drought (Figures 2.1 and 2.2) killed 50% (78 of 155) of white spruce seedlings after only a couple of hours of intense heat. By contrast, none of the sugar maple seedlings died and only 13% (20 of 155) developed some leaf necrosis. After this HW, all white spruce seedlings were replaced, and the affected sugar maple seedlings were removed from the experiment and not replaced.

2.3.2 Effects of successive drought on seedlings

2.3.2.1 Visual symptoms: Leaf necrosis

At the end of the experiment, anecdotal necrosis was observed in a few white spruce seedlings without significant differences among the treatments (Figure 2.3A). On the contrary, leaf necrosis was observed on sugar maple seedlings from the end of the first drought (Drought 1) on both controls and stressed seedlings (D_1), but D1 seedlings seemed to be the most affected (Figure 2.4). Worsening necrosis was observed after the second drought (Drought 2) for each treatment (Figures 2.3B and 2.4). However, the D_{1+0} and D_{1+2} seedlings were the most affected with a mean value of 40% and 30% of healthy leaves remaining, respectively, compared with 74% for the controls (Ctl) ($p = 0.009$ and $p = 0.001$ respectively) seedlings and 73% for the D_{0+2} seedlings ($p = 0.01$ and $p = 0.002$ respectively). Although no significant differences were found between the D_{1+0} and D_{1+2} treatments ($p = 0.42$), the D_{1+2} seedlings had a mean value of healthy leaves 10% less than D_{1+0} (Figure 2.4). These results suggest a tendency of the D_{1+2} sugar maple seedlings to accumulate the effects of the two successive droughts.

2.3.2.2 Relative water content

At the end of the experiment, measurements of RWC of white spruce seedlings showed mean values around 80% for each treatment ($p = 0.41$) (Figure 2.5A). Thus, none of the drought treatments seemed to have impacted the water content of white spruce needles. On the other hand, RWC of sugar maple seedlings measured at the end of the experiment showed an impact of the first drought (Drought 1) and to a lesser extent of the second drought (Drought 2) (Figure 2.5B). D_{1+0} and D_{1+2} seedlings presented lower mean RWC than controls (13% and 17%, respectively, $p = 0.005$ and $p = 0.023$). A similar trend was observed when comparing the D_{1+0} and D_{1+2} seedlings with D_{2+0} seedlings. No difference in RWC was observed between controls and D_{2+0} seedlings ($p = 0.15$). Finally, some very low RWC values for D_{1+2} seedlings compared with the other treatments seem to reveal a stress accumulation pattern, at least for some seedlings.

2.3.2.3 Ratio of dry shoot and root mass on initial live plant mass (standardized DM)

After the second drought (Drought 2), the mean of the standardized dry root mass of white spruce was between 0.017 and 0.025 for each treatment ($p = 0.18$) (Figure 2.6A). This result reflects that the drought treatments had no impact on white spruce root growth. However, the D_{1+0} seedlings showed a slight tendency to have a greater root biomass than D_{0+2} ($p = 0.065$), suggesting that the first drought could have promoted a root growth response in white spruce unlike the second drought (Figure 2.6A).

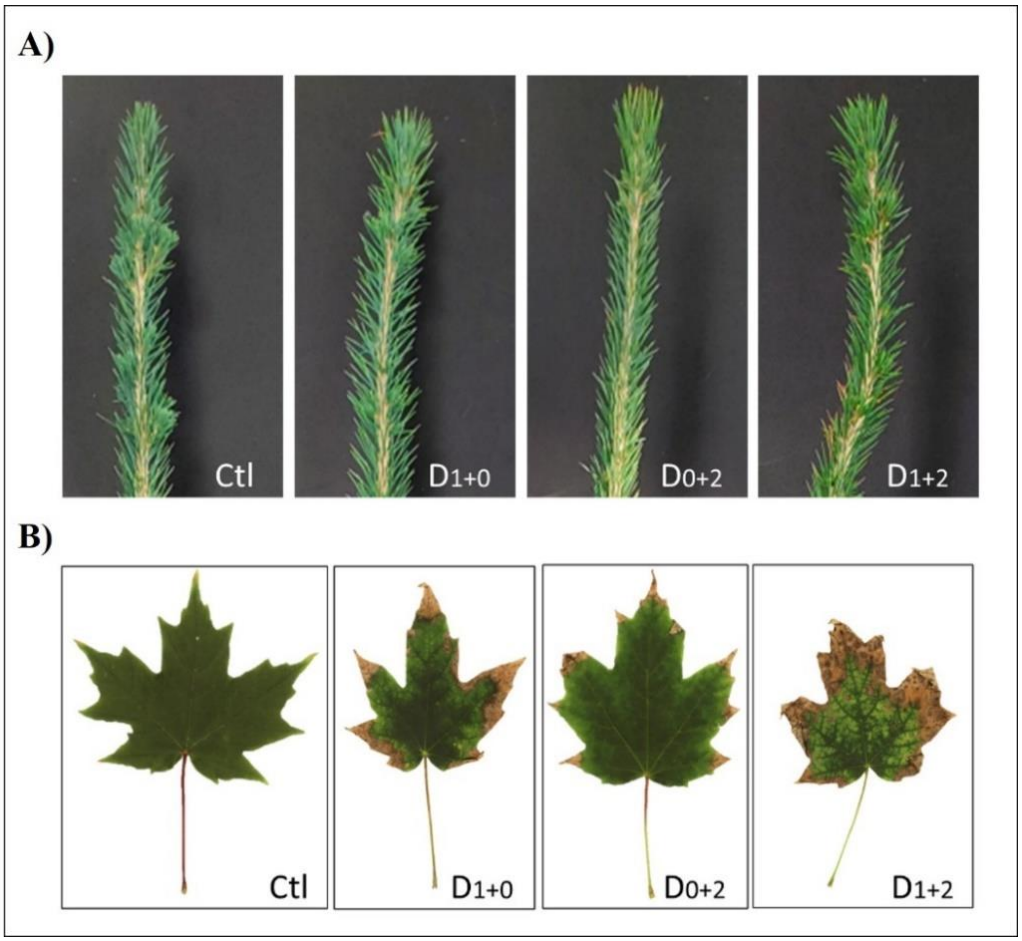

Figure 2.3. Necrotic damages. Pictures taken at the end of the experiment (after Drought 2) highlighting the representative necrotic damage to (A) the branches of white spruce (not subject to the heat wave) and (B) the leaves of sugar maple (subject to the heat wave) as a function of drought treatments. See Figure 2.1 and Methods for the meaning of treatments.

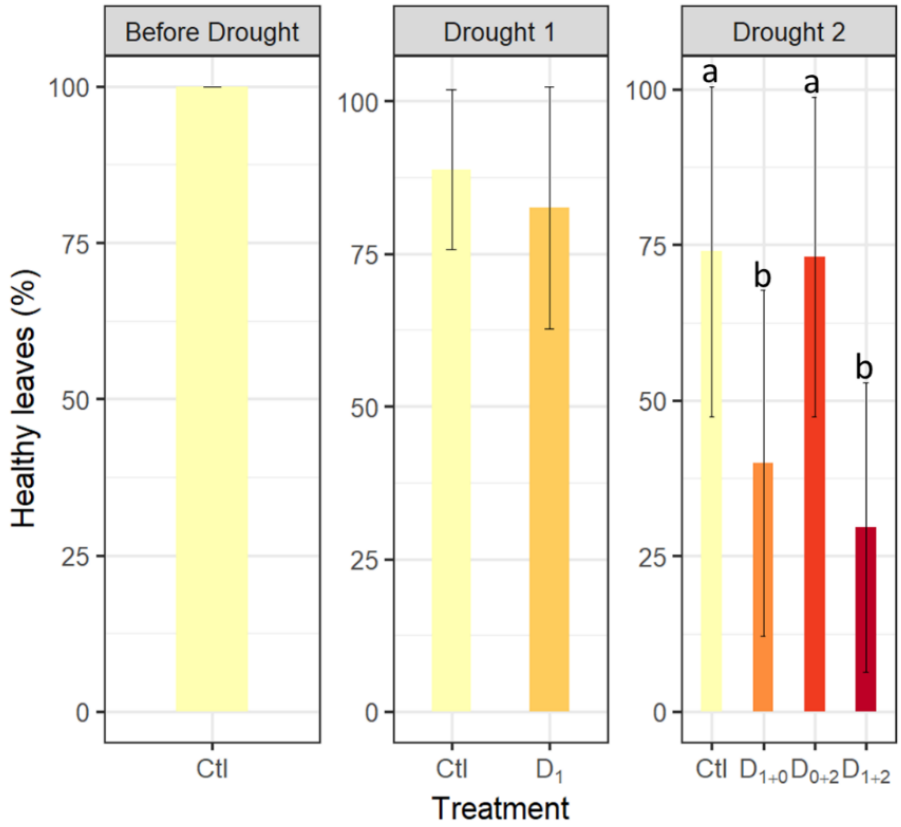

Figure 2.4. Changes over the course of the experiment in the percentage of healthy leaves. Bar plot of the evolution of the mean percentage of healthy leaves per sugar maple seedlings (subject to the heat wave) as a function of periods of experiments (Before drought, Drought 1, and Drought 2) and drought treatments (control [Ctl], D_1, D_{1+0}, D_{0+2}, and D_{1+2}). See Figure 2.1 and Methods for periods' explanations and the meaning of the treatments. Different letters show significant difference between treatments based on logistic regression analysis. Before drought, n = 40 per treatment, Drought 1, n = 35 per treatment, and Drought 2, n = 23 per treatment.

As for the standardized dry shoot mass, no difference was observed among treatments with mean values between 0.05 and 0.055 for each treatment (p = 0.76) (Figure 2.6C). Biomass measurements of sugar maple seedlings taken at the end of the experiment showed different trends than those observed for white spruce seedlings. D_{0+2} and control seedlings showed similar standardized dry root mass values (p = 0.56), while D_{1+0} and D_{1+2} seedlings showed standardized dry root mass values 1.5 and 1.6 times lower than the control (p = 0.002 both)

seedlings, respectively (Figure 2.6B). As for the standardized dry shoot mass, D_{1+0} seedlings ($p = 0.04$) and to a lesser extent D_{1+2} seedlings ($p = 0.056$) tended to stand out from the controls with a mean 1.3 times lower (Figure 2.6D). On the other hand, the D_{0+2} seedlings were not significantly different from the controls ($p = 0.14$) and from the other two treatments ($p > 0.5$). In summary, standardized shoot and root DM of sugar maple seedlings were primarily affected by the first drought (Drought 1).

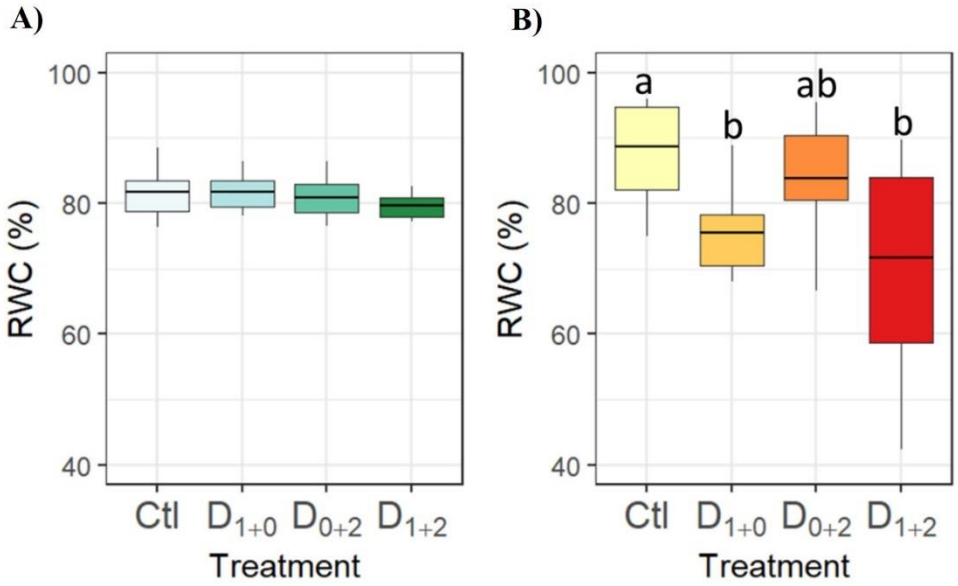

Figure 2.5. Relative water content (RWC %). Box plot of RWC as a function of drought treatments at the end of the experience (after Drought 2) in white spruce (not subject to the heat wave) (A) and sugar maple (subject to the heat wave) seedlings (B). Different letters indicate significant differences ($p < 0.05$) among treatments based on Kruskal–Wallis rank sum test and a pairwise Wilcoxon rank sum test. The limits of the box are the 25% and 75% percentiles, the separating line between the lower and upper part of the box is the mean, the lower and upper limits of the whiskers are the 10th and 90th percentiles, respectively, and points are beyond 1.5 the interquartile range (25th–75th percentiles). See Figure 2.1 and Methods for the meaning of treatments. n = 10–12 replicas per treatment and species.

2.3.2.4 Net photosynthesis rate

Mean values of net photosynthesis in white spruce seedlings measured after the second drought (Drought 2) at 400 µmol.m^{-2}.s^{-1} were around 3 µmol.m^{-2}.s^{-1} for each treatment and revealed no effect of any treatment for this variable ($p = 0.68$) (Figure 2.6E). On the other hand, differences were observed in sugar maple seedlings among treatments. D_{1+2} stood out from the other treatments with mean values 2.2 times lower than the controls (Ctl) ($p = 0.0008$) (Figure 2.6F). Even though the differences were not significant, D_{1+0} and D_{0+2} seedlings had mean values 1.4–1.3 times lower, respectively, than the controls (Ctl) ($p = 0.13$ and 0.4). D_{1+2} seedlings being the most affected seem to reveal a cumulative effect of the two droughts on net photosynthesis in sugar maple.

2.4 Discussion

2.4.1 Impact of the HW

Our experiment took place in a greenhouse where temperatures were not controlled. This experimental design was adopted to have natural temperature fluctuations. However, as previously explained (see Methods), a HW occurred before the first drought and caused 50% mortality in white spruce seedlings; all white spruce seedlings were subsequently replaced. This extreme and unpredictable event probably reflects the future summer climate in North America, which is expected to be more and more prone to HW events (Auclair et al., 1997; van Mantgem et al., 2009). The high drought tolerance observed in this study for white spruce applies only to the replaced seedlings (i.e., those that had not been subjected to the HW). Although our results demonstrate a good ability of white spruce to resist successive drought events, it should be highlighted that the effect of drought can be aggravated by the interaction with other stresses such as high temperatures (P. Lu et al., 2019). Thus, the intolerance to high temperatures of this species (Lapenis et al., 2022) could accentuate its vulnerability to drought and lead to a reduction in its distribution in the area most affected by global warming (Gagne et al., 2020). While white spruce showed a great intolerance to the HW, sugar maple did not sustain any loss, and only 13% of seedlings showed (slight) leaf necrosis. These results indicate a marked difference in tolerance to high temperatures between these two species. As mentioned, sugar maple seedlings that showed necrosis after the HW were removed from the experiment and the remaining seedlings were followed for the remaining of the experiment. Hence, we must

consider the possibility that the HW event influenced the responses of sugar maple to drought, thus underlining the complexity of the natural climatic conditions and stress interaction.

2.4.2 Responses of white spruce and sugar maple to drought and heat stress

Regarding white spruce, the results did not support either of our initial hypotheses. More precisely, our experimental conditions triggered neither a drought acclimation nor a stress accumulation response in this species, but high drought tolerance was observed. Such drought tolerance from white spruce was not expected since several studies have shown that decreasing soil water availability tends to decrease growth and increase mortality rates at various stages of development (Gagne et al., 2020; Hogg et al., 2017; Sullivan et al., 2021). Better drought tolerance than expected could be due to the colonization of mycorrhizal fungi from the tree nursery. Indeed, several studies have shown that such symbioses can improve the water balance and drought tolerance of coniferous seedlings (Pickles & Simard, 2017). Results from these studies are complex to compare as the sensitivity and the vulnerability of species to drought are highly dependent on experimental factors such as the severity or duration of the disturbance, the level of light exposure, or the level of neighborhood competition (Shovon et al., 2021). In addition, individual intrinsic factors such as the developmental stage (Cavender-Bares & Bazzaz, 2000), the genotype, and its origin (Depardieu et al., 2020) also can strongly influence drought tolerance. Sugar maple, on the other hand, showed a clear intolerance to drought potentially exacerbated by the heat stress event. Our results indicate that the first drought (Drought 1) had very deleterious effects on (1) the leaf RWC, (2) the net photosynthesis, and (3) the spread of leaf necrosis compared with controls. The deleterious effects of the first drought (Drought 1) were observed soon after the treatment and were maintained until the end of the experiment despite the rewatering.

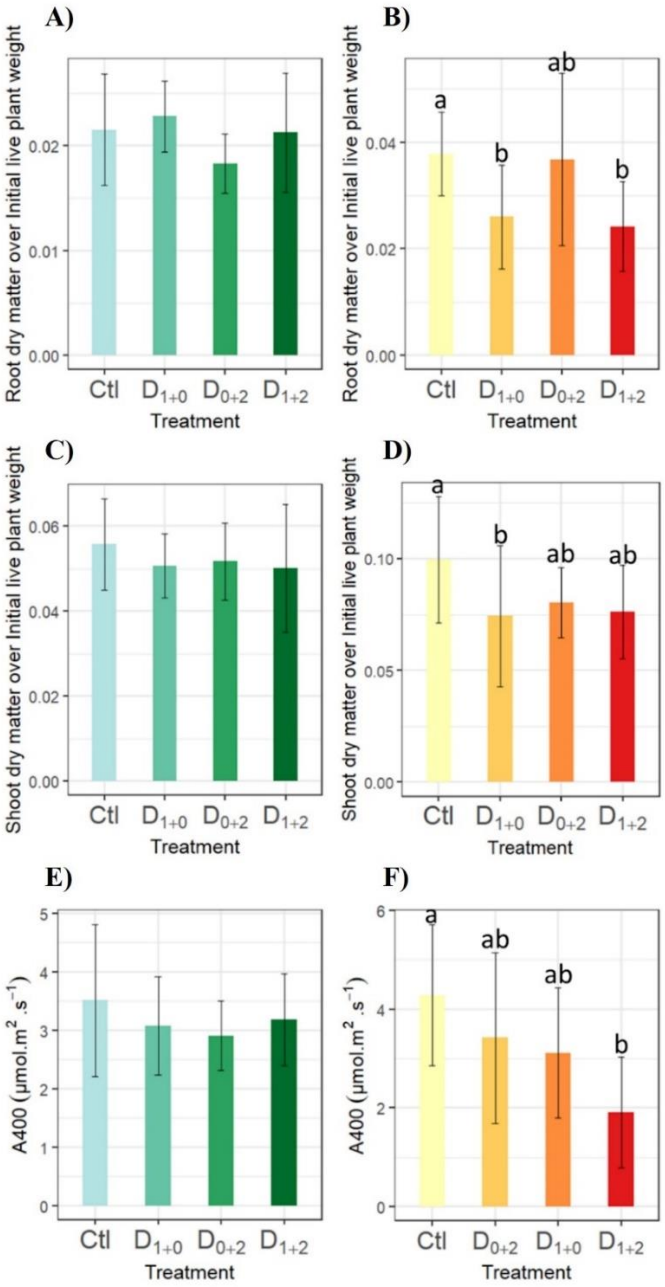

Figure 2.6. Bar plots of root dry matter over initial live plant mass of white spruce (not subject to the heat wave, HW) (A) and sugar maple (subject to the heat wave, +HW) seedlings (B) and shoot dry matter over initial live plant mass in white spruce (C) and sugar maple seedlings (D) as a function of drought treatments at the end of the experience (after Drought 2) and net photosynthetic rate (A400) measured at 400 µmol.m^2.s^{-1} in white spruce (HW) (E) and sugar maple (+HW) seedlings (F) at the end of the experiment (after Drought 2) as a function of drought treatments. Different letters on plots indicate significant differences ($p < 0.05$) among treatments based on ANOVA and Tukey's honestly significant difference post hoc test. See Figure 2.1 and Methods for the meaning of treatments.

In addition, the worsening leaf necrosis observed between the first and the second drought in the D_{1+0} seedlings suggests inability to recover and an aggravation of drought symptoms over time following the first treatment. However, sugar maple showed a better tolerance to the second drought compared with the first as the D_{0+2} seedlings showed a similar general response to those of the controls for all variables. The double-drought treatment, though, accentuated the decrease in net photosynthesis in D_{1+2} seedlings compared with other treatments, demonstrating a stress accumulation response. Moreover, although no significant difference was found for the other variables between the D_{1+2} and D_{1+0} seedlings, it is important to note that RWC and the percentage of healthy leaves in D_{1+2} seedlings also showed a stress accumulation trend. In sugar maple, these results demonstrate that a HW event and successive droughts during the same growing season did not trigger an acclimation response (refutes Hypothesis 1) but caused a stress accumulation response (confirms Hypothesis 2) for which a decrease in net photosynthesis is the best indicator.

2.4.3 Contrasting drought response of sugar maple

Our experiment was designed to produce an initial moderate drought (Drought 1) and a second more severe drought (Drought 2) to determine whether a single drought could cause a stress memory response. We also anticipated observing more damaging effects on seedlings following the second drought compared with the first because the second drought was more intense (D_{1+0} vs. D_{0+2}). However, our results showed an opposite trend in sugar maples, D_{1+0} seedlings were more impacted than D_{0+2}. We propose three hypotheses to explain these results. First, we suspect an interaction between the first drought (Drought 1) and the high temperatures recorded during the HW and this experimental period. In fact, the Drought 1 period was warmer than the Drought 2 period, which may have increased the severity of the first drought compared with the second. One of the responses often observed in plants following a drought is a decrease in their water loss by reducing their leaf transpiration (Skelton et al., 2017). This decrease is caused by an increase in stomatal closure, which also leads to a decrease in both CO_2 uptake and photosynthetic yield (McDowell et al., 2008). However, a sharp decrease in transpiration can also lead to an increase in leaf temperature, which is worse during high-temperature events (Bauweraerts et al., 2014), exacerbating the damage caused by drought (Ruehr et al., 2015).

Energy excess from heat or photons can trigger an overproduction of reactive oxygen species (ROS). ROS are highly energetic molecules responsible for photooxidative stress that damages cells (Wujeska et al., 2013). Thus, a strong increase in ROS can cause damage to plants such as the degradation of cell membranes and a homeostatic imbalance in cells. These cellular and molecular lesions can cause a dysfunction in the photosynthetic machinery (Teskey et al., 2015) and an increase in cell death manifesting as an increase in necrotic tissue (Vollenweider et al., 2016), as observed in our study. Second, the recovery time between the HW and the first drought was potentially too short and may have aggravated the response of D_{1+0} seedlings to the first drought. Indeed, although there was no visible effect on sugar maple immediately following the HW, it may have increased the vulnerability of the seedlings to drought. Third, the interaction between the developmental stage of seedlings and drought treatments may have played a role in the responses observed. Almost one month separated the two droughts: D_{1+0} seedlings were subjected to the first drought at the start of the growing season, while the D_{0+2} seedlings were not subjected to the second drought until the end of the season (Figure 2.1). Even this short developmental gap could explain the difference in drought tolerance between the D_{1+0} and D_{0+2} seedlings. Seedlings in the early growing season may show greater sensitivity to drought due to their less developed root system and lower sugar reserves (Cavender-Bares & Bazzaz, 2000; Niinemets, 2010). The greater vulnerability to drought of the D_{1+0} seedlings compared with the D_{0+2} seedlings could also be attributed to a trade-off in energy allocation to growth rather than defense processes (Lundgren & Des Marais, 2020; Vázquez-González et al., 2020).

2.5 Conclusion

Our study first highlighted the high vulnerability of white spruce to heat compared with sugar maple. While the sugar maple did not show any visible direct injuries from the heat event, we hypothesized that the interaction of heat and drought may have contributed to increasing its vulnerability to drought and induce a stress accumulation pattern, mainly observed in the net photosynthesis indicator. Moreover, our experiment showed the difficulty of triggering and detecting a stress memory pattern in tree species at the seedling stage with a semi controlled experimental design. Even though the study did not make it possible to observe a stress memory pattern in these two species, this does not mean that such a mechanism is not present. As a species of long life span subjected to many environmental changes throughout its life, it would

be surprising that trees have not developed a stress memory pattern. However, it is not clear which combination of intensity, duration, and even timing of stress can lead to the development of a stress memory. Moreover, it remains possible that some individuals in our study developed stress memory, but our experimental design did not allow us to identify them. We thus advise focusing on the fine-tuning of experimental parameters and conducting a longitudinal study to consider the intraspecific variability by using nondestructive vigor indicators like net photosynthesis.

Authors contributions: Z. Ribeyre designed and performed the experiments, analyzed the data, designed the Figures, and wrote the manuscript. P. Nolet and C. Messier supervised the project and provided critical feedback and helped shape the analysis and manuscript.

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Conflict of interest: The authors declare that there is no conflict of interest.

Data availability statement: Data (Ribeyre, 2022) are available from Zenodo: https://doi.org/10.5281/zenodo.7004519.

Crédit photo : Zoé Ribeyre

3) CHAPITRE 3

DE NOVO TRANSCRIPTOME ASSEMBLY AND DISCOVERY OF DROUGHT-RESPONSIVE GENES IN WHITE SPRUCE (*Picea glauca*)

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Résumé

Dans le contexte des changements climatiques, les forêts sont de plus en plus menacées par l'augmentation de la fréquence et de l'intensité des évènements de sécheresse. Il est donc crucial de mieux comprendre comment les arbres, notamment ceux avec une large aire de distribution et une forte importance économique et écologique, répondent à la sécheresse. Dans cette étude, nous avons examiné les réponses à l'échelle du transcriptome de semis de 2 ans d'épinettes blanches (*Picea glauca* (Moench) Voss) soumis à une sécheresse de 22 jours en serre afin d'identifier les gènes clés et les voies métaboliques impliqués dans la réponse à ce stress. Nous avons tout d'abord réalisé un assemblage transcriptomique *de novo* à partir d'échantillons d'épinettes blanches de divers stades de développement et soumis à des stress hydriques variés et nous avons utilisé cet assemblage comme référence pour nos analyses transcriptomiques. L'assemblage *de novo* du transcriptome a révélé un total de 33 287 transcrits (18 934 gènes uniques potentiels annotés) et 4 425 gènes uniques ont été identifiés comme étant potentiellement impliqués dans la réponse à la sécheresse à l'aide d'une analyse de l'expression différentielle. Plusieurs transcrits identifiés, dont les fonctions prédites sont associées à la photosynthèse, l'organisation de la paroi cellulaire et le transport de l'eau, étaient régulés à la baisse en réponse à la sécheresse, tandis que les transcrits liés à la réponse à l'acide abscissique et aux mécanismes de défense étaient majoritairement régulés à la hausse. Les résultats ont également souligné l'implication, encore très peu décrite, du métabolisme des lipides dans la réponse à la sécheresse chez les conifères, ainsi que des changements significatifs dans l'expression de plusieurs facteurs de transcription. Notre recherche est donc une étape fondamentale dans l'élucidation des mécanismes moléculaires impliqués dans la réponse à la sécheresse à court terme chez les semis d'épinettes blanches. En outre, elle constitue une source précieuse de nouvelles données génétiques à explorer dans le cadre de futures recherches fonctionnelles.

Mots-clés : Transcriptomique, tolérance à la sécheresse, conifère, stress hydrique, changements globaux, facteur de transcription, métabolisme des lipides

Abstract

Forests face an escalating threat from the increasing frequency of extreme drought events driven by climate change. To address this challenge, it is crucial to understand how widely distributed species of economic or ecological importance may respond to drought stress. In this study, we examined the transcriptome of white spruce (*Picea glauca* (Moench) Voss) to identify key genes and metabolic pathways involved in the species' response to water stress. We assembled a de novo transcriptome, performed differential gene expression analyses at four time points over 22 days during a controlled drought stress experiment involving 2-year-old plants and three genetically distinct clones, and conducted gene enrichment analyses. The transcriptome assembly and gene expression analysis identified a total of 33,287 transcripts corresponding to 18,934 annotated unique genes, including 4,425 genes that are uniquely responsive to drought. Many transcripts that had predicted functions associated with photosynthesis, cell wall organization, and water transport were down-regulated under drought conditions, while transcripts linked to abscisic acid response and defense response were up-regulated. Our study highlights a previously uncharacterized effect of drought stress on lipid metabolism genes in conifers and significant changes in the expression of several transcription factors, suggesting a regulatory response potentially linked to drought response or acclimation. Our research represents a fundamental step in unraveling the molecular mechanisms underlying short-term drought responses in white spruce seedlings. In addition, it provides a valuable source of new genetic data that could contribute to genetic selection strategies aimed at enhancing the drought resistance and resilience of white spruce to changing climates.

Keywords: Transcriptomics, drought tolerance, conifer, water stress, global change, transcription factor, lipid metabolism

3.1 Introduction

Climate change projections raise concerns about trees having to cope with intensified and frequent extreme events (Forzieri et al., 2022). Drought is currently causing heightened disruptions in forests, diminishing resilience and increasing mortality rates (Hartmann et al., 2022). Future climates may reduce the productivity of essential conifer species in forests, underscoring the importance of prioritizing resilient and productive species for warmer, drier conditions. In this regard, recent research efforts started to look at methods and approaches for the selection and breeding of more resilient conifers (e.g., Depardieu et al., 2020; Laverdière et al., 2022; Soro et al., 2023). However, in spite of recent progress (Baldi & La Porta, 2022; Depardieu et al., 2021; Haas et al., 2021; Stival Sena et al., 2018), there are still large gaps in our understanding of the complex molecular response of trees to drought at the transcriptome-wide level.

Drought response in long-lived woody plants such as conifers involves an complex network of genes and molecular mechanisms (Hamanishi & Campbell, 2011). Indeed, several major gene classes or families are involved in short-term physiological responses to drought, including cell growth, photosynthesis, water loss, phytohormones metabolism, stomatal aperture and closure, and the maintenance of osmotic balance (Moran et al., 2017). More specifically, the pivotal role of the phytohormone ABA as a precursor molecule in drought stress signaling is widely acknowledged in coniferous species (Haas et al., 2021). In addition, aquaporins (AQPs) and ion channels facilitate the transport of water and ions across cell membranes, playing a critical role in regulating water balance in trees (Y. Wang et al., 2020). Previous studies have revealed modifications in the regulation of genes responsible for synthesizing and transporting defense molecules such as flavonoids and terpenoids (Depardieu et al., 2021; Laoué et al., 2021), antioxidants involved in ROS scavenging (H. Fox et al., 2018; W. Li et al., 2021), osmoprotectants such as carbohydrates (W. He et al., 2020; L. Zhang et al., 2021), and proline (Sancho-Knapik et al., 2017; F. Xiao et al., 2021). Heat shock proteins (HSPs) play a pivotal role in safeguarding and stabilizing proteins under drought conditions (L. Zhang et al., 2021). Similarly, chaperone proteins like dehydrins, a subset of late embryogenesis abundant (LEA) proteins, maintain protein and cell membrane stability throughout the hydric constraint (Stival Sena et al., 2018; Velasco-Conde et al., 2012). In such conditions, alterations in the composition and structure of the cell wall are directed by the control of genes linked to the synthesis of cell

wall polysaccharides and membrane (Coleman et al., 2021; T. Wang et al., 2016). Finally, genes involved in transcriptional regulation networks, such as AP2/ERF, bZIP, TCP, WRKY, and MYB transcription factors, coordinate molecular responses to drought (Behringer et al., 2015; Du et al., 2018; W. Li et al., 2021; Lorenz et al., 2011).

White spruce (*Picea glauca* (Moench) Voss) is a conifer species widely distributed across Canada and the northern USA, known for its straight grained and strong wood, making it valuable for lumber and pulp production (Hassegawa et al., 2020; S. Zhang & Koubaa, 2008) in addition to its ecological importance. Its rapid growth in various environments makes white spruce an important species in forestry and reforestation efforts, representing a significant portion of Canada's forest inventory and being one of the most widely planted tree species (Mullin et al., 2011; S. Zhang & Koubaa, 2008). It is also considered to be a model conifer species for genetic and genomic investigations (Bousquet et al., 2021); several studies have shown the susceptibility of white spruce to drought, as demonstrated by a marked reduction in growth (Depardieu et al., 2020; Hogg et al., 2017; Soro et al., 2023; Sullivan et al., 2021), increased mortality (P. Lu et al., 2019; C. Peng et al., 2011), and changes in population abundance and distribution (D'Orangeville et al., 2018). Similarly to numerous conifers of the *Pinophyta* group, white spruce swiftly initiates the ABA pathway under drought conditions, leading to early stomatal closure (Brodribb et al., 2014). This process reduces water loss while also concurrently decreasing photosynthetic uptake, thereby posing a risk of carbohydrate depletion if the drought persists (McDowell et al., 2008). The importance of intraspecific genetic variation for drought response has also been recently highlighted in white spruce (Depardieu et al., 2020, 2024). Thus, characterization of its intraspecific variability at the molecular level appears essential to better delineate the tolerance threshold of stress and identify potential genetic traits governing a tree species' drought response and resilience (Gazol et al., 2023). Understanding white spruce's molecular response to drought is crucial for elucidating acclimation and adaptation mechanisms, informing sustainable forest management, and enhancing resilience to changing climates (Bousquet et al., 2021).

Recent studies have identified genes associated with drought adaptation in white spruce based on testing extensive lists of candidate genes rather than the entire transcriptome (Depardieu et al., 2021; Hornoy et al., 2015). Despite the rapid proliferation of genomic resources, including nuclear (Birol et al., 2013; Warren, Keeling, et al., 2015), mitochondrial, and chloroplast

genomes (Jackman et al., 2016), gene catalogs (Rigault et al., 2011), SNP catalogs (Pavy et al., 2008, 2013, 2016), and quantitative trait loci (QTL) analyses (Laoué et al., 2021; Pavy et al., 2017; Pelgas et al., 2011) brought about by advancements in Next Generation Sequencing (NGS) and high-throughput genotyping technologies, these resources still remain incomplete and fragmented (De La Torre et al., 2014; Neale & Wheeler, 2019). Considering the extensive gene flow linking natural populations of white spruce (Jaramillo-Correa et al., 2001), the relatively recent nature of local genetic adaptation to climate following Holocene recolonization (Prunier et al., 2011), and the highly multigenic nature of local adaptation to climate in spruces (Hornoy et al., 2015; Prunier et al., 2011), it is anticipated that there may be dozens to hundreds of genes potentially involved in drought response and resilience. Consequently, it is imperative that genome-wide and/or transcriptome-wide studies are conducted to elucidate the molecular bases of these polygenic traits more comprehensively.

This study was carried out in white spruce and had three main objectives. First, we assembled a *de novo* transcriptome based on RNA sequencing (RNA-Seq) of samples sourced from distinct developmental stages of white spruce and subjected to short and long-term drought and to defoliation. This approach aimed to capture a broad sampling of expressed genes, specifically emphasizing the response to drought conditions. Second, transcriptomic analyses were conducted on the foliage of white spruce seedlings during a 22-day greenhouse drought experiment to identify key drought-responsive genes involved in short-term water stress acclimation in this species. Third, enrichment analyses of differentially expressed genes were performed to highlight the main metabolic pathways involved in response to short-term water stress. We also explore the intraspecific variation in drought-responsive genes using three clones. Given the large hydraulic safety margin of white spruce and its drastic reduction in gas exchange during drought (Sullivan et al., 2021), we hypothesize that under severe and short-term drought conditions, this species will prioritize the regulation of water management processes from the onset of treatment, at the expense of growth-related processes. Consequently, we expect to observe an up-regulation of genes involved in water homeostasis and transport, alongside a down-regulation of genes associated with photosynthesis and growth.

3.2 Material and methods

3.2.1 De novo transcriptome assembly and functional annotation of the white spruce transcriptome assembly, GCAT 4.0

3.2.1.1 Plant material

A *de novo* transcriptome was assembled from RNA-Seq data obtained from three distinct experiments involving the collection of *Picea glauca* foliage. A total of 16 samples came from a common garden experiment belonging to the International Diversity Experiment Network with Trees (IDENT) network, where eight trees had been subjected to water exclusion and eight others to summer irrigation since 2014 ("Experiment 1", see Methods S1 and Table S2.1 in Supporting information for details). The access of IDENT site and sampling permission was provided by the Forest Research and Monitoring Section of the Ontario Forest Research Institute. Six other samples came from a greenhouse experiment with a budworm-induced biotic stress treatment ("Experiment 2"; Methods S1 and Table S2.1 in Supporting information). The inclusion of data from Experiment 2 was motivated by the reported points of convergence in the signaling networks involved in responses to abiotic and biotic stresses in plants (Fujita et al., 2006; Leisner et al., 2023). Six samples were from a greenhouse drought stress experiment on young clonal seedlings including three water-stressed and three well-watered seedlings (control seedlings in "Experiment 3"; Methods S1 and Table S2.1 in Supporting information), as previously described in Stival Sena et al. (2018). A total of 28 samples were used for *de novo* transcriptome assembly. Information about sample pool collections, RNA extraction and integrity assessment, library construction, and sequencing can be found in the Supporting Information file (Methods S1 and Table S2.1).

3.2.1.2 RNA de novo transcriptome assembly

Quality of RNA-seq raw sequence data was first checked using FASTQC v0.11.9 (Andrews, 2017). Raw reads were cleaned using Trimmomatics.0.39 (A. M. Bolger et al., 2014, p. 201) to remove poorly sequenced nucleotides and remaining adaptor sequences. Clean reads were further filtered for length longer than 30 bp. For each of the 28 samples, filtered reads were used to produce a transcriptome assembly using the A5 pipeline (Coil et al., 2015) that integrate an overlap-based assembler to correct base-call errors (SGA tool (Simpson & Durbin, 2012))

and a de Bruijn graph assembler (IDBA-UD (Y. Peng et al., 2012)) to produce contigs that are latter scaffolded using SSPACE ((Boetzer et al., 2011)). Transcriptome assemblies were then scaffolded with one another using LINKS 1.8.6 (Warren, Yang, et al., 2015). The resulting consensus assembly was then scaffolded again with a previously published *Picea glauca* transcriptome assembly (Rigault et al., 2011) using LINKS 1.8.6 and sequences shorter than 500 bp were removed as they were not likely to code for functional proteins. The completeness of this new assembly, hereafter named GCAT 4.0, was then evaluated using BUSCO (Benchmarking Universal Single-Copy Orthologs) v5.4.3 with -m transcriptome option and sequence comparison with the *Embryophyta* and *Viridiplantae* reference databases (odb10) (Manni et al., 2021). The number of open reading frames (ORFs) and other complementary statistics were performed using the TRAPID web server (https://bioinformatics.psb.ugent.be/trapid_02/) and the PLAZA version 4.5 database (https://bioinformatics.psb.ugent.be/plaza/versions/plaza_v4_5_dicots/) (Bucchini et al., 2021).

3.2.1.3 Functional annotation of transcripts

Functional annotation for the new transcriptome assembly was retrieved by sequence similarity searches using BLASTx of OmicsBox (Conesa et al., 2005; Götz et al., 2008) (cut-off E-value of $\leq 10^{-5}$) against the Refseq database from the NCBI (Accessed September 29th, 2022). The description of protein signatures was obtained after detection of homologous protein domains of translated sequences following a search of the Interpro database using the OmicsBox. Gene Ontology (GO) annotations including GO molecular function, GO biological process and GO cellular component terms were also obtained for each individual transcript using GO Annotation tool in OmicsBox. To obtain a complete annotation of the *de novo* assembly GCAT 4.0, BLASTx analyses were performed against public databases such as PlantTFDB and *Viridiplantae*, using DIAMOND-aligner v.2.0.14 (Buchfink et al., 2021). Analysis parameters were set to "sensitive" mode, k-1, b1.2 and an E-value of $\leq 10^{-5}$. The OmicsBox assembly annotation has been deposited and is publicly available (https://github.com/ZoeRibeyre/De-novo-transcriptome-assembly-and-discovery-of-drought-responsive-genes-in-white-spruce.git). Putative genes were annotated against the transcriptomes of *Arabidopsis thaliana, Populus trichocarpa and Malus domestica* (data downloaded from PLAZA 5.0, sub-sections

Locus FASTA Data - Protein files - Selected transcript (Van Bel et al., 2022)) using Blastx with an E-value cut-off set to $\leq 10^{-5}$.

The presence of transcription factors (TFs) was additionally corroborated by analyzing BLASTx results against the plant transcription factor database PlantRegMap/PlantTFDB v5.0 (http://planttfdb.gao-lab.org/; (Jin et al., 2017; Tian et al., 2020) and the Refseq database, and based on protein signatures detected using OmicsBox . The number of putative unique genes contained in the *de novo* transcriptome assembly GCAT 4.0 was determined by BLASTn analysis against the latest white spruce reference genome publicly available on NCBI (WS77111v2, Accessed on July 2022).

3.2.2 Drought stress experiment and transcriptome analysis

3.2.2.1 Plant material, water treatment, and RNA sequencing

Transcriptomic analyses, composed of differential expression and enrichment analyses, were performed on raw RNA-seq data from 2-year-old white spruce foliage submitted to a greenhouse water stress experiment published by Stival Sena et al. (2018) (referred to in this study as "Experiment 3"). The seedlings were represented by three genetically unrelated 2-year-old clones (C8, C11, and C95). The seedlings were watered twice per week for two months before the experiment. Then, following a completely randomized design, half of the plants were watered (controls) and the other half were withheld from water (stressed) for 22 days. The newly formed foliage (needles) was sampled at 0, 14, 18, and 22 days (6 samples per condition and time point, n=48) (see Experiment 3, Figure 3.1 and Table S2.1). Needles were frozen in liquid nitrogen immediately after sampling and stored at −80 °C until RNA extraction. At each sampling day, the midday water potential (branch) of four plants per genotype in control and stressed treatments was measured using a Scholander pressure chamber (Model 610, PMS Instruments, Albany, OR, USA). Water potential measurements show a sharp decline starting on day 14, which intensifies on day 18 and even further by day 22, reflecting both the severity of the imposed drought and the trees' physiological response to this stress (see Sena Stival et al., 2018). More detailed information about sample pool collections, RNA extraction and integrity assessment, library construction, and sequencing can be found in the Supporting Information file, specifically in Methods S1 and Table S2.1, within the Experiment 3 section.

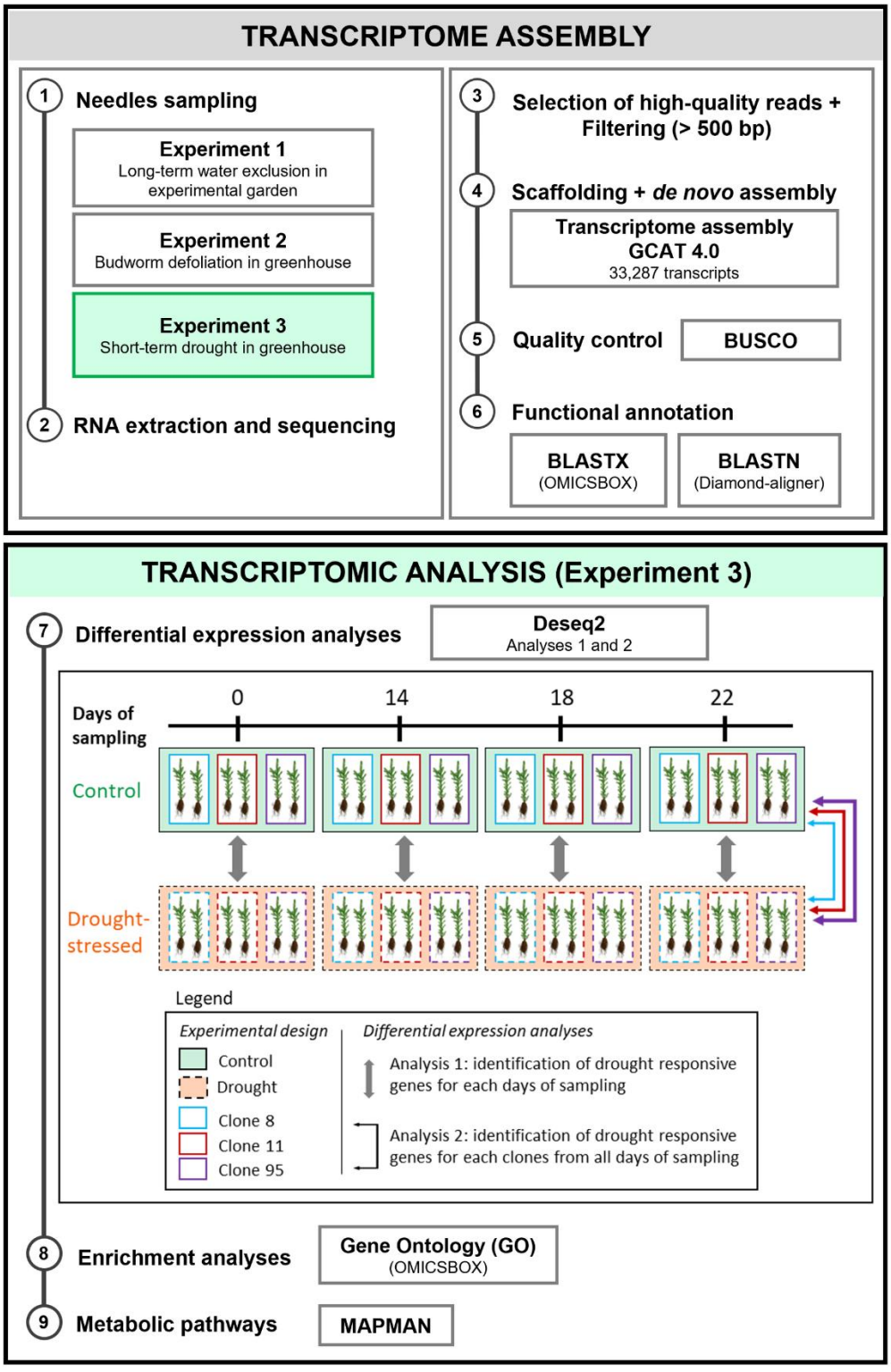

Figure 3.1. Experimental design and analysis pipeline used in this study. The analysis steps are presented in chronological order for the two boxes *Transcriptome assembly* and *Transcriptomic analysis*. The tool used for each type of analysis is reported.

3.2.2.2 Differential expression and enrichment analyses

Differential expression analyses between drought-stressed and control seedlings were carried out to identify transcripts involved in conifer drought response. A first set of analyses was carried out at each time point (Analysis 1, Figure 3.1) to identify the transcripts significantly up- or down-regulated throughout drought intensification. Considering the insufficient number of replicates to perform a clone-by-clone analysis for each time point, the transcripts differentially expressed for each clone were determined by comparing water stress versus control conditions for all time points (Analysis 2, Figure 3.1). Differential expression analyses were conducted by pseudo-aligning high-quality reads against the assembly GCAT 4.0 using Kallisto v0.48.0 (Bray et al., 2016). Read counts were normalized using DESeq2 (Love et al., 2014) and DESeq-normalized expression values were then used to calculate the fold change for a given transcript expressed as a log2-fold change (LFC). Differentially expressed transcripts (DETs), and corresponding genes (DEGs), between drought-treated and control samples were then identified using the R package DESeq2 (Love et al., 2014) with an absolute threshold of 2 for LFC and an adjusted p-value of 0.05.

Gene ontology (GO) enrichment analyses were performed on significant DETs using the OmicsBox and a Fisher's exact test (Götz et al., 2008). GO enrichment analyses were based on lists of DETs whose expression was significantly regulated at each time point. Venn diagrams were generated to highlight unique and shared DEGs between time points and clones using Venn diagrams (ggVennDiagram v1.2.2 R package, (C.-H. Gao et al., 2021); Venndetail v1.16.0 R package, (K. Guo & McGregor, 2024).

The functions and the regulation of DEGs were visualized using metabolic pathway diagrams from MapMan v3.6.0RC1 (Schwacke et al., 2019; Thimm et al., 2004). MapMan manages a hierarchical tree structure that describes different functional categories or "Bins" according to the MapMan nomenclature. The Mercator4 online tool was used to create the mapping file required to run MapMan from a FASTA file (the *de novo* assembly transcriptome GCAT 4.0) by assigning sequences to the corresponding Bin terms (M. Bolger et al., 2021). This analysis was conducted at the gene level. All available metabolic pathway diagrams were downloaded from the MapMan interface and visualized following the analyses. We selected the pathway diagrams for metabolism (X4.5 Metabolism Overview R5.0) and photosynthesis (X4.5

Photosynthesis R5.0) as those had the most DEGs identified in our study. To improve understanding of the results in the context of drought-related response, we graphically synthesized parts from the Cellular_response_overview pathway and abiotic results from the Biotic Stress pathway by removing sections with very few or no DEGs.

3.3 Results

3.3.1 Statistics and quality assessment of the new white spruce de novo assembly, GCAT 4.0

The *de novo* transcriptome assembly conducted in this study encompasses a total of 33,287 unique transcripts, corresponding to 18,934 unique genes, as determined through BLASTn analysis against the reference genome of white spruce (Tables S2.2 and S2.3). A total of 33,283 potential open reading frames (ORFs) with an average length of 852 base pairs (bp) were identified using the TRAPID pipeline (Bucchini et al., 2021). The contig N50 stands at 1,816 bp, and the contig N90 is 746 bp (Table S2.2). Sequence length distribution showed that the transcriptome assembly encompassed a wide range of transcript sizes: 56.2% spanning from 1,000 bp to 4,000 bp, 40.7% ranging from 500 bp to 1,000 bp, and 3.1% exceeding 4,000 bp (Figure 3.2A), and a median length of 1,173 bp and a mean length of 1,488 bp (Table S2.2). Within the 425 *Viridiplantae* odb10 BUSCO groups, 94.1% were identified as complete and single-copy, 2.6% as complete and duplicated, 3.1% as fragmented, and only 0.2% were absent (Figure 3.2B). Additionally, among the 1,614 *Embryophyta* odb10 BUSCO groups, 82.9% were categorized as complete and single-copy, 4.5% as complete and duplicated, 4.0% as fragmented, and 8.6% were found to be missing (Figure 3.2C). BLASTx analysis of the *de novo* assembly against public databases revealed sequence homology rates of 65.27% with UniProt, 43.5% with PlantTF, 80.62% with *Viridiplantae* NR, and 79.41% with the NCBI RefSeq database (Table S2.4).

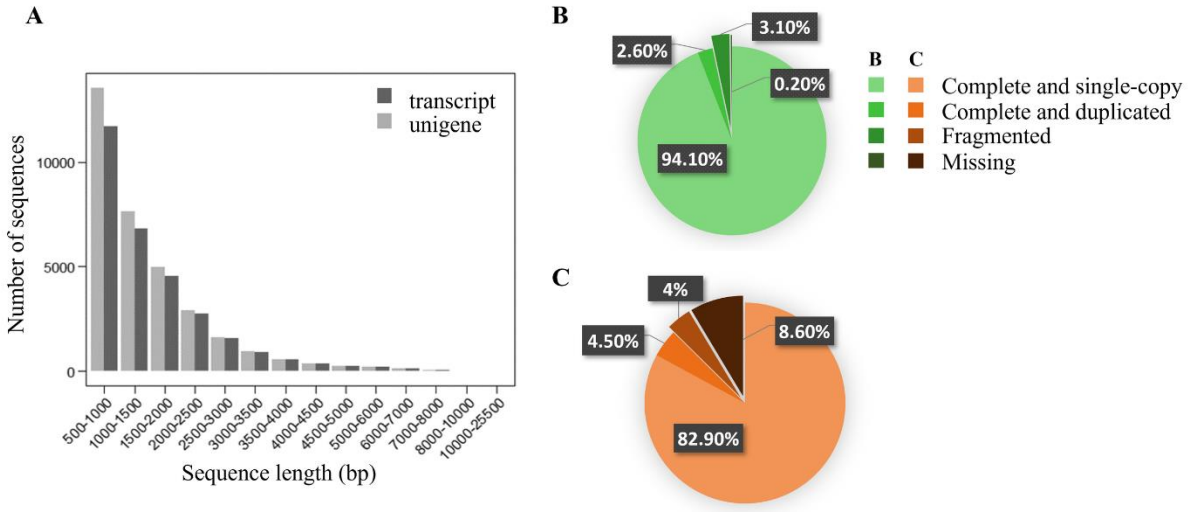

Figure 3.2. Characteristics and quality assessment of the *de novo* assembly GCAT 4.0. (A) Distribution of the number of transcript and unigene sequences as a function of the sequence length expressed in base pairs (bp). The two pie charts represent the results of the BUSCO analysis using (B) the *viridiplantae* database (Viridiplantae_(odb10) and (C) the *embryophyta* database (Embryophyta_(odb10)).

3.3.2 Temporal dynamics in gene expression in response to drought

Analysis of differentially expressed genes (DEGs) identified 4,425 out of the 18,934 detected unigenes in response to drought, with 1,370 up-regulated unigenes and 3,055 down-regulated unigenes (Figure 3.3 and Table S2.5). As the water stress intensifies over time, an increasing number of both up-regulated and down-regulated genes were observed, showing an initial response affecting a few genes followed by changes in a very large number of genes expressed; 16 DEGs were identified on day 0, followed by 88 genes on day 14. Subsequently, the number of regulated genes escalated to 1,620 on day 18, reaching a substantial peak of 4,186 on day 22 (Figure 3.3B). The DEGs were not the same from the beginning to the end of the treatment. Specifically, an overlap of 37% was observed exclusively for up-regulated genes between days 18 and 22 (Figure 3.3D), while a 23% overlap was observed for down-regulated genes (Figure 3.3C).

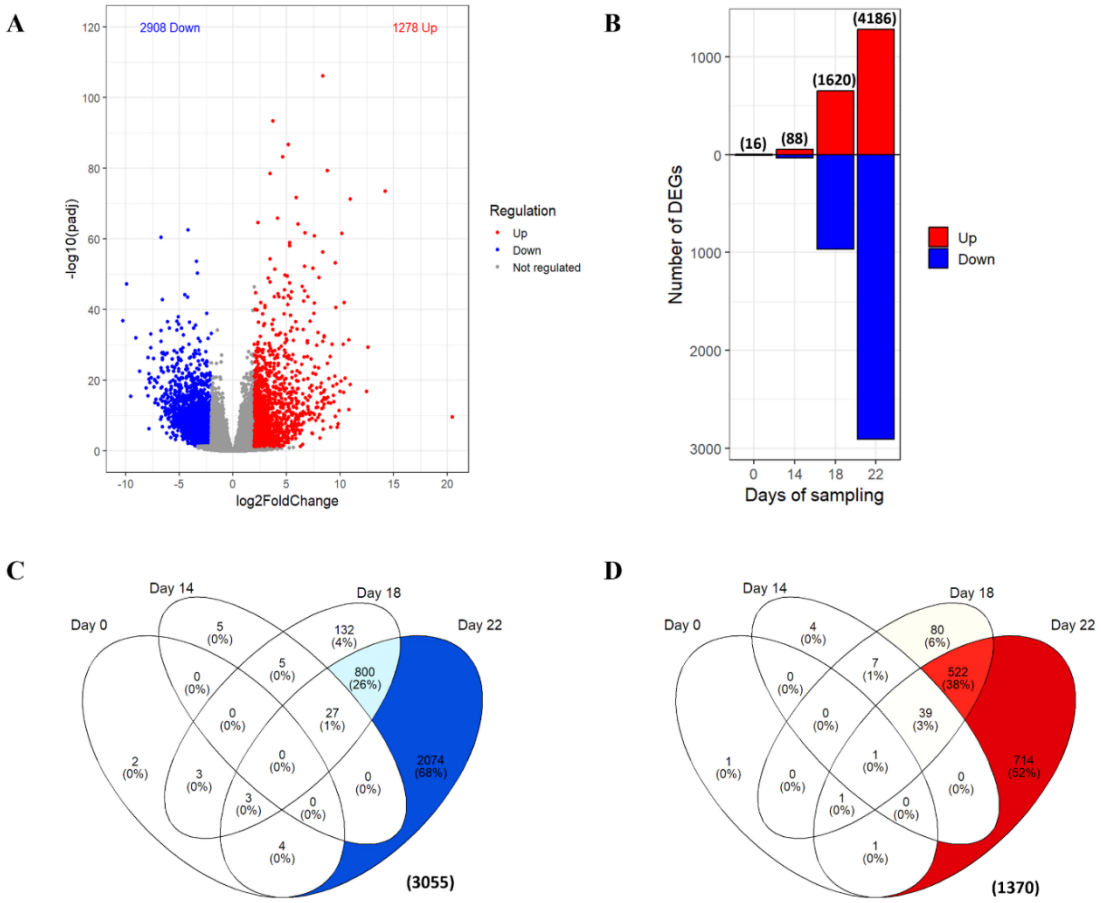

Figure 3.3. Differentially expressed unigenes (DEGs) in response to drought in white spruce. (A) Volcano plot control versus water stressed white spruce trees on day 22. Down-regulated unigenes (FDR ⩽ 0.05 and a log2FC ⩽ -2) are shown in blue, while up-regulated unigenes (FDR ⩽ 0.05 and a log2FC ⩾ 2) are represented in red. Genes whose expression is not significantly altered by drought are identified by grey dots. (B) The number of differentially expressed unigenes (DEGs) is shown as a function of their regulation (in red, upwards, and in blue, downwards) for the four sampling days. The Venn Diagrams depict the overlaps of (C) downregulated and (D) upregulated differentially expressed genes across the days of sampling. The intensity of the color is positively correlated with the number of unigenes.

3.3.3 Identification of key functions involved in short-term drought response

The MapMan analysis performed on all sampling days illustrated the key metabolic pathways involved in the water stress response of white spruce seedlings. It showed that 42.8% of the 4,425 drought-responsive genes were assigned to Bins belonging to 29 major functional groups (Tables S2.6.1 and S2.6.2). The most represented functions among unigenes encompassed enzymatic classification (35.76%), solute transport (10.2%), RNA biosynthesis (9.24%), protein modification (6.02%), cell wall organization (4.28%), protein homeostasis (4.44%), phytohormone action (3.54%), photosynthesis (2.8%), carbohydrate metabolism (2.64%), and lipid metabolism (2.59%) (Table S2.6.2). Furthermore, the level 3 Gene Ontology (GO) annotation identified highly represented biological processes (BP) such as transmembrane transport (204 DEGs), signaling (107 DEGs), carbohydrate metabolism (168 DEGs), and lipid metabolism (107 DEGs). Notably, both photosynthesis and cell wall biosynthesis/organization processes had a substantial proportion of down-regulated DEGs (95.6 and 78.1%, respectively). The GO annotation also revealed a substantial presence of molecular functions (MF), such as oxidoreductase activity (385 DEGs), hydrolase activity (344 DEGs), and catalytic activity (260 DEGs) (Figure 3.4A and Table S2.7).

The GO enrichment analysis performed on differentially expressed transcripts (DETs) indicated that BP and MF changed between the first half of the experiment (0-14 days) and the latter half (18-22 days) in the experiment. Before day 18, MFs associated with the regulation of molecular function, cellular process, and catalytic activity were enriched. In contrast, up-regulated DETs on day 18 and day 22 were associated with responses with osmotic stress, hormone stimuli including abscisic acid (ABA), reactions to external stimuli, defense mechanisms, and carbohydrate and lipid metabolism. The photosynthesis process was among the enriched BPs linked to down-regulated DETs at day 22 (Figure 3.4A and 3.4B and Figure 3.5A and 3.5B). Several molecular functions were also enriched in both up-regulated and down-regulated DETs, particularly involving catalytic activity and oxidoreductase activity. However, the observed enrichment of lyase and antioxidant activities was unique to up-regulated DETs (Figure 3.4B, MF panel).

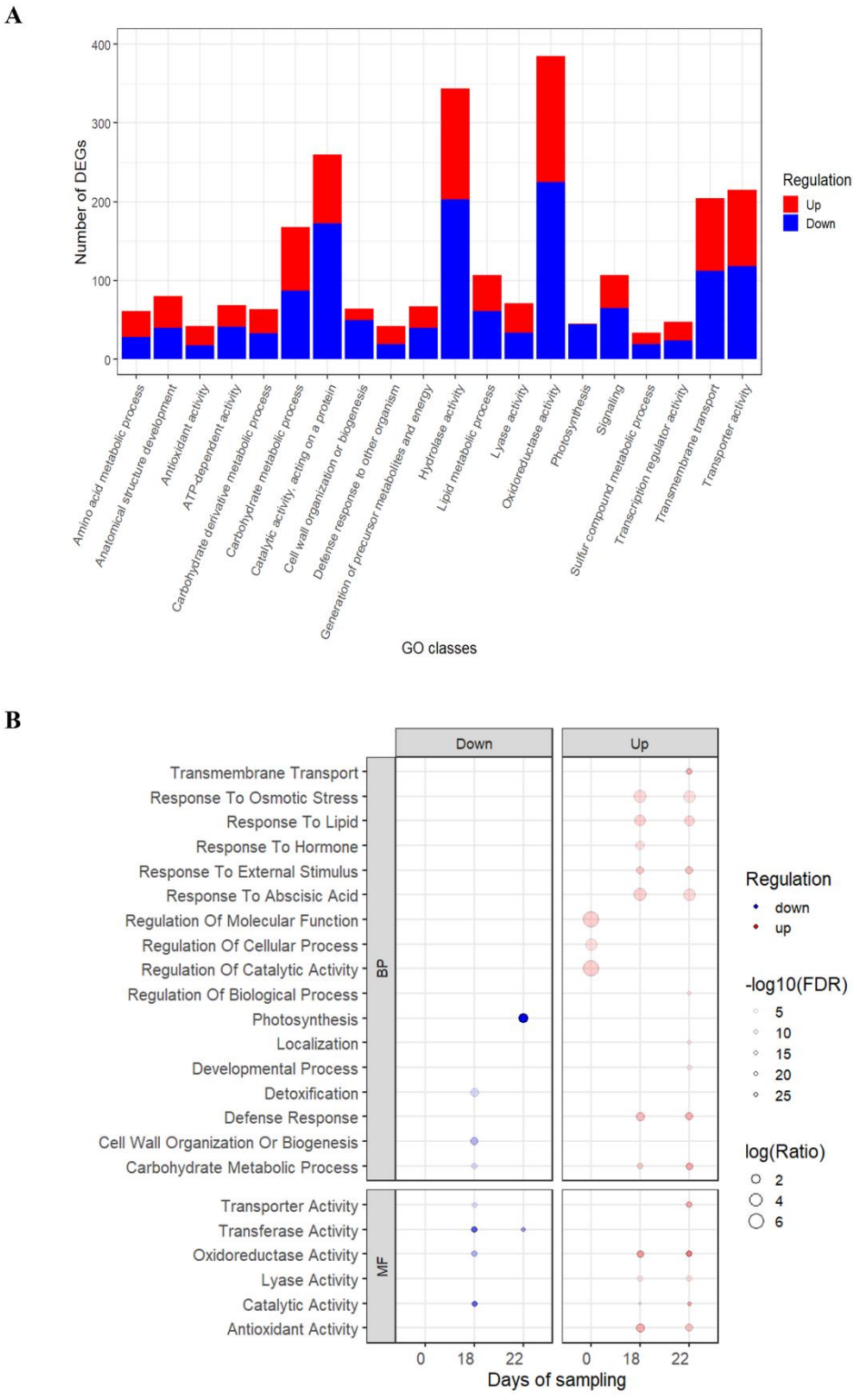

Figure 3.4. Results of gene ontology enrichment analyses as a function of drought exposure time. (A) The barplot represents the gene ontology (GO) annotation from OmicsBox of unique DEGs regulated on all time points. (B) The scatterplots represent the enriched GO terms belonging to the biological process (BP) and molecular function (MF), as a function of exposure time to the water stress treatment (Days of sampling). Significantly enriched GO terms are shown with the transparency gradient based on -log10(FDR). The size

of the dots indicates the ratio of the number of annotated sequences in the sample to the reference transcriptome GCAT 4.0. Enriched GO terms associated with up- and down-regulated sequences are shown in red and blue, respectively. Day 14 showed no significant enrichment and has been withdrawn from the graph for clarity.

3.3.4 Gene expression profiles at peak water stress: insights into drought-responsive pathways

A distinct MapMan analysis was conducted only on DEGs at day 22 (Figure 3.5), which had by far the most water stress responsive DEGs (4,186 DEGs; Figure 3.3A), and included 68% of the total down-regulated DEGs and 52% of the total up-regulated DEGs (Figure 3.3C and 3.3D). The data included DEGs associated with cell wall organization (76 unigenes), with a prevalent down-regulation observed in photosynthesis metabolism (55 unigenes), lipid metabolism (46 unigenes), carbohydrate metabolism (47 unigenes), and secondary metabolism (34 unigenes), primarily connected to terpenoids and phenolic compounds (Figure 3.5A and 3.5B). The redox homeostasis process was well-represented with 41 DEGs (Figure 3.5C and Tables S2.6.2 and S2.6.3). Furthermore, the identification of InterPro domains indicated many DEGs encoding Leucine-rich repeat and kinases proteins, alpha-beta hydrolases, AAA+ ATPases, and cytochrome P450 specifically on day 22. Finally, members of heat shock proteins (HSPs), dehydrins, major intrinsic proteins, and late embryogenesis abundant proteins (LEA) were also detected (S1.2 Figure).

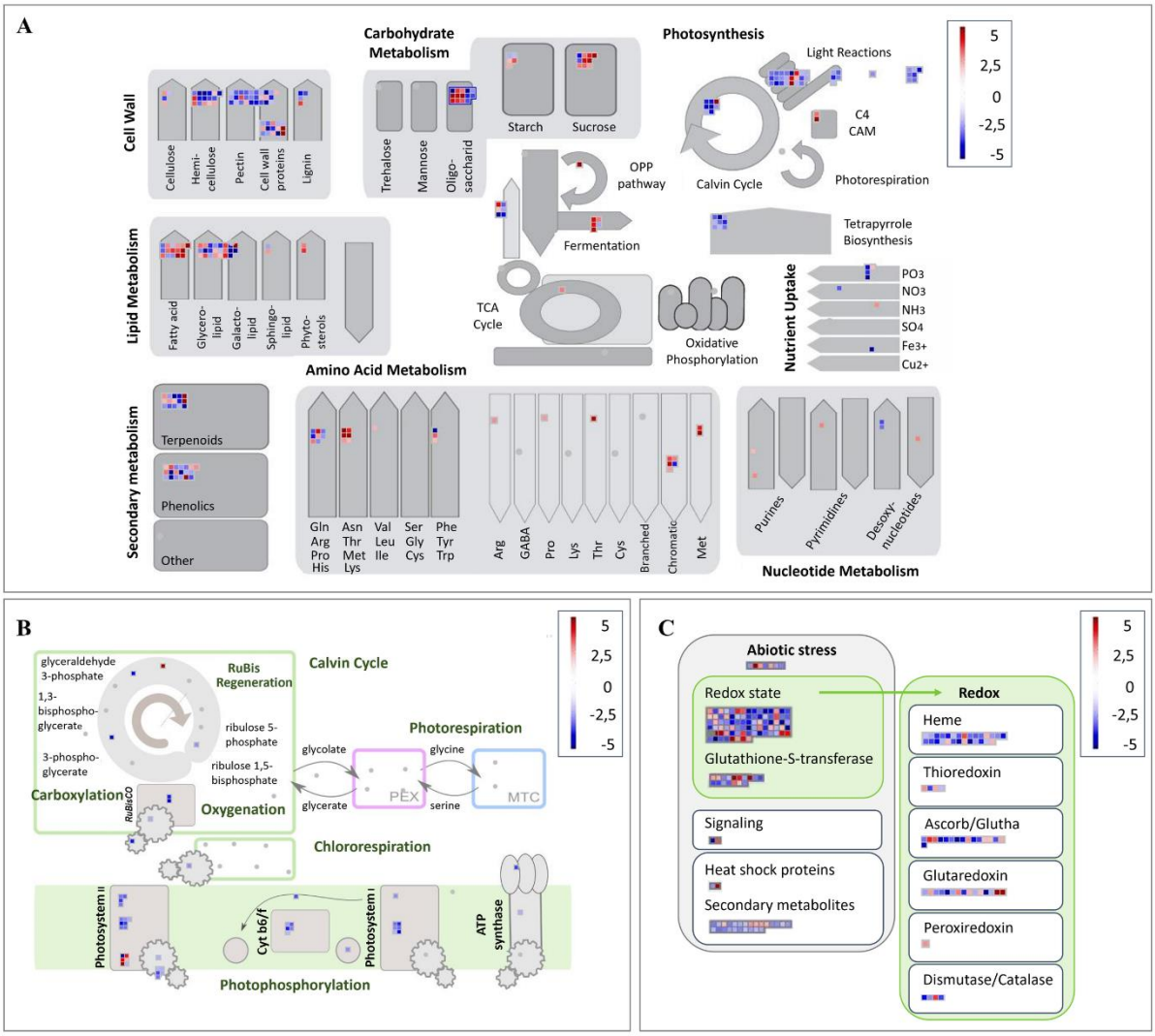

Figure 3.5. Pathways based on MapMan classification of differentially expressed genes (DEGs) involved in drought stress responses after 22 days in white spruce seedlings. Expression profiles of DEGs involved in metabolism overview (A), photosynthesis (B), abiotic stresses and redox homeostasis (C) are presented. The schematic representation of panel (C) was obtained after modifying MapMan's original pathways (biotic stress and cellular response overview pathways) to improve and more concisely synthesize the results obtained in the context of our specific short-term drought experiment. The scale bar represents the up- (red) and down- (blue) regulation of gene expression based on log2FC scores.

3.3.5 Identification of drought-responsive transcription factors

A total of 389 potential transcription factors (TFs) were differentially expressed in the present study, 103 of which are considered highly regulated with a LFC greater than 2 or less than -2 with 62 up-regulated and 42 down-regulated unigenes classified into 17 classes. The most represented classes were the RING type zinc fingers (26 DEGs), followed by NAC (15 DEGs) and AP2/ERF (14 DEGs) (Figure 3.6A, Tables S2.8.1 and S2.5.2). Notably, the RING and C2H2 type zinc finger genes, as well as the WRKY genes, had a predominantly up-regulated expression under drought. The AP2/ERF and AUX/IAA classes contained an equal number of genes with both up and down regulation, while the CBF/NF, PLATZ, and PHD type zinc finger subfamilies exclusively had up-regulated genes. The data showed that most changes in the expression of these TFs occur after 18 days of drought with a notable increase in the magnitude of the LFC (Figure 3.6B and Table S2.8.2). The most up-regulated TFs after 18 days including three NACs, two AP2/ERF, two zinc fingers and one CBF/NF.

3.3.6 Intraspecific genetic variation of drought-responsive genes

The present study used three genetically unrelated clones, and a clone-to-clone analysis identified 638 up-regulated and 63 down-regulated differentially expressed genes, indicating intraspecific gene expression differences under drought (Figure S1.3 and Table S2.9). Overall time points, no down-regulated DEGs were shared among clones (Figure S1.3B), and only 21% of up-regulated DEGs were common among all three clones (Figure S1.3A). Shared DEGs were involved in BP of defense mechanisms and macromolecule metabolism covering carbohydrates, lipids, and amino acids (Figure S1.4A), and were also associated with MF of catalytic activities including transferase, oxidoreductase, lyase, isomerase, and hydrolase activities (Figure S1.4C). GO enrichment analysis showed that the most enriched BP or MF was similar across all three clones, including catalytic and antioxidant activities, as well as defense response processes (Figure S1.4).

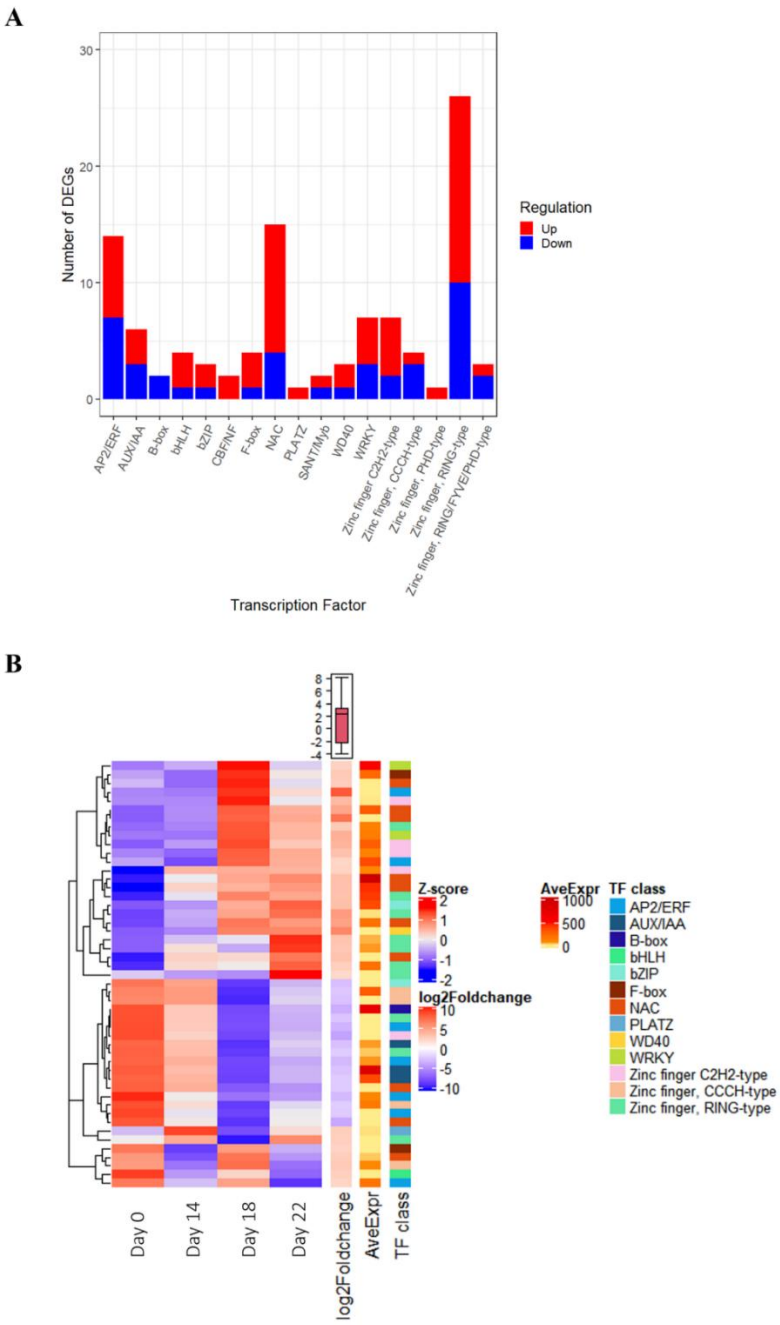

Figure 3.6. Main classes of transcription factors (TFs) unigenes significantly regulated in response to drought. (A) Histogram showing the number of up (red) or down (blue) regulated genes for the most represented classes of drought-responsive TFs. (B) Heatmap showing the expression of TFs belonging to key TF classes in the response to drought conditions. To the right of the heatmap is the log2 fold change (log2Foldchange), which corresponds to the level of regulation of transcription factor expression when it was detected significantly regulated for a given time point. In cases where a TF was up-regulated at more than one time point, the log2foldchange was averaged over multiple time points and plotted in the heatmap. The complete list of drought-responsive TFs is presented in Table S2.8.

3.4 Discussion

This study presents an expanded white spruce transcriptome under water stress, enhancing transcriptomic resources, and characterizing key regulated genes in this conifer model species. The new transcriptome assembly allowed for a great characterization of key genes that are regulated under drought conditions. Our transcriptomic analysis describes the regulation of genes in white spruce after 22 days of water stress, revealing a significant increase in differentially regulated genes (DEGs) compared to controls, with over 4,000 DEGs by day 22. This robust regulation underscores the intensity of the treatment and the strong response in white spruce. The gene expression data suggests that the treatment disrupted numerous physiological processes, as expected for this drought-sensitive species. We identified several drought-responsive genes associated with photosynthesis, growth, water transport, sugar and lipid metabolism, and defense mechanisms.

3.4.1 Quality of the new transcriptome assembly

A new transcriptome assembly of white spruce has been generated based on needles representing different developmental stages (seedlings and saplings) and exposed to various conditions as extreme drought stress. This new assembly complements the previously published and 2011 dated representation of genes expressed under such environmental conditions in white spruce (Figure 3.1). The completeness achieved in the GCAT 4.0 assembly is consistent with similar investigations conducted on various conifer species (Breidenbach et al., 2020; I. H. Lee et al., 2019; Ojeda et al., 2019; Visser et al., 2023). Our assembly approach yielded a high proportion of complete and single-copy genes, with minimal redundancy of complete genes (Figure 3.2). The transcriptome assembly representing roughly 62.26% of the 30,410 estimated genomic gene count (Gagalova et al., 2022), highlighting the substantial representation of genes, especially considering that the assembly exclusively originated from needle tissue. The GCAT 4.0 transcriptome assembly represents a robust foundation that complements the previously published assembly to investigate the molecular pathways involved in the response of white spruce needles to drought stress.

3.4.2 Signaling and hormonal response to drought

In response to water deficit, plants initiate a cascade of hormonal and signaling pathways that orchestrate both molecular and physiological responses toward drought tolerance. These processes involve the activation of genes responsible for the synthesis and signaling pathway of the stress hormone abscisic acid (ABA), which is facilitated by a variety of protein kinases and tyrosine phosphatases (Klápště et al., 2020). Our transcriptomic analysis identified 115 to 385 putative protein kinases with drought-responsive expression with approximately two-thirds being down-regulated. We also observed an up-regulation of three tyrosine phosphatases (Table S2.5). Isohydric species, like white spruce, activate early stomatal closure in response to drought (Brodribb et al., 2014), with ABA playing a key role in reducing water loss (Brodribb & McAdam, 2013; Brunner et al., 2015). While it has been traditionally suggested that ABA biosynthesis and signaling occur in the roots before being transported to the leaves to initiate stomatal closure in drought-stressed plants, recent research indicates that these mechanisms may start directly in the leaves of pine and spruce species (Mitchell et al., 2017; Pashkovskiy et al., 2019). In our study, biological processes (BP) related to ABA were enriched on days 18 and 22 (Figure 3.4B). The differentially expressed genes (DEGs) associated with ABA biosynthesis and signal transduction were primarily identified at days 18 and 22, but some were also detected within the first 14 days of treatment. Specifically, we identified two up-regulated gene related to NCED3 (9-cis-epoxycarotenoid dioxygenase 3), a key enzyme involved in ABA synthesis and previously observed in the drought stress response of *Picea abies* (Haas et al., 2021) and *Pinus massoniana* (Du et al., 2018). Additionally, we found one up-regulated gene associated with the ABA receptor PYL, which plays a role in inhibiting PP2C (2C-type protein phosphatases), known as a negative regulator of the ABA-signaling enhancer SNF1-related protein kinase (SnRK2) (Table S2.5) (Du et al., 2018; Haas et al., 2021). Our findings support the significance of ABA-related genes in the response of white spruce and suggest that the intensity and duration of the stress amplify this signaling pathway.

On days 18 and 22, several up-regulated DEGs related to hormones other than ABA, particularly auxin (12 DEGs) and ethylene (6 DEGs) (Table S2.5) were observed. We identified three putative up-regulated AUX/IAA sequences, known to be involved in early auxin signaling and regulated in response to drought (Luo et al., 2018). We observed five up-regulated and four down-regulated genes belonging to the SAUR (small auxin upregulated RNA) -like auxin-

responsive protein family, which may influence tree drought tolerance by establishing leaf auxin concentration gradients and regulating stomatal closure (S. Li, Yan, et al., 2023). The expression of five putative Dormancy/auxin-associated proteins, which play pivotal roles in responding to stress and impacting plant growth and development, was also detected (Table S2.5) (Souza et al., 2019). Our findings indicate the regulation of genes that may play a role in initiating hormonal signal transduction under drought conditions, which is expected to affect growth and photosynthesis in white spruce.

3.4.3 Negative impact of drought on photosynthesis, growth, and water transport

The numerous down-regulated genes linked to photosynthesis, particularly showing a more pronounced decline after 18 and 22 days of drought treatment (Figure 3.4 and Figure 3.5B), suggest an abrupt disruption of photosynthesis as the drought stress intensifies. Water availability significantly impacts photosynthesis, often causing a limitation in CO_2 uptake due to reduced stomatal and mesophyll conductance (Perdomo et al., 2017). Alterations in photosynthesis can also be attributed to metabolic disruptions induced by oxidative stress, leading to the degradation of cellular membranes, components of the electron transport chain, and photosynthetic pigments, among others (Drake et al., 2017b; Lei et al., 2022). Here, three DEGs were associated with rubisco activity, including two encoding Ribulose-1,5-bisphosphate carboxylase/oxygenase and one related to rubisco activase (Table S2.5), which plays a pivotal role in the assimilation and fixation of CO_2 (Perdomo et al., 2017). We observed a down-regulation of genes associated with critical components of the electron transport chain, including one DEG related to the cytochrome b6f complex, seven DEGs associated with Photosystem I (PSI), and four DEGs linked to Photosystem II (PSII). The cytochrome b6f complex expedites the movement of electrons between these two photosystems, resulting in the formation of a proton gradient that drives the synthesis of adenosine triphosphate (ATP) (Foyer et al., 2012). In plants, PSI and PSII play pivotal roles in capturing light energy and facilitating the transfer of electrons within the electron transport chain (J. E. Johnson & Berry, 2021). In line with previous studies, our findings suggest that prolonged periods of water stress can adversely affect both PSI and PSII (Shimakawa & Miyake, 2018; Zlobin et al., 2019). We also observed a decrease in the expression of 13 DEGs associated with photosynthetic pigments such as chlorophyll a, chlorophyll b, and carotenoids (Table S2.5), which is in line with previous research conducted on conifers (Lei et al., 2022; Schiop et al., 2017; Zlobin et al.,

2019). Our study highlights a significant disruption of photosynthesis in white spruce under drought conditions, which may be due to both reduced CO_2 uptake and damage to numerous components within the photosynthetic chain.

Water stress in trees leads to reduced growth, even before a decline in photosynthesis occurs (Granda & Camarero, 2017; Piper et al., 2017), which involves decreased cell wall expansion due to turgor loss and osmotic imbalances, as well as a decline in cell division and wall construction (Gall et al., 2015). In our study, two potential osmotin/thaumatin-like (OTL) proteins had decreased expression. These proteins play a role in maintaining cellular osmolarity during stress, as indicated by (de Jesús-Pires et al., 2020), suggesting a probable osmotic adjustment in white spruce under drought conditions. The regulation of water transport and cell turgor pressure relies on specialized water channels called aquaporins (AQPs). Consistent with the substantial decrease in water potential measured in the same white spruce seedlings subjected to the same drought experiment (Stival Sena et al., 2018), the down-regulation of ten aquaporins, specifically plasma membrane intrinsic proteins (PIPs), indicated a reduction of water transport in needles. These observations align with previous research in spruces and pines (Du et al., 2018; Laur & Hacke, 2014; Lorenz et al., 2011) and support a water conservation mechanism by the reduction in AQPs expression during water stress in conifers. The enrichment of down-regulated transcripts related to cell wall organization or biosynthesis (Figure 3.4A and 4B) highlights the reduction in cell division and wall construction under drought conditions. We identified 19DEGs linked to both the cellulose synthase (CesAs) involved in cellulose synthesis within primary cell walls, and cellulose synthase-like (CSLs) families recognized for their contribution to secondary cell wall synthesis (T. Wang et al., 2016). Consistent with previous findings in water-stressed *Abies alba* seedlings, we observed a decreased expression of genes encoding xyloglucan endotransglucosylase/hydrolase (XTH), a crucial enzyme involved in plant cell wall reconstruction (Behringer et al., 2015; Cheng et al., 2021). These findings emphasize the disruption of several crucial growth-related processes in white spruce induced by drought.

3.4.4 Regulation of the carbohydrate and lipid metabolisms

We reported an enrichment of carbohydrate metabolism under drought conditions on days 18 and 22 (Figure 3.5A). A common defense mechanism in drought-affected trees is to reallocate

carbon resources away from growth and toward storage of non-structural carbohydrates (NSCs), such as starch and soluble sugars, e.g., sucrose. The concentration of these compounds increases in root and woody tissues and contributes to maintaining osmotic balance (Hartmann & Trumbore, 2016; Piper et al., 2017); the compounds may serve as carbon precursors for the synthesis of defense compounds and act as signaling molecules (Jeandet et al., 2022). In our study, five differentially expressed genes (DEGs) were associated with sucrose synthase and eight with the sucrose and hexose transporters SWEETs (Table S2.5). This suggests a modulation of sucrose levels in white spruce seedlings under drought conditions, as previously shown in Norway spruce and pine (Ivanov et al., 2019; Pashkovskiy et al., 2019). While competition for a limited pool of available resources has long been considered the driving force behind the trade-off between growth and defense (Figueroa-Macías et al., 2021), recent findings in *Arabidopsis thaliana* suggest that the incompatibility between growth and defense may also be due to the antagonistic nature of the molecular pathways regulating these two processes (Neuser et al., 2019).

Lipids play essential roles in cell membrane structure, energy storage, and signaling (Kim, 2020). Various conifer species, such as those found in the *Larix*, *Pinus*, and *Picea* genera, possess substantial lipid reserves (Hoch et al., 2003; Tomasella et al., 2019), but our understanding of lipid metabolism in conifers under water deficit conditions remains limited. Lipid metabolism was altered in response to drought stress in our experiment, primarily affecting glycerolipid metabolism (Figure 3.5A). Glycerolipids are crucial for thylakoid lipid bilayer formation and efficient photosynthesis, and decreased levels of these molecules have been linked to reduced photosynthesis in higher plants (Kobayashi et al., 2016). Drought induced the regulation of genes associated with fatty acid metabolism, leading to the up-regulation of putative malate synthases (3 DEGs), citrate synthases (2 DEGs), and isocitrate lyase (1 DEG) (Table S2.5). These enzymes play a crucial role in the glyoxylate cycle, providing essential precursors for gluconeogenesis, the process of converting non-carbohydrate precursors into carbohydrates (Walker et al., 2021). While most research on conifers under water stress has traditionally focused on sugar metabolism, lipid metabolism has frequently been underemphasized. Nonetheless, our findings highlight a shift in the regulation of genes associated with lipid metabolism, underscoring its active role in drought responses in white spruce. This aspect merits deeper exploration in coniferous species.

3.4.5 Drought-responsive genes coding for protective defense and stress resistance and resilience

A strong representation of antioxidant activity was observed among the DEGs in our study (Figure 3.4A and 3.4B and Figure 3.5A, 3.5B and 3.5C), with increased expression of putative glutathione peroxidases (3 DEGs), glutathione S-transferases (10 DEGs), peroxidases (10 DEGs) and catalases (3 DEGs) (Table S2.5). Many protective molecules such as antioxidants proteins, late embryogenesis abundant proteins (LEA), heat shock proteins (HSPs), and other types of molecules are involved in drought responses of coniferous species (Baldi & La Porta, 2022). Reactive oxygen species (ROS) can act as signaling molecules initially during stress, but prolonged or intensified stress increases ROS production, disrupting redox balance and causing oxidative stress (Mukarram et al., 2021). Oxidative stress damages various structures and molecules, such as membrane lipids, proteins, photosynthetic pigments, and nucleic acids (Bilska et al., 2019; Chan et al., 2016; Corpas et al., 2020). This damage seems to be avoided by trees through the production of protective enzymes and molecules to maintain homeostasis and counteract oxidative stress. The balance of antioxidant enzymes plays a major role in ROS scavenging mechanism in plants (Sofo et al., 2015; Vaish et al., 2020), consistent with a role in drought response in conifers (Du et al., 2018; H. Fox et al., 2018; Lei et al., 2022). Our study also identified numerous cytochrome P450 genes (CYTs) that were down-regulated (42 DEGs) and up-regulated (7 DEGs) under stress conditions (Table S2.5). In contrast, previous observations in *Pinus elliottii* showed only up-regulation of CYTs (Y. Zhang et al., 2023). CYTs play a crucial role in drought response by contributing to antioxidant activities and defense response in plants (Pandian et al., 2020; Tahmasebi et al., 2023). *CsCYT75B1*, a gene of *Citrus sinensis*, was associated with flavonoid metabolism and was highly expressed after drought stress, contributing to drought tolerance by elevating ROS scavenging activities (Rao et al., 2020). Due to the interaction of CYTs whose expression is induced with other key genes in response to water stress (Tahmasebi et al., 2023), the pivotal role of CYTs will require further investigation in white spruce and coniferous species.

HSPs and LEA proteins are chaperone proteins that protect cells from abiotic stress by stabilizing proteins and membranes under stress (Baldi & La Porta, 2022; Moran et al., 2017, p. 201). Drought-responsive genes coding for HSPs (10 DEGs) and Chaperone DnaJ-domain proteins (11 DEGs) were identified in our study. DnaJ proteins are the main co-chaperones

modulating the Hsp70 functions (Kampinga & Craig, 2010), and overexpression of the *VaDJI* gene coding for a DnaJ protein conferred ABA insensitivity and drought tolerance in transgenic tobacco (Gautam et al., 2023). In addition, the expression of 13 LEA genes (Table S2.5), including four up-regulated putative dehydrins (Table S2.10, GCAT3.3 genes) belonging to the LEA sub-group II (Y. Liu et al., 2017) was noted. *Pinaceae* dehydrin induction appears to occur after a certain period of drought (Moran et al., 2017; Perdiguero et al., 2012) which could indicate an increasing role of these genes in stress protection as the stress intensity rises. As previously observed in white spruce, we found that the expression of *PgDhn33, PgDhn35* and *PgDhn16* was strongly induced, while the expression of *PgDhn37* was repressed (Table S2.10). Interestingly, the expression of two key NLRs or NBS-LRRs (nucleotide-binding, leucine-rich-repeat) genes, known to play a central role in plant resilience to stress and linked to resistance pathogens in conifers (J.-J. Liu & Ekramoddoullah, 2007), were induced under drought conditions (GQ03714_K21, GQ03512_J05), as previously reported in white spruce (Table S2.10; (Van Ghelder et al., 2019).

3.4.6 Key transcription factors involved in the transcriptional control of drought-responsive genes

Several classes of transcription factors (TFs) including AP2/ERF, NAC, WRKY, MYB, and zinc finger homeodomain TFs were drought-responsive in our study, consistent with other reports in conifer species (Cobo-Simón et al., 2023; Du et al., 2018; H. Fox et al., 2018; W. Li et al., 2021). However, in *Arabidopsis thaliana*, NAC TFs were reported to be mainly up-regulated in response to drought (M. Wang et al., 2022). Interestingly, a drought-responsive gene annotated as CCCH-type zinc finger (GQ03707_G19) and a WRKY (GQ04107_D16) in our study were also reported as key genes involved in drought adaptation in white spruce (Depardieu et al., 2021). The two MYB sequences identified in this study had homologies with putative *Arabidopsis thaliana* proteins known to enhance protection against oxidative damage or to be involved in growth, phenylpropanoid biosynthesis, and the ABA signaling pathway (Agarwal et al., 2020; D. Lu et al., 2014; Wyrzykowska et al., 2022). Drought-induced AP2/ERF genes in our study were close homologs to ethylene responsive elements in other species (Kitajima et al., 2000), and to improve drought tolerance in conifers (J. Zhang et al., 2023). DREB subfamily genes within the AP2/ERF group, induced in response to drought stress, are known to activate downstream stress resistance genes and enhance plant drought

resistance independently of the ABA signaling pathway, as observed in *Arabidopsis thaliana* (Rehman & Mahmood, 2015). Finally, we observed contrasting expression patterns among WRKY members under drought conditions. Similar findings were reported in *Pinus massoniana*, where some WRKY genes responded to drought stress induced by exogenous ABA, resulting in improved drought tolerance in transgenic tobacco plants (Sun et al., 2022). Numerous zinc finger TFs were identified in white spruce (Table S2.8), and homologs found in the PlantTFDB database indicate a potential role in stomatal aperture, ROS production and drought tolerance (Ding et al., 2015; Hsu et al., 2014).

3.4.7 Intraspecific genetic variation in gene regulation under drought stress: findings and future avenues

Intraspecific genetic variation in drought response is crucial for selection and adaptation in tree populations faced with environmental change (Schueler et al., 2021). Recent studies in white spruce have highlighted the role of genetic variation among populations (Depardieu et al., 2020), as well as the genomic and transcriptomic basis for drought response and resilience (Depardieu et al., 2021; Stival Sena et al., 2018). In our study, differences among the three clones in DEGs underline most of the variance during water stress conditions. Only 21% of the up-regulated and none of the down-regulated genes were common to the three clones, suggesting that the gene network involved in drought response varies widely between genotypes. Alternatively, biological processes and metabolic functions of DEGs were highly similar between genotypes (Table S2.9). Dissimilar gene networks and similar metabolic pathways involved in water-stress response among genotypes were also observed for two clones of loblolly pine with opposite phenotypes for drought tolerance (W. Li et al., 2021). Our results are also congruent with those of the fir *Abies pinsapo* with contrasting gene expression patterns among post-drought phenotypes (Cobo-Simón et al., 2023). Future studies contrasting drought-induced responses between genotypes of various species of conifers and gymnosperms will likely help to appreciate the variance in key metabolic pathways underlying conifer drought responses and improve selection strategies to cope with climate changes. From a prospective standpoint, exploring transcriptome-wide expression within conifer species with diverse ecological preferences holds promise for unraveling the nuanced modulation of gene expression in response to drought. Also, it appears important to investigate responses of epigenetic nature,

which are likely to bear an important role in acclimation and adaptation to drought, in addition to modulation of transcriptome-wide expression (Moran et al., 2017).

3.5 Conclusion

Our study has provided new and valuable transcriptomic data for understanding how white spruce responds to water stress conditions. By conducting a transcriptomic analysis and monitoring white spruce over time during water stress, we have identified specific gene sets at different time points (days 0, 14, 18 and 22) that shed light on the response to drought. The genes we identified are involved in major known biological processes, including hormonal responses, photosynthesis, growth, cell wall organization, water transport, carbohydrate metabolism, and defense mechanisms, all of which are essential for drought tolerance and acclimation. Our results at the transcriptome level confirm the hypothesis that white spruce seedlings appear to prioritize maintaining their hydraulic balance over growth during a short drought. Furthermore, the results also highlight the ability of white spruce seedlings to activate defense mechanisms, such as antioxidant defenses and chaperone proteins, under such stress conditions. To further understand the drought response of this species, it is crucial to validate these transcriptomic observations using other omics approaches, such as proteomics and metabolomics. One particularly interesting finding is the significant regulation of lipid metabolism, a process that has not been extensively studied in conifers and requires further investigation. Given the role of lipids as energy reserves and precursors of defence compounds, future studies should focus on elucidating their contribution to tree responses to drought, by targeting the key molecular pathways involved during water stress through enzyme activity measurements and lipid profiling. We believe that future research should prioritize the identification and the comparison of key genes and mechanisms involved in drought response and post-drought recovery of plants. This information could be instrumental in shaping effective genetic selection strategies to enhance white spruce's resistance and resilience in the face of drought induced by climate change. Ultimately, this knowledge can inform forest management practices aimed at supporting conifer regeneration and growth in increasingly challenging dry conditions.

3.6 Supplementary data

3.6.1 Supplementary figures

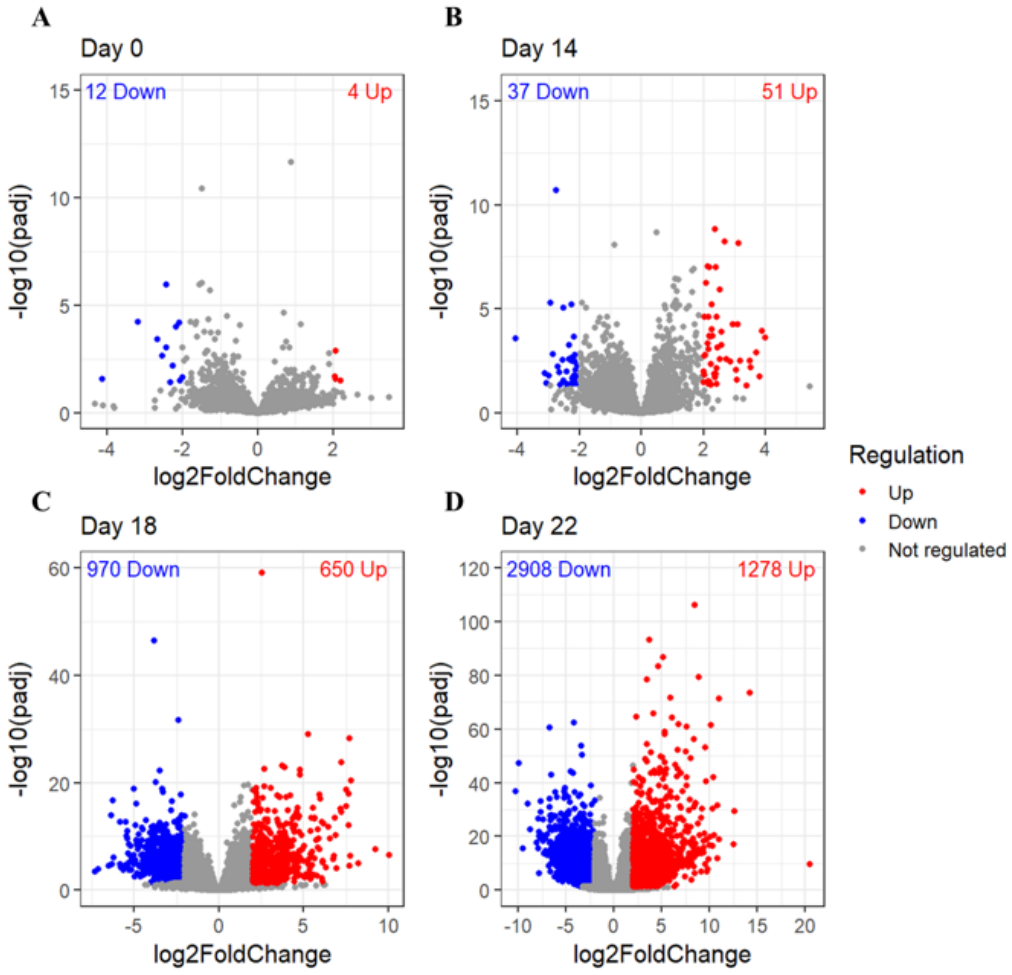

Figure S1.1. Volcano plots of differentially expressed genes (DEGs). Volcano plots of DEGs are presented for days 0, 14, 18 and 22 (A-D). Unigenes in blue are under-represented in stressed seedlings (FDR $\leqslant$ 0.05 and a log2FC $\leqslant$ -2), while transcripts in red are over-represented in stressed seedlings (FDR $\leqslant$ 0.05 and a log2FC $\geqslant$ 2). Unigenes in gray are not significantly different between the two groups.

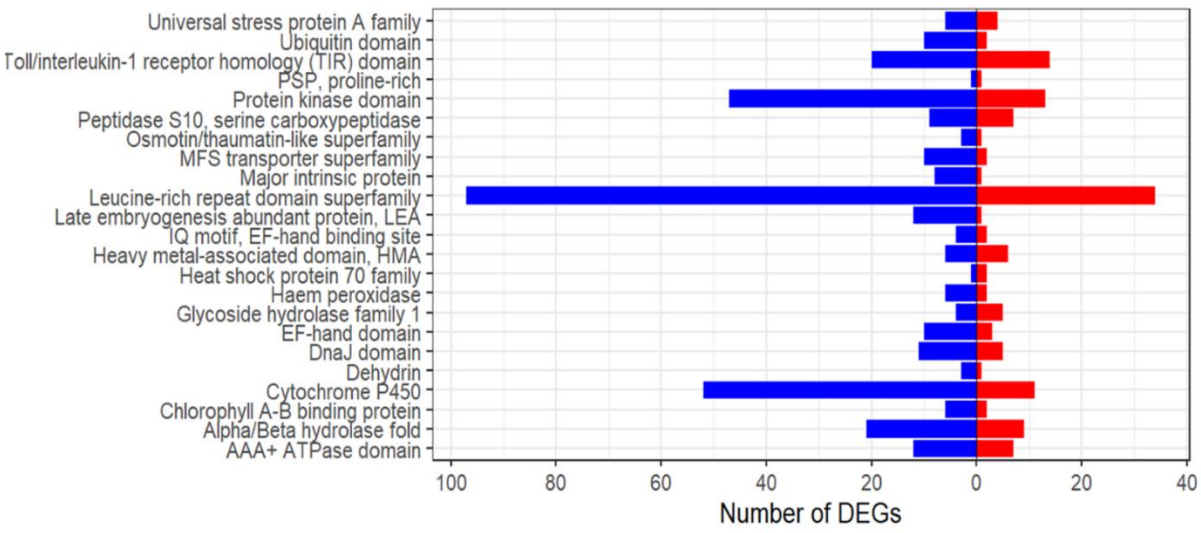

Figure S1.2. Major protein families involved in the drought response among the differentially expressed genes (DEGs) at day 22. The plot represents the number of up- and down-regulated DEGs at day 22 encoding selected protein families with known roles in plant drought response.

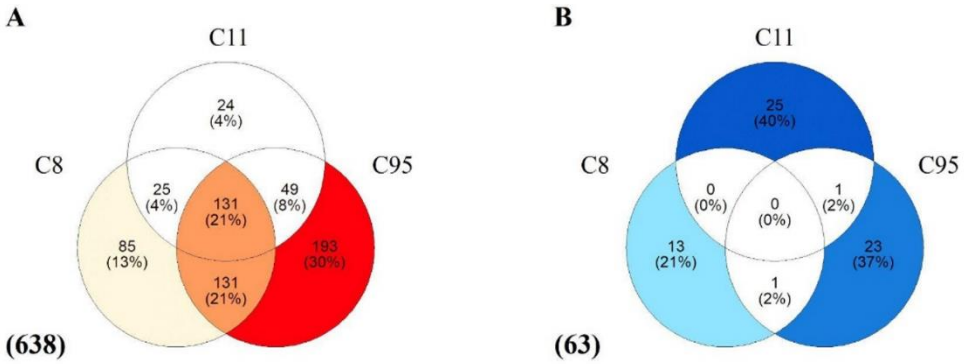

Figure S1.3. Unique and shared differentially expressed genes (DEGs) among clones. The Venn diagrams depict the overlaps of (A) up-regulated (red) and (B) down-regulated (blue) DEGs across the C8, C11, and C95 clones. The total number of DEGs are shown in bold brackets.

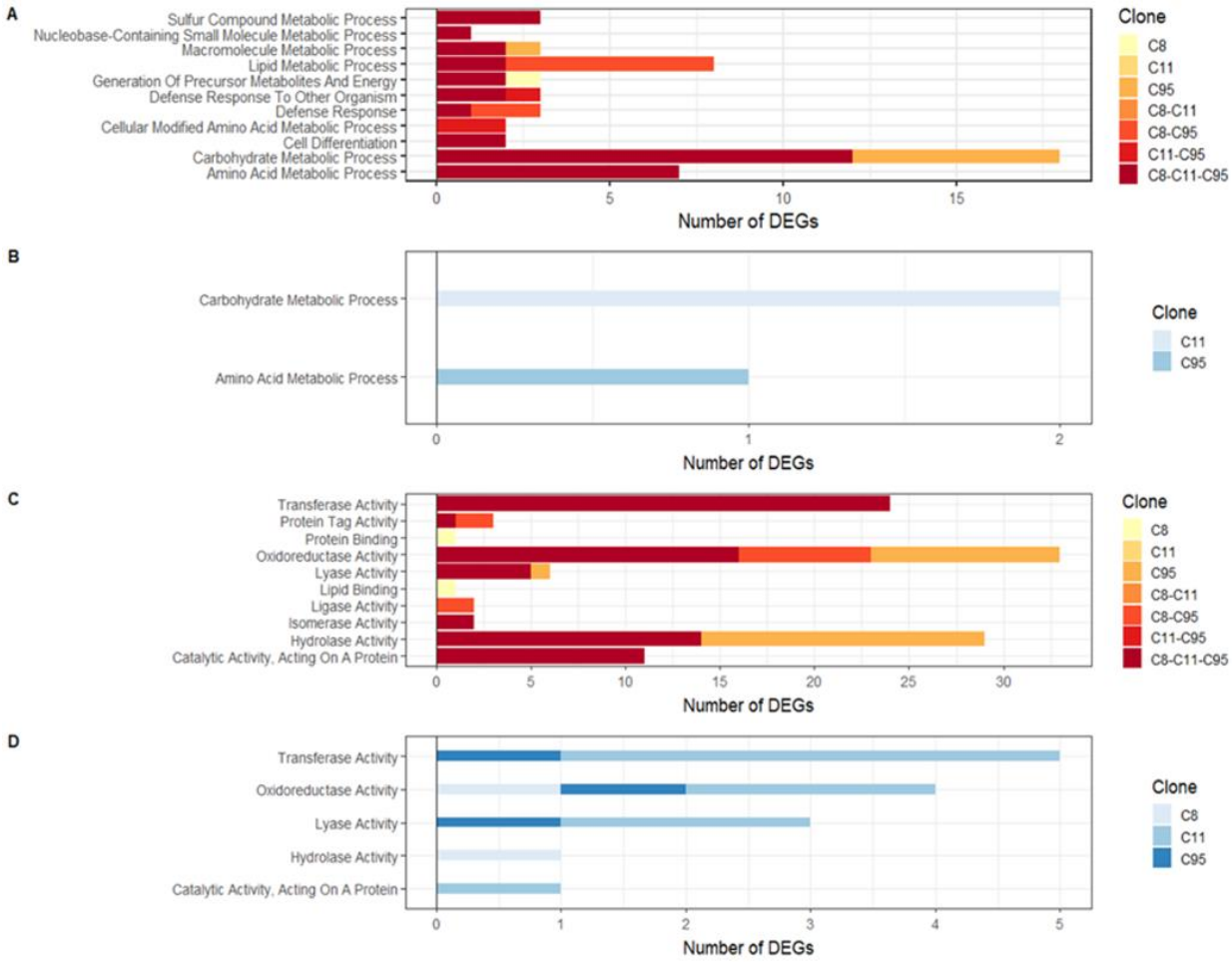

Figure S1.4. Gene Ontology (GO) annotation of unique and shared differentially expressed genes (DEGs) among clones. GO annotation of biological process (BP) of (A) up- and (B) down-regulated DEGs of clones and GO annotation of molecular functions (MF) of (C) up- and (D) down-regulated DEGs. Unique DEGs correspond to C8, C11 or C95 and the shared DEGs among clones correspond to C8-C11, C11-C95 and C8-C11-C95.

3.6.2. Supplementary tables

Table S2.1. Summary of plant material used for *de novo* transcriptome assembly and transcriptomic analyses.

Table S2.2. Statistics summary of the *de novo* transcriptome assembly GCAT 4.0.

Table S2.3. Complete functional annotation of the *de novo* transcriptome assembly GCAT 4.0 based on the OmicsBox analysis. The Excel file contains four sheets; Caption, S2.3.1 (Blast2GO annotation and BLASTX), S2.3.2 (BLASTN WS77111v2), S2.3.3 (BLASTN GCAT3.3).

Table S2.4. Summary of BLASTX results of the *de novo* transcriptome assembly GCAT 4.0. The table gathers the results of BLASTX expressed in percentage realized with different public databases. The refSeq_29sep22 database (NCBI) was used in Blast2GO pro suite for our analyses. The meaning of variables: DB: database; DB sequences (%): the percentage of matched sequence; DB unique sequences (%): the percentage of unique matched sequence; Unigenes (%): the percentage of matched unigene; Unique unigenes (%): the percentage of unique matched unigenes. The genome used is the reference genome of white spruce WS77111v2 (https://www.ncbi.nlm.nih.gov/datasets/genome/GCA_000966675.3).

Table S2.5. List of differentially expressed transcripts (DETs) as a function of time points and their corresponding genes (DEGs) under drought. The Excel file contains two sheets: Caption and S5 (results of DEG analysis and annotation according to time points).

Table S2.6. MapMan analyses performed on differentially expressed genes (DEGs) from all sampling days and on DEGs from day 22. The Excel file contains five sheets; Caption, S2.6.1 (export results of all sampling days), S2.6.2 (the main Bin categories of all sampling days), S2.6.3 (export results of day 22) and S2.6.4 (the main Bin categories of day 22).

Table S2.7. Gene Ontology (GO) enrichment of differentially expressed transcripts (DETs) as a function of time points under drought. The Excel file contains three sheets; Caption, S2.7.1 (GO enrichment according to time points) and S2.7.2 (selected GO enrichment according to time points).

Table S2.8. Transcription Factors (TFs) annotation of differentially expressed transcripts (DETs) and their corresponding genes (DEGs). The Excel file contains three sheets; Caption, S2.8.1 (TFs annotation among all transcripts) and S2.8.2 (TFs annotation among selected DEGs).

Table S2.9. List of differentially expressed transcripts (DETs) and Gene Ontology (GO) enrichment as a function of clones and their corresponding genes (DEGs) under drought. The Excel file contains three sheets; Caption, S2.9.1 (results of DEG analysis and annotation according to clones) and S2.9.2 (GO enrichment according to clones).

Table S2.10. Cross-checking information from previously published studies on differentially expressed transcripts (DETs) and their corresponding genes (DEGs). The Excel file contains two sheets; Caption and S2.10 (list of key drought-responsive genes previously observed in published studies).

3.6.2. Supplementary methods

Methods S1. Methodological details of experiences used for *de novo* transcriptome assembly.

Data availability statement: White spruce RNA-seq raw data sets from IDENT samples used for de novo transcriptome assembly have been deposited at National Center for Biotechnology Information (NCBI) under the project name PRJNA1078812 (SRA accession numbers: SAMN40018916 to SAMN40018931). The raw RNA-seq data from Stival Sena et al. (2018) have been deposited at National Center for Biotechnology Information (NCBI) under the project name PRJNA1200035. The *de novo* transcriptome assembly is available in the Dryad repository: https://doi.org/10.5061/dryad.bcc2fqzm4. The supplementary tables are available in Github repository: https://github.com/ZoeRibeyre/De-novo-transcriptome-assembly-and-discovery-of-drought-responsive-genes-in-white-spruce.git

Author contributions: Z. Ribeyre, C. Depardieu, J. Mackay and G. Parent designed the study and C. Messier contributed to the development of the IDENT plantation. Z. Ribeyre, C. Depardieu, J. Prunier, G. Parent and J. Mackay designed methods and carried out the experiments. Z. Ribeyre, J. Prunier, C. Depardieu and G. Pelletier performed the analyses and discussed the results. Z. Ribeyre and C. Depardieu wrote the manuscript with contributions and feedback from J. Prunier, J. Mackay, J. Bousquet, G. Parent, C. Messier, P. Nolet, G. Pelletier and A. Droit. All authors contributed to the article and approved the submitted version.

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Crédit photo : Zoé Ribeyre

4) CHAPITRE 4

INSIGHTS INTO DROUGHT RESPONSES: COMPARATIVE TRANSCRIPTOMICS OF SUGAR MAPLE AND WHITE SPRUCE IN SHORT-TERM AND LONG-TERM PERSPECTIVES

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Résumé

Pour élaborer des stratégies sylvicoles dans le but de préserver les forêts, il est nécessaire d'avoir une meilleure compréhension des réponses des arbres à la sécheresse. En écologie forestière, la plupart des études transcriptomiques se sont concentrées sur des conditions expérimentales contrôlées, avec notamment un stress hydrique à court terme instauré sur des semis d'arbres. Cependant, de telles conditions ne reflètent pas fidèlement la dynamique des écosystèmes naturels. Les études transcriptomiques comparatives entre les angiospermes et les gymnospermes permettent de mettre en avant les caractéristiques moléculaires communes et spécifiques aux deux taxons qui pourraient être exploitées pour améliorer la tolérance des arbres à la sécheresse. Cependant, les études transcriptomiques qui comparent les effets de la sécheresse à court et à long terme chez les angiospermes et les gymnospermes sont encore limitées. Pour combler cette lacune, nous avons analysé des données de séquençage de l'ARN provenant d'expériences de sécheresse à court terme en serre et d'une expérience en jardin commun à long terme sur l'érable à sucre (*Acer saccharum* (Marsh)) et l'épinette blanche (*Picea glauca* (Moench) Voss), deux espèces canadiennes importantes sur les plans écologique et économique. Nous avons utilisé des assemblages transcriptomiques *de novo* comme références génomiques pour analyser les changements transcriptomiques de ces espèces dans différentes conditions de sécheresse. L'assemblage *de novo* du transcriptome de l'érable à sucre réalisé dans le cadre de cette étude comprenait 23 702 transcrits (15 560 gènes uniques potentiels). L'analyse a révélé des stratégies d'acclimatation à la sécheresse distinctes pour ces deux espèces, avec toutefois quelques réponses moléculaires partagées comme une induction des processus de transport transmembranaire. L'érable à sucre a présenté des similitudes dans les réponses moléculaires entre la sécheresse à court et à long terme, mais avec des patrons de régulation très distincts. En revanche, l'épinette blanche présentait des réponses moléculaires plus variables entre les deux traitements hydriques. Des gènes potentiellement impliqués dans la réponse à la sécheresse chez les deux espèces ont été identifiés et constituent de bons candidats pour des études fonctionnelles. Cette étude met en évidence des différences dans les réponses moléculaires entre l'érable à sucre, un angiosperme, et l'épinette blanche, un gymnosperme, soumis à des conditions de stress hydriques similaires. Ces résultats ouvrent la voie à des recherches plus approfondies sur les bases génétiques de l'acclimatation à la sécheresse chez les arbres.

Mots-clés : Arbres forestiers, érable à sucre, épinette blanche, sécheresse, long terme transcriptomique, acclimatation, comparaison intra et interspécifique

Abstract

Understanding tree responses to drought is crucial for developing effective strategies to maintain forest health in the context of climate change. In forestry research, most transcriptomic studies have focused on short-term drought stress in tree seedlings under controlled conditions, which does not accurately capture the dynamics in natural settings. Comparative transcriptomic studies between gymnosperms and angiosperms may provide insights into common and species-specific molecular traits, which could be leveraged for silvicultural practices to improve drought tolerance. However, comparative studies analyzing transcriptomic responses to short- and long-term drought in angiosperms and gymnosperms are limited. To address this gap, we analyzed RNA-sequencing data from both short-term drought experiments in greenhouses and a long-term field experiment using sugar maple (*Acer saccharum* (Marsh)) and white spruce (*Picea glauca* (Moench) Voss), two ecologically and economically important Canadian tree species. We used *de novo* transcriptome assemblies as genomic references to analyze transcriptomic changes in these species at various developmental stages under different drought conditions. We developed a *de novo* assembly for sugar maple which included 23,702 transcripts (15,560 putative unique genes). Our analysis revealed distinct drought acclimation strategies for sugar maple and white spruce, with some overlapping molecular responses. Both species showed an induction of transmembrane transport processes under prolonged water stress. Sugar maple exhibited a more consistent response between short-term and long-term drought, with significant differences in the regulatory patterns. In contrast, the white spruce response was more variable between the two water treatments. We also identified promising candidate genes in both species that could be further studied for their role in drought response. This study underscores the differing molecular responses of sugar maple, an angiosperm, and white spruce, a gymnosperm, when subjected to similar water-stress conditions. These findings should pave the way for further research into the genetic basis of drought acclimation in trees.

Keywords: Forest trees, sugar maple, white spruce, drought stress, interspecific comparison, acclimation

4.1 Introduction

In recent decades, changes in precipitation patterns and an increase in severe droughts have significantly heightened tree mortality and reduced biomass productivity in Canadian forests (Bonsal et al., 2011; Hartmann et al., 2022; C. Peng et al., 2011). In this context, evaluating the sensitivity and acclimation potential of trees along with resilience to long-term variations in water availability hold considerable importance (Gessler et al., 2020; Kramer et al., 2020). Trees have a range of physiological and molecular mechanisms to cope with drought stress. Initially, they minimize water loss through stomatal control, which can be regulated by hormonal signals and vapour pressure deficit or actively mediated negative feedback responses of stomata to reduced leaf turgor (Rodriguez-Dominguez et al., 2016). In prolonged drought conditions, trees may rely on phenotypic plasticity to enhance their long-term survival, for example, by reducing photosynthetic and growth rates, strengthening cell walls, decreasing leaf size, and altering root development (Schönbeck et al., 2022; Zweifel et al., 2020).

The effects of drought vary between gymnosperm and angiosperm trees and may depend on the legacy of past disturbances and the severity and timing of the drought (Anderegg, Schwalm, et al., 2015; DeSoto et al., 2020; H. Li et al., 2021; X. Wu et al., 2022). Key differences between angiosperms and gymnosperms in response to drought include water balance control, carbon management, secondary metabolism, and defense mechanisms (Baldi & La Porta, 2022; Carnicer et al., 2013; Choat et al., 2018; D. M. Johnson et al., 2012). These physiological differences are partly attributable to xylem characteristics, such as non-structural carbohydrate content, parenchyma cell fraction, or sapwood capacitance (Carnicer et al., 2013; Michelot et al., 2012; Sala et al., 2012). Many conifers, the largest group of gymnosperm trees, rely on more conservative hydraulic strategies with a higher safety margin and reduced stomatal sensitivity to water stress compared to angiosperms, due to their ability to isolate embolized tracheids (Carnicer et al., 2013; Choat et al., 2018; Díaz-Sala et al., 2013; D. M. Johnson et al., 2012). Although many angiosperms are more susceptible to cavitation, they have efficient recovery mechanisms that support drought resilience (Brodersen & McElrone, 2013; Klein et al., 2018; Trifilò et al., 2019).

This physiological differentiation between angiosperms and gymnosperms has emerged following their evolutionary split at least 300 million years ago (Pavy et al., 2012). Conifers

feature large genomes spanning 18 to 35 Gb for Pinaceae, with abundant non coding DNA including transposable elements (De La Torre et al., 2014; Prunier et al., 2015). Widely distributed conifer species generally exhibit multigenic adaptation to local climates (Hornoy et al., 2015; Prunier et al., 2011) and considerable intraspecific genetic variation, enhancing their potential for drought resilience (Depardieu et al., 2020). In contrast, angiosperm trees generally possess much smaller genomes (less than 1 Gb), marked by frequent duplication events fostering high speciation rates and the evolution of functional traits that enhance their competitiveness, especially in their conductive system and defense mechanisms (De La Torre et al., 2020; Dodsworth et al., 2015). Despite these genomic differences, comparable numbers of gene and gene families, and high levels of coding gene sequence similarities (ranging from 58 to 61%) are reported across gymnosperms and angiosperm trees (Prunier et al., 2016; Rigault et al., 2011). Recently, shared adaptive genes have been identified between gymnosperms and angiosperms, indicating the potential occurrence of molecular-level adaptive convergence across broad taxonomic categories despite their ancient divergence (Pavy et al., 2023).

The two target species of our study are sugar maple (*Acer saccharum* [Marsh.]), a dicot angiosperm, and white spruce (*Picea glauca* [Moench] Voss), a gymnosperm from the coniferales, both of significant economic and ecological importance in Canada (Godman et al., 1990; Hassegawa et al., 2020; Mullin et al., 2011; Pitel & Yanai, 2014). They are both characterized as late successional tree species, relatively slow growing, and vulnerable to drought conditions (Hogg et al., 2017; Moreau et al., 2020; C. Peng et al., 2011; Putnam & Reich, 2017). Projections of future climate in Canada raise concerns of potential increased vulnerability to drought and potential shifts in the distribution of their respective populations (Aubin et al., 2018). With the rapid advancement of next-generation sequencing (NGS) technologies, sequencing woody species genomes has become more accessible. Genomic resources (Birol et al., 2013; Jackman et al., 2016; Warren, Keeling, et al., 2015) and transcriptomic data (Raherison et al., 2015; Ribeyre et al., 2025; Rigault et al., 2011) are well developed in white spruce. In sugar maple, an initial genome assembly was recently developed (McEvoy et al., 2022), transcriptome sequencing was used for genic microsatellite marker development (Harmon et al., 2017), and gene expression was studied in response to short-term water stress (Mulozi et al., 2023).

Understanding gene expression responses to both long-term (multiple years) and short-term (days to weeks) drought will shed light into the acclimation potential of trees and their resilience to water stress (Cobo-Simón et al., 2023; Robertson et al., 2022). Short-term changes in gene expression may provide insights into initial sensing and rapid response events, while long-term changes may unveil mechanisms of acclimation to drought with relevance to plant performance under stress (Cohen et al., 2010). However, to date, nearly all studies have focused on short-term responses in controlled environments contrasting well-watered and non-watered plants (Yao et al., 2021). In natural settings, trees gradually experience escalating drought stress, leading to varying physiological and transcriptional responses at different levels of water limitation. Furthermore, a comparative approach between sugar maple and white spruce may broaden our understanding by identifying common or group-specific genetic traits across tree species.

We developed a comparative investigation of transcriptomic expression profiles of sugar maple and white spruce subjected to six years of rain exclusion in a field experiment as well as short-term, greenhouse drought treatments. Our first objective, was to develop a *de novo* assembly of the sugar maple transcriptome similar to recent work in white spruce (Ribeyre et al., 2025). This allowed us to compare the transcriptional networks under short-term and long-term drought conditions by RNA-sequencing in sugar maple (Mulozi et al., 2023) and white spruce (Ribeyre et al., 2025) saplings. Our second objective was to identify drought responsive genes for the two species, exposed to both short- and long-term drought. Our third objective was to identify and compare the molecular pathways between the two species which are involved in putative acclimation processes.

4.2 Materials and methods

This study employs both intraspecific and interspecific comparative transcriptomic approaches to investigate the functions, key genes, and drought response strategies of sugar maple and white spruce under long- and short-term water stress (Figure 4.1).

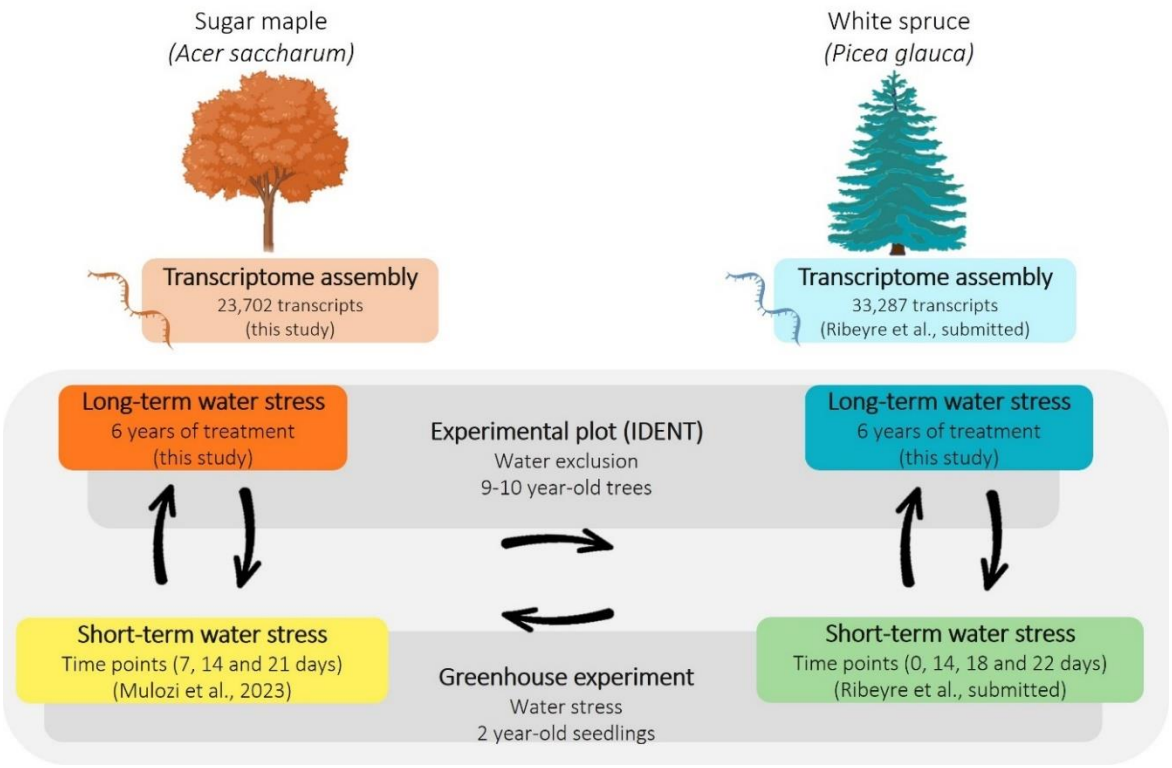

Figure 4.1. Experimental design overview. This study integrates data from four distinct datasets: two datasets derived from an unpublished long-term water exclusion field experiment involving sugar maple and white spruce, alongside two datasets sourced from previously published studies on sugar maple (Mulozi et al., 2023) and white spruce (Ribeyre et al., 2025), conducted under short-term greenhouse conditions. Arrows denote the comparison approach between species and experiments within this study. The color scheme utilized to differentiate species and experimental conditions will remain consistent throughout the article.

4.2.1 Experiments and data set origins

4.2.1.1 Long-term water exclusion

Experimental design and plant material

Sugar maple leaves and white spruce shoots were collected in September 2021 from an experimental site established in 2013 as part of the International Diversity Experiment Network

with Trees (IDENT) at the Ontario Forest Research Institute Nursery and Arboretum in Sault Ste. Marie, Ontario, Canada (46.546501°N, -84.45565°W, 220 m a.s.l.). Monoculture stands of sugar maple and white spruce were arranged in a randomized complete block design and divided into two water treatments initiated in spring 2014 (Belluau et al., 2021). Half of the plots were irrigated weekly from June to August and served as the control, while the remaining plots were subjected to rain exclusion. Sampling was conducted within four irrigated and four rain exclusion blocks per species. Leaf and needle samples were collected from three randomly selected trees per plot, for a total of ten samples per water treatment for each species. Sampling was conducted between 10 am and 12 noon. Foliage samples were immediately frozen in liquid nitrogen after removal and stored at -70°C until RNA extraction.

RNA extraction, RNA-seq libraries synthesis and sequencing

RNA was extracted from foliar samples by finely grinding 15 to 20 mg of tissue in liquid nitrogen. The extraction was performed using the ReliaPrep™ RNA Tissue Miniprep System from Promega, which utilizes guanidine thiocyanate (GTC) and 1-Thioglycerol. The total RNA concentration was determined using a Thermo Scientific™ NanoDrop™ OneC (http://www.nanodrop.com/support) and then stored at -70°C. Ten samples per treatment were selected for sugar maple, and eight samples per treatment were chosen for white spruce for RNA sequencing. The quality assessment, RNA-seq library preparation, and sequencing were conducted at the Genome Quebec Innovation Center, located at McGill University in Montreal, Quebec, Canada. Sequencing was carried out using an Illumina NovaSeq6000 S4 Sequencing platform (Pair End, 2x100 pb).

Morphological measurements

Height (H) and basal diameter at 1 cm above ground (BD) of trees were measured in 2016, 2018 and 2020. We sampled three maple sugar leaves and two upper segments of white spruce current-year shoots from the same trees sampled for RNA extraction to determine the leaf and needle size and the specific leaf area (SLA). During sample selection we ensured that the selected leaves and needles were fully expanded and well illuminated, without significant herbivore or pathogen damage. Upon arrival at the laboratory, samples were stored in a dark, cool environment. After oven drying at 48°C for 72 hours, three leaves per sugar maple and 20 needles per white spruce were weighed to determine dry mass. The leaf and needle surface areas

were assessed using WinFolia and WinSeedle software, respectively. Subsequently, SLA was computed as the leaf area divided by the leaf dry mass (Garnier et al., 2001). A multifactorial repeated measures ANOVA followed by a Bonferroni post hoc multiple comparison test was used to test the effect of water treatment (T), species (S), and year (Y) and interactions on H and BD. We verified that the trees sampled were representative of all trees in the experimental stand (Figure S2.1). A multifactorial ANOVA was performed to test the effect of water treatment and species on leaf and needle size and SLA. Statistical analyses were performed with the 4.3.0 version of the R software.

4.2.1.2 Short-term water stress: data features

The RNA-seq data used to assess the effects of short-term water stress on potted sugar maple and white spruce came from two separate greenhouse experiments. The experiment on sugar maple seedlings, as described by Mulozi et al. (2023), involved subjecting the seedlings to 21 days of water stress, with four samples collected for both control and stress conditions at 7, 14, and 21 days of treatment (n=24). The experiment on white spruce seedlings, as described by Stival Sena et al. (2018), involved subjecting the seedlings to 22 days of water stress. Six samples were collected for both control and stress conditions at 0, 14, 18, and 22 days of treatment (n=48). RNA was extracted and sequenced from foliar samples (Figure 4.1, Table S3.1).

4.2.2 Generation and annotation of the sugar maple transcriptome assembly

4.2.2.1 Plant material and de novo assembly

The *de novo* assembly of the sugar maple transcriptome was built on leaf RNA-seq data from ten seedlings for each water treatment from the IDENT experiment, and further incorporated a transcriptome assembly obtained from adult trees growing in uncontrolled conditions (Harmon et al., 2017) (Table S3.1). RNA-seq raw sequence quality was assessed using FASTQC v0.11.9 (Andrews, 2017). Then, Trimmomatics 0.39 (A. M. Bolger et al., 2014) was used to clean the raw reads from nucleotides with low quality sequencing and residual adaptor sequences. The resulting clean reads shorter than 30 bp were then filtered out. High-quality reads were independently assembled into a transcriptome for each sample using the SGA (Simpson & Durbin, 2012) and IDBA-UD assemblers (Y. Peng et al., 2012) implemented in the a5 pipeline

(Coil et al., 2015). Individual transcriptomes were all scaffolded within one consensus transcriptome using LINKS 1.8.6 (Warren, Keeling, et al., 2015). Using the same tool, a final refinement incorporated scaffolds from a previously published transcriptome assembly (Harmon et al., 2017). Sequences shorter than 500 bp were excluded from this last assembly build due to their presumed lack of coding potential for functional proteins. The evaluation of the completeness of the *de novo* assembly was performed using BUSCO (Benchmarking Universal Single-Copy Orthologs) v5.4.3 with the -m transcriptome option and sequence comparison with the *Embryophyta* and *Viridiplantae* reference databases (odb10) (Manni et al., 2021). The TRAPID web server and the PLAZA version 4.5 database (Bucchini et al., 2021) were used for the analysis of the number of open reading frames (ORFs) and other statistics (Table 1).

Table 1. Quality assessment and statistics of the *de novo* transcriptome assembly of sugar maple. Tables summarizing (A) the quality assessment conducted by BUSCO analysis using the Viridiplantae database (Viridiplantae_odb10) and the Embryophyta database (Embryophyta_odb10), and (B) statistics of the sugar maple *de novo* transcriptome assembly generated in this study.

A)

Database	Complete and Single	Complete and Duplicated	Fragmented	Missing
Embryophyta	86.1%	1.4%	6.6%	5.9%
Viridiplantae	90.4%	0.9%	6.1%	2.6%

B)

Assembly statistics	
Total assembly length	35,928,678
Number of sequences	23,702
Mean sequence length	1,515 bp
Median sequence length	1,153 bp
N50	1,888 bp
L50	5,821
Sequences with ORF	23,431
Average ORF length	302 bp
Percent of homologous sequences (genome)	85.31
Number of putative unigenes	15,560
Percent of annotated sequences (BLASTx)	89.74
Percent of annotated sequences (GO classification)	67.3

4.2.2.2 Functional annotation of the transcriptome assembly

The functional annotations of the sugar maple *de novo* transcriptome assembly were obtained by sequence similarity searches using BLASTx analysis with OmicsBox software (Götz et al., 2008) against the NCBI Refseq database (accessed September 29, 2022) with an E-value threshold of ≤10^-5. Protein signatures were identified through homologous protein domain searches within translated sequences via the Interpro database using OmicsBox software. Gene

ontology (GO) annotations were assigned to each transcript using the same software (Table S3.2). Furthermore, BLASTx analyses against public databases, including *Viridiplantae*, Swissprot and PlantTFDB, databases from PLAZA 5.0 (Van Bel et al., 2022), were conducted for homologous sequence identification and sugar maple transcriptome annotation (Table S3.3). DIAMOND-aligner version 2.0.14 (Buchfink et al., 2021) was employed for BLAST analyses, configured in "sensitive" mode with parameters set to k-1, b1.2, and an E-value threshold of $\leq 10^{\wedge -5}$. Additionally, sequence homology between the transcriptome assembly and the sugar maple coding DNA sequence genome (available at https://treegenesdb.org/org/Acer-saccharum), was determined through BLASTn analysis with an E-value threshold of $10^{\wedge -5}$ (Table S3.3).

4.2.3 Transcriptomic analyses in context of long-term and short-term water stress

4.2.3.1 Differential expression analyses

Differential expression analyses between the stressed and control trees were performed using RNA-seq high-quality reads from the long-term IDENT experiment in both sugar maple and white spruce. In addition, differential expression analyses were performed on raw RNA-seq data retrieved from the short-term water stress experiments of the two species to compare the gene expression profiles of the stressed and control seedlings. For the differential expression analyses, high-quality reads were pseudo-aligned to the sugar maple *de novo* assembly of this study and the white spruce assembly (Ribeyre et al., 2025) using Kallisto v0.48.0 (Bray et al., 2016). Read counts were normalized and the differential analyses were performed using the R package DESeq2 (Love et al., 2014). The DESeq-normalized expression values were used to compute the log2 fold change (LFC) between stressed and unstressed conditions for each transcript. Significant differentially expressed transcripts (DETs) were identified by applying a likelihood ratio test (LRT) approach with a false discovery rate (FDR) correction (Love et al., 2014). DETs were considered significantly differentially expressed if they met the criteria of an FDR threshold of 0.05 and an LFC of 1. Sequence homology of long- and short-term experiment transcripts was determined by BLASTn analysis performed against the sugar maple coding DNA sequence genome (see material and methods, Table S3.3) and the latest white spruce reference genome publicly available on NCBI (WS77111v2, Accessed on July 2022).

4.2.3.2 Gene ontology functional analyses

To distinguish between unique or co-expressed differentially expressed transcripts (DETs) and their regulation across the four experiments (Figure 4.1), we employed ggVennDiagram (v1.2.2 R) (C.-H. Gao et al., 2021), and Venndetail (v1.16.0 R) (K. Guo, 2018/2019) packages. Gene ontology (GO) functional annotation was conducted on the lists of unique and co-expressed DETs for each species, using OmicsBox (Götz et al., 2008). In this study, we have developed a rigorous computational method to compare functional annotations based on GO terms across species and under experimental drought conditions, and to determine the predominant regulatory pattern of DETs associated with specific GO classes (e.g., primarily up-regulated, down-regulated, or equally up- and down-regulated). First, for each species and experimental condition, we normalized the count of DETs that are up- and down-regulated, or regulated in both directions for each identified GO term (at levels 2, 3, and 4) against the total number of DETs:

$$(1) \qquad P_{ij} = n_{ij}/N_{tot}$$

where P_{ij} represents the normalized DET number for the GO class of interest *i*, where *j* is the observed regulation type for each DET. For instance, *j* could refer to whether a DET is down-regulated, up-regulated, or both down- and up-regulated. The variable n_{ij} signifies the number of DETs associated with the GO class *i* for the regulation type *j*. N_{tot} corresponds to the total number of transcript sequences, considering all types of regulations. Second, we aimed to determine the principal pattern of transcript regulation for each GO class. To achieve this, we introduced a regulation scoring mechanism for each P_i, contingent upon the specific regulation type *j*. DETs that showed up-regulation were given a multiplier of 1 for P_i. Conversely, DETs displaying down-regulation were given a multiplier of -1. In cases where DETs were regulated in both directions, P_i was multiplied by 10^{-10}, a number close to zero chosen to center the desired scale of variation within the specific context of our study. We then calculated the total sum of the products of the normalized numbers of DETs associated with each GO class and their respective regulation scores using the formula:

$$(2) \qquad SP_{\mathrm{i}} = \sum (P_{\mathrm{iup}}\, x\, 1 + P_{\mathrm{idown}}\, x\, (-1) + P_{\mathrm{iupdown}}\, x\, 10^{-10})$$

where SP_i represents the final normalized score providing information on the predominantly observed regulatory pattern for a given GO class *i*. P_{iup} signifies the normalized DET number for the GO class of interest i for up-regulated DETs, P_{idown} represents the normalized DET number for the GO class of interest i for down-regulated DETs, and $P_{iupdown}$ represents the normalized DET number for the GO class of interest i for DETs that were regulated in both directions. In practical terms, if the value of SP_i is positive, it indicates that for the studied GO class, the majority of DET expression is induced in response to stress. The larger the proportion of up-regulated DETs for the GO class of interest, the greater the positive value will be. Conversely, a negative value of SP_i will indicate a majority of down-regulated DETs for a given GO class, with increasingly negative values for a growing proportion of DETs whose expression is reduced under drought conditions (Table S3.4).

4.3 RESULTS

4.3.1 Characteristics and quality assessment of the sugar maple transcriptome assembly

In this study, we produced a *de novo* assembly of the sugar maple transcriptome, and the BUSCO analyses performed using the *Embryophyta* and *Viridiplantae* databases indicated that 86.1-90.4% of the transcriptome sequences are complete and single, 0.9-1.4% were complete and duplicated, 6.1-6.6% were fragmented, and 2.6-5.9% were missing (Table 1A). The transcriptome assembly comprises 23,702 sequences with a median length of 1,153 bp. 23,431 potential ORFs with an average length of 302 bp were detected using TRAPID (Bucchini et al., 2021). BLASTn analysis performed against the reference genome of sugar maple revealed a sequence homology of 85.31% and 15,560 putative unigenes. 89.74% of sequences were successfully annotated by BLASTx analysis using the NCBI *Viridiplantae* database, while 67.3% of sequences were successfully annotated with GO terms (Table 1B, Table S3.3).

4.3.2 Morphological responses to long-term water stress in sugar maple and white spruce

Radial growth, as indicated by basal diameter (BD), was significantly influenced by treatment, species, and the year of measurement (Figure 4.2A). Significant interactions were observed between treatment and year ($p = 0.017$) and between species and year ($p < 0.01$). The Bonferroni post-hoc analysis revealed that sugar maple BD is influenced by the interplay between treatment

and year, whereas white spruce, in contrast, does not demonstrate such dependency (Figure 4.2A, Table S3.5). Height was also strongly affected by treatment, species, and the year of measurement, with significant interactions between treatment, species, and year (Figure 2B). The post-hoc analysis showed that the height of both sugar maple and white spruce was negatively impacted by the low water treatment (Figure 4.2B, Table S3.5). The area of sugar maple leaves was impacted by the treatment, while the area of white spruce needles was unaffected after 6 years of treatment (Figure 4.2C). Furthermore, the specific leaf area (SLA) remained unchanged in both species under drought conditions (Figure 4.2D, Table S3.5).

4.3.3 Key gene ontology classes in sugar maple: long-term versus short-term water stress

During long-term water exclusion, sugar maple trees exhibited 618 DETs (386 unigenes), while under short-term water stress, sugar maple seedlings displayed 4,379 DETs (3,606 unigenes) (Figure 4.3A; Table S3.6). Out of the 618 DETs detected, 514 transcripts were exclusively expressed in long-term drought conditions, with 64.0% and 36.0% being up- and down-regulated, respectively. Of 104 total DETs identified as co-expressed between long-term and short-term conditions, 20.19% were found to be up-regulated, 45.19% were down-regulated, and 34.62% were regulated either up or down depending on the experiment. 4,275 DETs were exclusively identified under short-term water stress, with 51.11% being up-regulated, 48.84% down-regulated, and 0.02% having regulation dependent on sampling time (Figure 4.3A).

We successfully assigned GO terms to 55.5% and 71.0% of long- and short-term DETs, respectively, were successfully assigned to GO terms (Tables S3.4 and S3.6). A consistent similarity in major biological processes (BPs) and molecular functions (MFs) was observed across GO classes at levels 2, 3, and 4, spanning categories unique to both long-term and short-term drought responses, as well as those co-expressed (Figure 4.3B-C). Notably, DETs associated with BP terms related to localization, transmembrane transport, cellular component organization, or biogenesis, along with the MF term transporter activity, showed a predominant up-regulation pattern in the long-term. Conversely, DETs annotated for BPs such as response to stress, cellular response to stimulus, and cell communication also showed a contrasting regulatory trend. These DETs were primarily down-regulated in the long term, while they showed both up- and down-regulation in the short term, with a tendency towards up-regulation.

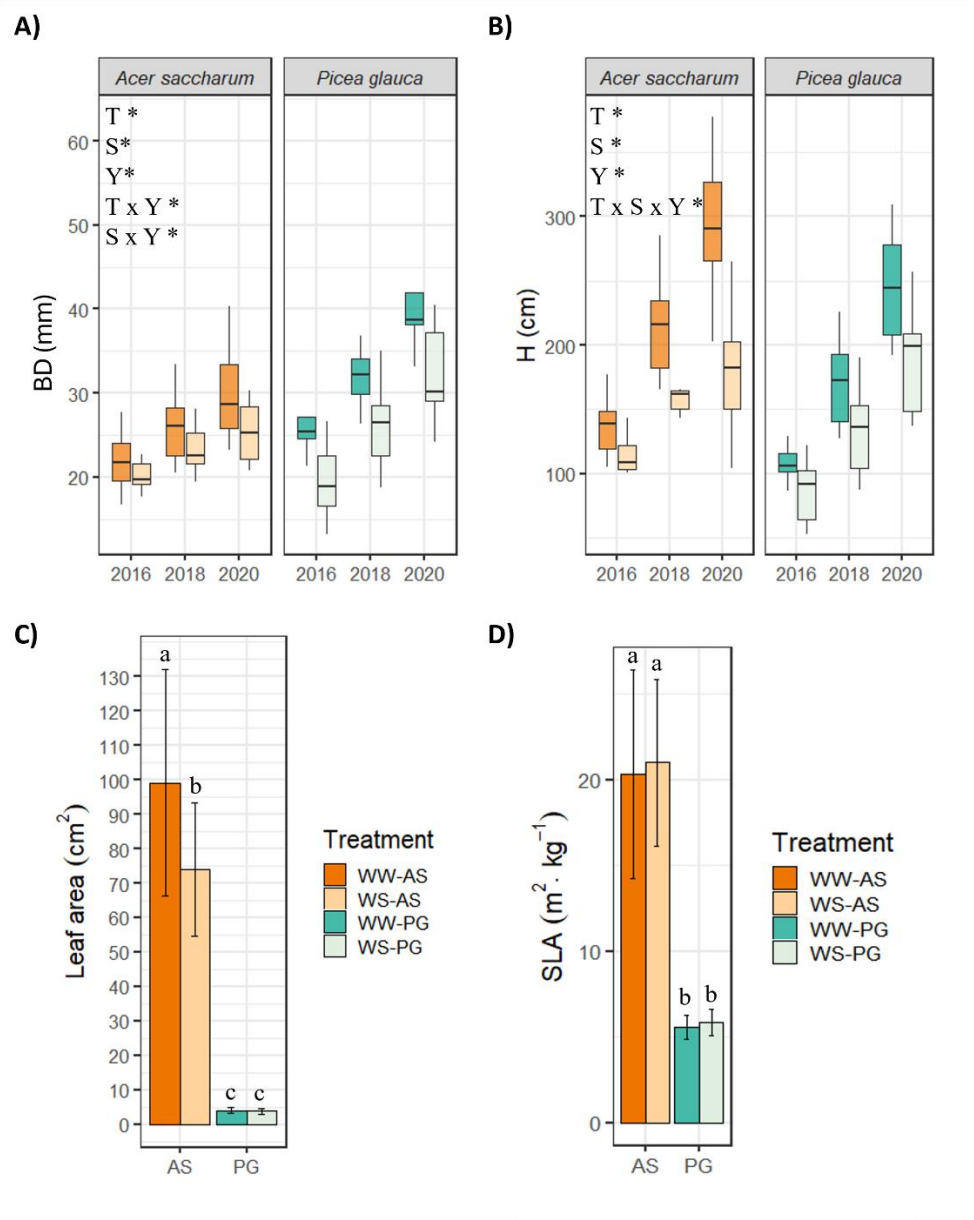

Figure 4.2. Morphological measurements of trees under long-term water exclusion. The diameter at breast height (BD, in mm), height (H, in cm), leaf area (in cm²), and specific leaf area (SLA, in $m^2.kg^{-1}$) measurements presented in this Figure are derived from trees that were studied for long-term drought transcriptomic data. BD (A) and H (B) were measured in 2016, 2018, and 2020 on *Acer saccharum* (AS) and *Picea glauca* (PG) subjected to summer irrigation (well-watered, WW) or water exclusion (water stress, WS) since 2014. Leaf area (C) and SLA (D) as a function of species and treatment (well-watered, WW and water stress, WS) were measured for individuals sampled in 2021. Results of multifactorial repeated measures ANOVA are indicated in panels (A) and (B): T = water treatment, S = species, Y = year, X = interaction, * = significant difference ($p < 0.05$). Results of multifactorial ANOVA are indicated in panels (C) and (D) by letters ($p < 0.05$).

Despite the similar BP and MF profiles identified for co-expressed DETs under both long-term and short-term water stress, the regulatory patterns of some specific GO terms differed substantially (Figure 4.3C). The DETs identified as both long and short term predominantly exhibit a pattern of either downward or contrasting regulation in the two experiments (Figure 4.3C).

4.3.4 Key gene ontology classes in white spruce: contrasting long-term and short-term responses

In white spruce, an even larger difference was found between short- (12,188 DETs, 8,181 unigenes) and long-term water stress (88 DETs, 80 unigenes). The Venn diagram highlighted that 35 of the 88 DETs (11 up-regulated and 24 down-regulated) were exclusively expressed in the long-term experiment, while 12,135 DETs out of 12,188 were exclusively expressed in the short-term experiments (with 3,866 up-regulated, 8,230 down-regulated, and 39 showing both regulations dependent on sampling time) (Figure 4.4A).

We successfully assigned GO terms to 48.9% and 46.7% of DETs under long-term and short-term water stress, respectively (Table S3.6). Despite some similarities in the major biological process (BP) and molecular function (MF) GO classes at levels 2, 3, and 4 among DETs uniquely identified in both experiments, we observed a greater variety of GO terms in the short-term context (Figure 4B).

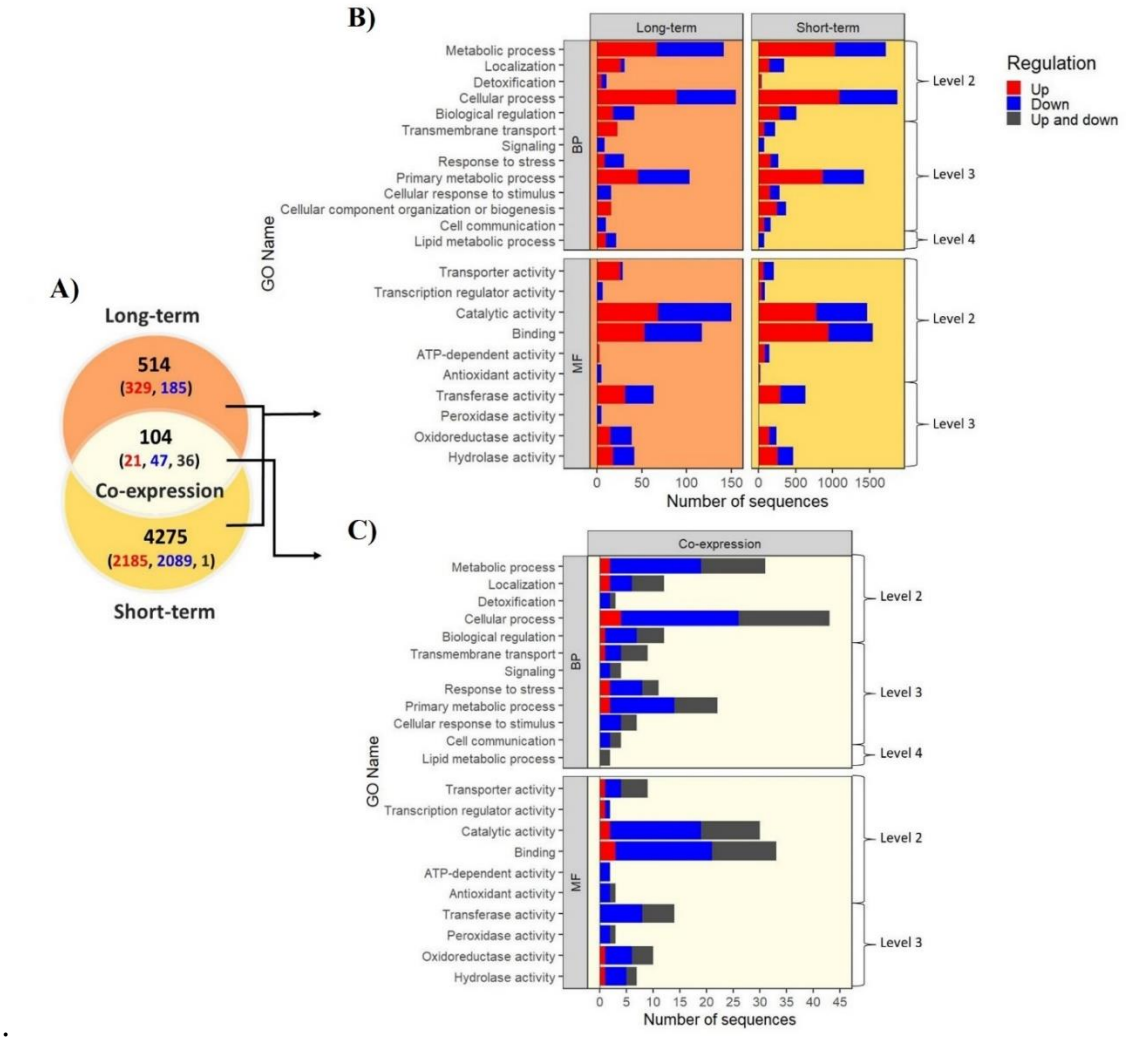

Figure 4.3. Key biological processes and molecular functions related to drought-responsive genes in sugar maple subjected to short-term or long-term water stress. (A) Venn diagram showing the differentially expressed transcripts (DETs) during the long-term and short-term water stress experiments, as well as the DETs co-expressed by the two experiments (Co-expression). The total number of DETs is shown in bold black, and the numbers of up- and down-regulated DETs are shown in red and blue, respectively. The number of DETs both up- and down-regulating is shown in grey. Histograms grouping the main gene ontology (GO) annotations by level 2, 3, and 4 of biological processes (BP) and molecular functions (MF) of (B) DETs identified in the long-term or short-term water stress experiment and (C) co-expressed DETs of the two experiments. The histogram is expressed as a function of the number of sequences of DETs. The upregulated and downregulated transcripts are indicated in red and blue, respectively, and the DETs that are regulated positively or negatively according to the different stress time points studied in the context of the short-term experiment (B) or according to the two experiments (C) are represented in gray.

BPs related to energy metabolism (such as sugar and lipid metabolism), stress response, photosynthesis, signaling, and cell wall organization or biogenesis were uniquely associated with short-term DETs. Among the shared processes and functions, a distinct regulatory pattern emerged for certain GO terms. Specifically, DETs annotated with BPs such as localization and transmembrane transport, along with MFs related to transporter activity, showed exclusive up-regulation in the long-term, whereas short-term associated DETs exhibited a mixed pattern of both up- and down-regulation. Moreover, MFs related to ATP-dependent activities, transferase, and oxidoreductase activities were uniquely linked to down-regulated DETs in the long-term, showing a more diverse regulation in the short-term (Figure 4.4B). Co-expressed DETs demonstrated a prevailing pattern of contrasting regulatory trends between the two experimental setups (Figure 4.4C). Among the GO classes, six contain down-regulated DETs, including developmental process, cellular process, transferase activity, oxidoreductase activity and hydrolase activity, which exclusively comprise down-regulated DETs.

4.3.5 Identifying key drought-responsive genes: insights from long- and short-term transcriptomic studies

Some genes were found to have significant regulation changes both in the long- and short- term drought, suggesting they may play a major role in complex regulatory networks under water-limited conditions. When considering the DETs resulting from both types of drought experiments for each species, a total of 89 unigenes (104 DETs) in sugar maple (Figure 4.5A) and 53 unigenes (53 DETs) in white spruce (Figure 4.5B) can be designated as candidate key genes. Among these candidate key genes, we identified 28 potential transcription factors (TFs) in sugar maple (Table 2) and 19 potential TFs in white spruce (Table 3) belonging to ERF, MYB, bHLH, zinc finger, FAR1, NAC, B3, Dof, WRYK, NF-YC, NF-YA, and HD-ZIP classes. Other key genes were found to be involved in stress response, transport, photosynthetic metabolism, carbohydrate metabolism, and catalytic activity (Tables 2 and 3).

Table 2. Putative key genes of sugar maple identified from shared differentially expressed genes between long- and short-term experiments. The gene IDs correspond to the gene identifiers of the sugar maple coding DNA sequence genome. Highly relevant genes induced in both experimental conditions are in bold.

Function	Putative genes	Gene ID	Regulation	
			Long-term	**Short-term**
Transcription factor	ERF	ACSA_06709	down	down
		ACSA_03474	down	down
		ACSA_04146	down	down
		ACSA_10532	up	down
		ACSA_12006	down	down
	B3	ACSA_13101	up	down
	bHLH	ACSA_11199	down	down
		ACSA_13718	down	down
		ACSA_12765	**up**	**up**
		ACSA_01728	up	down
		ACSA_15608	down	up
	FAR1	**ACSA_13557**	**up**	**up**
		ACSA_09983	down	up
		ACSA_04955	down	down
		ACSA_11208	down	down
	NAC	ACSA_05333	down	up
		ACSA_00563	up	down
	MYB	ACSA_13410	up	down
		ACSA_02807	up	down
		ACSA_14156	down	down
	MYB-related	ACSA_11101	up	down
		ACSA_11043	down	down
	NF-YA	**ACSA_11397**	**up**	**up**
		ACSA_11672	**up**	**up**
	Zinc finger	ACSA_39966	up	down
		ACSA_38165	down	down
		ACSA_34109	down	updown
		ACSA_01344	down	up
Transport	ABC transporter	ACSA_38634	down	down
		ACSA_19491	down	down
	Amino acid transporter	ACSA_22515	up	down
	MFS transporter	ACSA_24227	up	down
		ACSA_35016	down	down
		ACSA_03113	up	down
	WAT1-related protein	**ACSA_23663**	**up**	**up**
Response to stress	Peroxidase P7	ACSA_21018	down	down
		ACSA_16985	down	down
	Osmotin/thaumatin-like	**ACSA_14571**	**up**	**up**
	Cytochrome P450	ACSA_24824	down	down
		ACSA_24143	down	up
Photosynthesis	PsbP	ACSA_35985	down	down
	Haem oxygenase-like	ACSA_18520	up	down

Table 3. Putative key genes of white spruce identified from shared differentially expressed genes between long- and short-term experiments. The gene IDs correspond to the gene identifiers of the two white spruce genomes GCAT3.3 and WS77111v2. Highly relevant genes induced in both experimental conditions are in bold.

Function	Putative genes	Gene ID		Regulation	
		WS77111v2	**GCAT3.3**	**Long-term**	**Short-term**
Transcription factor	MYB-related	JZKD02S1614953.1	GQ04002_K05.1	up	down
		JZKD02S1270654.1	GQ03103_G21.1	down	down
		JZKD02S0336860.1	GQ02816_I07.1	down	down
		JZKD02S1164445.1	GQ03009_O18.1	down	up
	NAC	JZKD02S0498183.1	GQ03239_A01.2	down	down
		JZKD02S0009249.1	GQ03410_L17.3	down	down
		JZKD02S1432885.1	GQ03704_E09.1	up	down
	bHLH	JZKD02S0082054.1	GQ03011_B21.1	down	down
		JZKD02S0861591.1	GQ03717_C20.1	up	down
		JZKD02S0645113.1	GQ03607_K13.1	down	down
	Dof	JZKD02S1248791.1	GQ03713_M01.1	down	down
		JZKD02S0727291.1	GQ03713_M01.1	down	down
	B3	JZKD02S0129744.1	GQ03801_F22.2	down	down
		JZKD02S1308418.1	WS00728_F17.1	down	down
	ERF	JZKD02S1332693.1	GQ03719_B03.1	up	down
	FAR1	JZKD02S0223815.1	GQ04006_M16.1	down	down
	HD-ZIP	JZKD02S1213105.1	GQ03113_I23.1	down	down
	NF-YC	JZKD02S1642449.1	GQ03201_J21.1	down	down
	WRKY	JZKD02S0538852.1	WS0073_J02.1	up	down
Response to stress	Jacalin-like lectin domain	JZKD02S0080109.1	GQ03405_C23.1	up	down
	Polyketide synthase, type III	JZKD02S0027673.1	GQ0207_H16.1	up	down
	Plant EC metallothionein-like	JZKD02S1316431.1	GQ02510_E13.1	down	up
		JZKD02S1386491.1	**GQ02510_E13.1**	**up**	**up**
	S-adenosylmethionine decarboxylase	JZKD02S1033128.1	GQ03212_B13.3	up	down
	DMR6-like oxygenase 2-like	**JZKD02S0813268.1**	**GQ03116_B03.1**	**up**	**up**
Transport	Proline	JZKD02S1634530.1	GQ03418_B03.1	up	down
	Amino acid/polyamine transporter I	JZKD02S1489867.1	NA	up	down
Catalytic activity	Leucine rich repeat	JZKD02S0799405.1	WS0014_F02.1	down	down
		JZKD02S0941915.1	GQ02809_D01.1	down	down
		JZKD02S0430656.1	GQ02010_I02.1	down	down

Carbohydrate metabolim	Carbohydrate binding module family 20	JZKD02S1302632.1	GQ03207_I05.1	up	down

Gene expression data from short- and long-term stress revealed four distinct regulation profiles (Figure 4.5). One profile involves genes consistently down-regulated under drought conditions, indicating potential involvement in mitigating stress effects (39 in sugar maple, Figure 4.5A; 25 in white spruce, Figure 4.5B). 18 genes in both sugar maple (Figure 4.5A) and white spruce (Figure 5B) were down-regulated in response to short-term water stress, but showed long-term up-regulation, suggesting they play a role in maintaining or restoring normal cellular functions despite ongoing water stress. Lastly, 16 genes in sugar maple (Figure 4.5A) and two genes in white spruce (Figure 4.5B) were induced in both the short and long term and may be designated as genes of primary interest. This includes six genes in sugar maple, such as one osmotin-thaumatin-like protein (ACSA_14571), one WAT1-related protein (ACSA_23663), one FAR1 TF (ACSA_13557), one bHLH TF (ACSA_12765) and two NF-YA TFs (ACSA_11397; ACSA_11672) (Table 2). In addition, one putative metallothionein-like S protein (JZKD02S1386491.1) and one putative DMR6-like oxygenase 2 (JZKD02S0813268.1) in white spruce were also identified (Table 3).

4.3.6 Species divergence in drought-response regulatory functions

Our findings revealed a clear difference in the regulatory pattern of sugar maple under short- and long-term water stress, whereas white spruce exhibited a more consistent response (Figure 4.6). In sugar maple, DETs associated with BP categories such as response to stimulus, regulation of biological processes, metabolic process, and primary metabolic process, and those associated with MF categories such as binding, especially small molecule binding and ion binding, were predominantly down-regulated under long-term water stress but up-regulated under short-term water stress (Figure 4.3B, Figure 4.6). For both species, DETs related to localization, transmembrane transport, and transporter activity contained a high proportion of up-regulated DETs under long-term stress conditions. In contrast, these GO categories

contained a higher proportion of DETs whose expressions were significantly reduced in response to short-term water stress (Figure 4.3B and 4.4B, Figure 4.6). DETs related to cellular processes and photosynthesis in sugar maple were predominantly up-regulated in both the long- and short-term, whereas a predominance of down-regulation was observed in white spruce (Figure 4.6A-B).

4.4 Discussion

In this study, we conducted a *de novo* assembly of the sugar maple transcriptome using RNA-seq data from leaves of seedlings and saplings subjected to short-term and long-term water-stress conditions, thereby developing a novel genomic resource to investigate the responses triggered during drought. Additionally, our metadata analysis provided a comparative overview of molecular functions associated with drought-responsive genes in sugar maple and white spruce, two phylogenetically and ecologically distant tree species, under long-term versus short-term water stress conditions. The study highlighted putative acclimation strategies, while showing some convergence in the molecular responses to drought between the two species. Sugar maple showed an overall functional overlap in response to the length and severity of water stress, but regulatory patterns differed between short- and long-term treatments. In contrast, white spruce showed a more divergent molecular response between the two treatments.

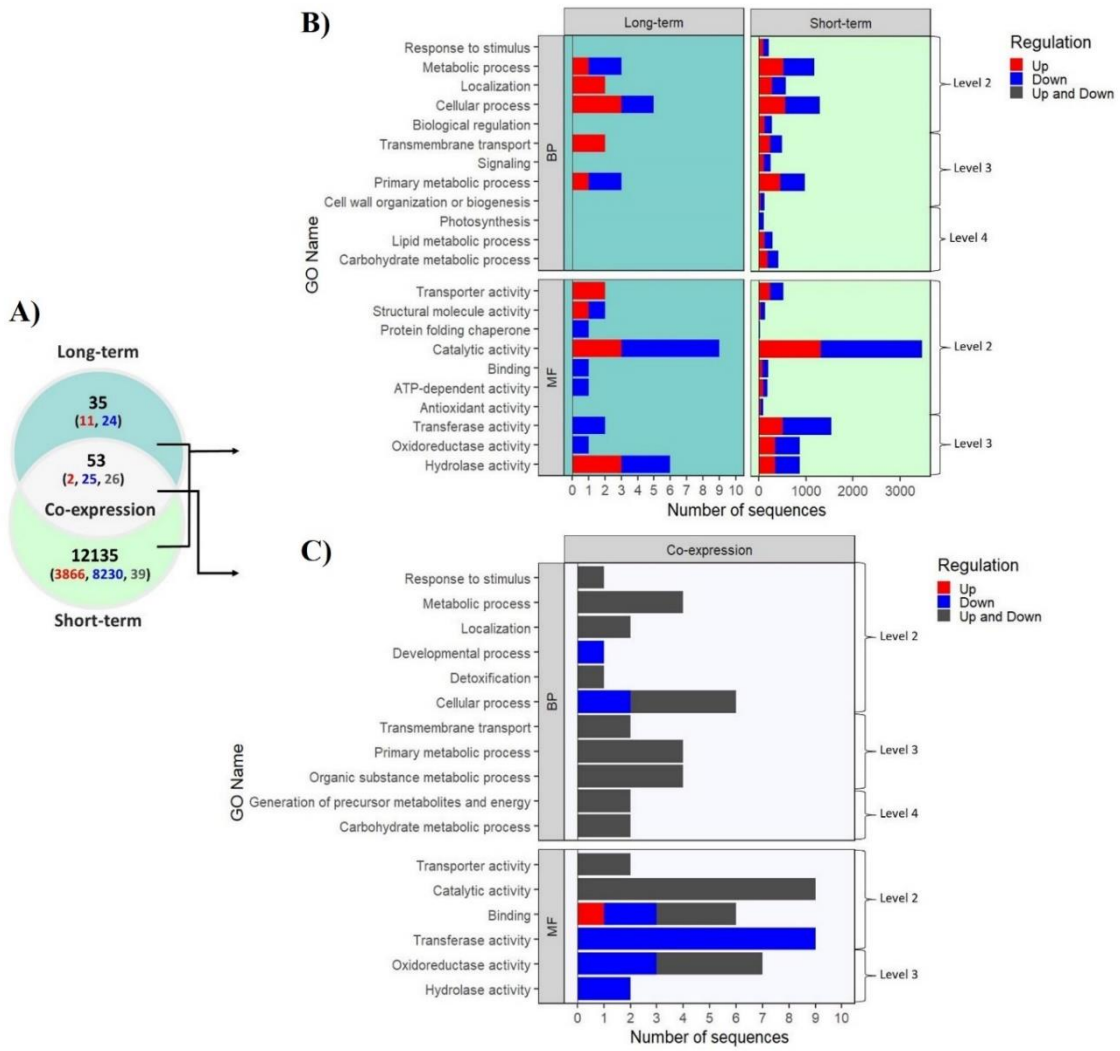

Figure 4.4. Key biological processes and molecular functions related to drought-responsive genes in white spruce subjected to short-term or long-term water stress. (A) Venn diagram showing the differentially expressed transcripts (DETs) during the long-term and short-term water stress experiments, as well as the DETs co-expressed by the two experiments (Co-expression). The total number of DETs is shown in bold black, and the numbers of up- and down-regulated DETs are shown in red and blue, respectively. The number of both up- and down-regulated DETs is shown in grey. Histograms grouping the main gene ontology (GO) annotations by level 2, 3, and 4 of biological processes (BP) and molecular functions (MF) of (B) DETs identified in the long-term or short-term water stress experiments and (C) co-expressed DETs of the two experiments. The histogram is expressed as a function of the number of sequences of DETs. The up-regulated and down-regulated transcripts are respectively indicated in red and blue. The DETs that are positively or negatively regulated according to the different stress time points studied in the context of the short-term experiment (B) or according to the two experiments (C) are shown in gray.

4.4.1 Phenotypic variations of trees submitted to long-term water exclusion

At our field experimental site, the long-term water exclusion treatment led to a 25% reduction in site precipitation (Belluau et al., 2021), causing significant negative effects on aboveground growth in both sugar maple and white spruce as well as reducing the area of sugar maple leaves (Figure 4.2). Under drought conditions, a lower total leaf area reduces total tree water loss and lessens the need for leaf-level water-conserving strategies such as increasing water use efficiency (McDowell et al., 2002). A recent study conducted at the same experimental site also revealed a decrease in the density of fine surface roots in these two species (Jaeger et al., 2023). This reduction in aboveground and surface root growth is a common acclimation response observed in trees when water availability is limited (Brunner et al., 2015). The decrease in leaf area observed in sugar maples is also an expected response from this highly plastic species, aiming to reduce evapotranspiration under drought conditions (X. Guo et al., 2020, 2023). The two species reacted differently to the same conditions over time, with white spruce showing earlier growth reduction than sugar maple (Figure 4.2). Both sugar maple and white spruce are often described as isohydric species that reduce stomatal conductance to avoid xylem cavitation as an initial response in to water stress, which could reduce photosynthesis and inhibit tree growth (Roman et al., 2015; Sullivan et al., 2021; Yi et al., 2017). However, recent observations suggest that different drought-response strategies along a spectrum from isohydric to anisohydric water regulation may occur within the same species (Depardieu et al., 2024). Under mild drought conditions, sugar maples may have sufficient water access and exhibit more anisohydric behavior, allowing them to keep their stomata open and maintain high photosynthetic rates for extended periods despite declining leaf water potential (Guillén et al., 2022). Collectively, these observations indicate that the water exclusion treatment had a significant impact on the trees at the phenotypic level.

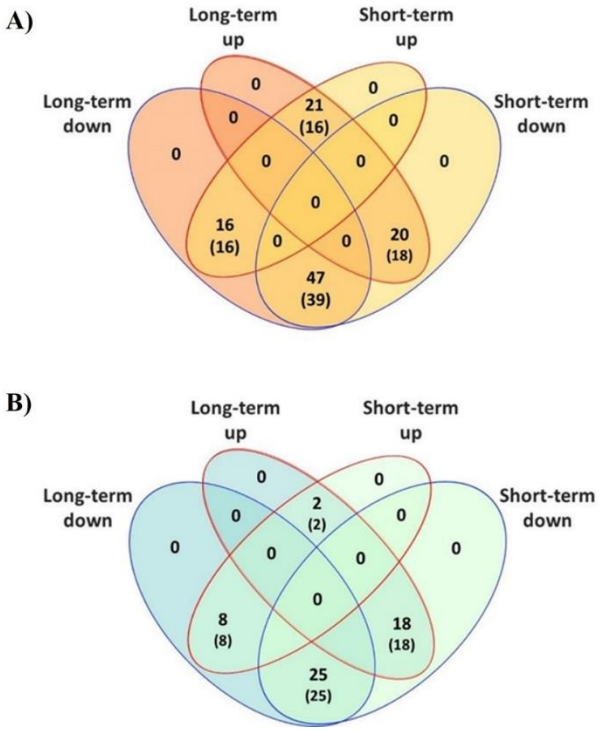

Figure 4.5. Overlap of differentially expressed transcripts (DETs) and corresponding unigenes regulatory profiles in water-stressed sugar maple and white spruce. Venn diagrams showing the overlap of DETs and corresponding unigenes between the four regulation profiles identified in the two drought experiments, long-term and short-term, for sugar maple (A) and white spruce (B). For each overlap, the number of DET is reported, along with the number of corresponding unigenes in parentheses. The up and down regulation profiles are indicated by the outlines of the circles, appearing in red and blue, respectively.

4.4.2 Contrasting transcriptome regulation in sugar maple and white spruce under long- and short-term water stress

In response to short-term water stress, 18.47% of the sugar maple transcriptome was significantly regulated compared to 36.61% of the white spruce transcriptome (Table S3.6). Short-term water stress experiments induced a stronger transcriptome regulatory response for both species compared to the long-term experiments (7 and 140 times more for sugar maple and white spruce, respectively, Table S3.6, Figures 4.3 and 4.4). This stronger transcriptional regulation under short-term conditions is due to the severity an rate of the applied water stress, as confirmed by previously collected physiological data showing a significant decrease in water

potential in white spruce seedlings during the short-term drought experiment (Stival Sena et al., 2018) and a strong increase in oxidative stress in sugar maple seedlings (Mulozi et al., 2023). It's worth noting that these short-term experiments were conducted on relatively young, potted trees grown in artificial media. Seedlings are generally more sensitive to drought than older trees with larger, well-established root systems (Fernández de Simón et al., 2020; Lasky et al., 2015; Niinemets, 2010). In the case of moderate stress applied over several years of growth, trees might experience increased vulnerability and reduced competitiveness due to the cumulative negative effects of stress (Mitchell et al., 2016). In this context, they may develop long-term acclimation responses involving gradual and profound morphological, physiological, and molecular changes (D'Odorico et al., 2021; S. Li, Lu, et al., 2023). Our results highlight a significant difference in transcriptomic regulation under long-term water stress conditions between the two species, with 88 DETs (80 unigenes) identified in white spruce and 618 DETs (386 unigenes) in sugar maple (Table S3.6, Figure 4.3 and 4.4). These results indicate that the two species exhibit different levels of sensitivity to long-term water stress, likely due to different physiological and molecular strategies for coping with this challenge.

4.4.3 Functional convergence and divergence between long- and short-term water stress in both species

Notwithstanding some obvious differences mentioned above, our study suggests a similarity in various biological processes and molecular functions induced by both long-term and short-term water stress (Figures 3 and 4), indicating that trees use common molecular pathways to respond to water stress. Different stresses of varying intensity have been shown to trigger similar genetic and functional networks, emphasizing the versatility of certain gene families and the conservation of stress response mechanisms (Gamboa-Tuz et al., 2018; X. Li et al., 2019). The functional convergence between short-term and long-term responses was notably more pronounced in sugar maple than in white spruce (Figures 3B and 4B). This observation might be explained by the low number of differentially expressed transcripts identified in the long-term experiment for white spruce, suggesting a lower level of gene regulation in response to the long-term stress.

In addition to functional similarities, our findings also reveal distinct regulation patterns between long-term and short-term water stress experiments (Figure 4.6). Sugar maples exposed

to long-term stress exhibit a high proportion of down-regulated transcripts associated with cellular and membrane protection (Figures 4.3B and 4.6; Table S3.7). Similarly to drought-induced genes, genes whose expression is reduced under stress conditions also play important roles in the survival and eventual development of the plant during water stress (Ingram & Bartels, 1996). The down-regulation of transcripts homologous with gene coding for five putative peroxidases, one catalase and two glutathione-S-transferases (GSTs) (Table S3.7) could be interpreted as an attempt for sugar maples to minimize cellular damage and conserve energy during drought conditions. After prolonged exposure, the regulation of genes involved in the stress response may be reduced due to the high energy cost and, consequently, subjected to trade-off in favor of other pathways, such as those associated with resource management and distribution (Leisner et al., 2023). The transmembrane transport and transporter activity GO classes predominantly contain drought-induced transcripts homologous with three putative MSF (major facilitator superfamily), three MATE (multidrug and toxic compound extrusion), and three OPT (oligopeptide transporter) transporters as well as three putative MscS (mechanosensitive ion channel) and auxin transporters in sugar maple under long-term drought conditions (Table S3.7, Figures 4.3B and 4.6).

Similarly, in white spruce, the expression of genes associated with transmembrane transport and transporter activity was primarily induced in the long term (Figure 4.4B, Figure 4.6), including gene coding for one putative proline, two ABC (ATP-binding cassette), and two amino acid transporters (Table S3.8). The regulation pattern of these transporters likely indicates an improvement in the tree's allocation of available resources to maintain optimal cellular homeostasis and enhance the distribution of nutrients and water within cells (Gill et al., 2021; Pinto & Ferreira, 2015).

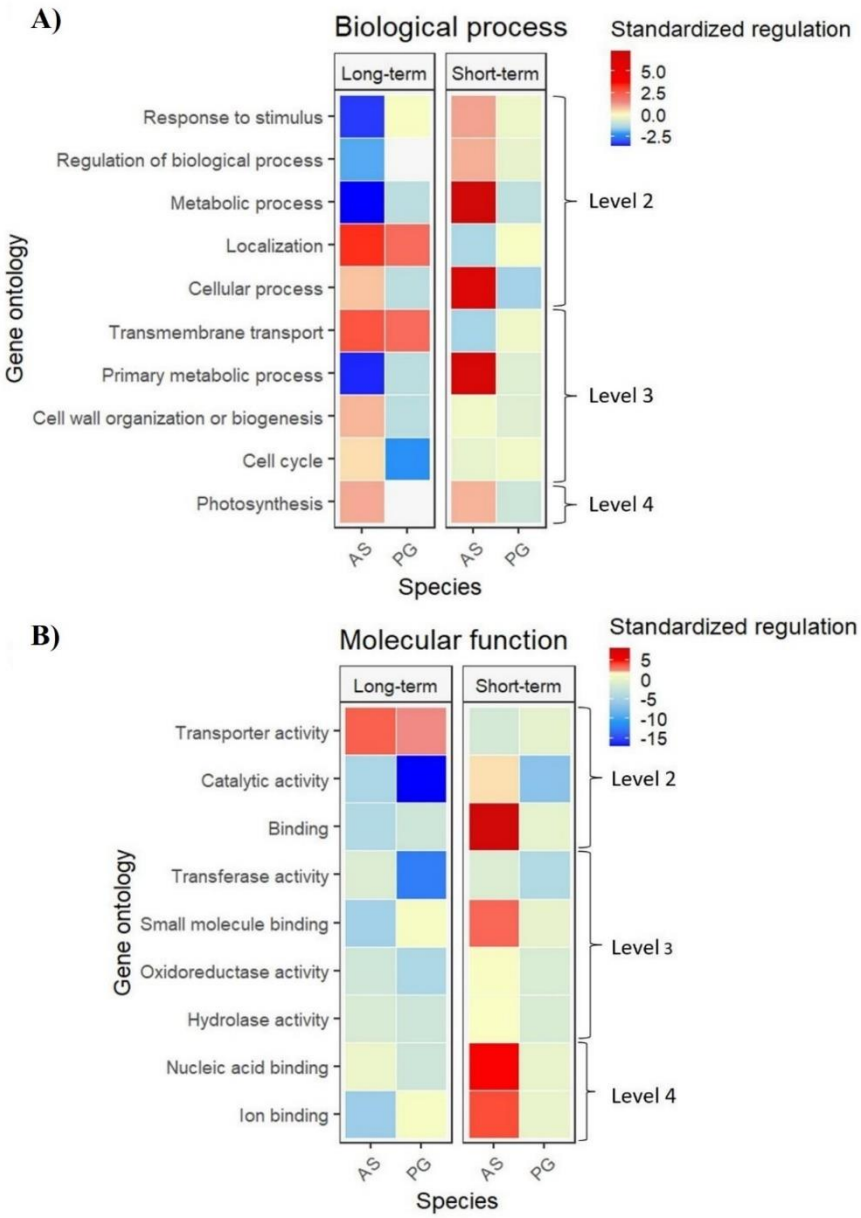

Figure 4.6. Comparative regulation of biological processes and molecular functions between sugar maple and white spruce. The heat maps illustrate the gene ontology (GO) levels 2, 3, and 4 for (A) biological processes and (B) molecular functions in sugar maple (AS) and white spruce (PG), subjected to long-term and short-term water stress experiments. The color gradient signifies the standardized regulation of DETs for each GO term across species and experiments. Within this gradient, red indicates that DETs are predominantly up-regulated, blue signifies that DETs are predominantly down-regulated, and yellow denotes that DETs are equally up- and down-regulated. White indicates that the GO term has not been identified.

Overall, the comparison of GO regulation patterns shows that sugar maple regulates its transcriptome more significantly (either up or down, depending on the GO classes considered) than white spruce (Figure 4.6), which could indicate a higher capacity for molecular adjustment to acclimate to drought conditions. This pattern is echoing the more primitive architecture of conifers compared to angiosperms, for a large array of traits including wood anatomy and morphological features (Gernandt et al. 2011).

4.4.4 Discovery of genes relevant for drought response and tolerance

To identify genes that may play a role in drought response and tolerance, we looked for transcripts that are differentially expressed under both long-term and short-term drought conditions (Figure 4.5, Table 2 and 3, respectively). Our approach assumes that genes expressed in response to two different intensities and/or durations of drought represent a potential genetic basis for drought response in a species. It is generally accepted that genes whose expression is induced under stress play an active role in tolerance, making them prime targets for functional studies. Although a gene that is down-regulated may also contribute to the drought response, its role is more indirect (Ingram & Bartels, 1996; Shinozaki & Yamaguchi-Shinozaki, 2007). A gene that is down-regulated in the short term but up-regulated in the long term is expected to promote an adaptive response and could therefore enhance tree acclimation to prolonged stress conditions. Our results highlight common molecular functions under water stress conditions and the potential importance of short-term stress in shaping long-term acclimation in angiosperm and gymnosperm trees. For both species examined, we identified 42 putative key genes in sugar maple and 31 in white spruce, each selected for their pertinent functions in the context of drought. Among these, six genes in sugar maple (Table 2) and two in white spruce (Table 3) are highly relevant for the drought response, as they exhibited induced expression under both short-term and long-term stress conditions. Among theses putative key genes for drought response, we identified transcription factors (TFs) that play a predominant role in activating or inhibiting gene regulation (Yao et al., 2021). Twenty-eight drought-responsive TFs were identified in sugar maple (Table 2) and 19 TFs in white spruce (Table 3), and they belong to families characterized for their role in the response to drought such as MYB, ERF, bHLH, NACs, NF-Yx and FAR1 (An et al., 2020; Dai et al., 2022; C. Li et al., 2021; Sobreiro et al., 2021; Yao et al., 2021, p. 20). Specifically, the up-regulated key gene FAR1 TF identified in sugar maple (ACSA_13557) has a homolog in *Arabidopsis thaliana* (AT2G25625), which

plays a crucial role in chloroplast disruption induced by stress (S. Wang & Blumwald, 2014). A bHLH family TF (ACSA_12765) was also up-regulated. It has a homolog in *Arabidopsis thaliana* (AT4G39900), which is not well characterized functionally in this plant but was found to be induced in response to cold and osmotic stress (Luhua et al., 2013), bHLH members from peanut (C. Li et al., 2021), apple (Q. Zhao et al., 2020) and desert poplar (Dong et al., 2014) significantly improved drought tolerance either by regulating stomatal development and photosynthesis in the transgenic plants or by maintaining ROS homeostasis at the cellular level. Then, two up-regulated NF-YA TFs (ACSA_11397, ACSA_11672) were identified; one has a homolog in *Arabidopsis thaliana* (AT5G01600), which encodes a chloroplast ferritin protein (FER1). Under stressful conditions, iron ions (Fe) can react with hydrogen peroxide (H_2O_2) to produce reactive oxygen species (ROS). In response to excess Fe and to protect cells, the gene AtFER1 in *Arabidopsis thaliana* encodes a ferritin protein that regulates iron storage (Tissot et al., 2019).

Among the genes common to both long-term and short-term water stress experiments in sugar maple, those involved in response to stress are predominantly down-regulated in the long-term context (Table 2). This group includes two putative peroxidases P7, known for their role in antioxidant activity (Baldi & La Porta, 2022), and two putative cytochrome P450 genes. However, a putative up-regulated osmotin/thaumatin (ACSA_14571) was also identified. It represents a potentially relevant gene in drought response associated with the maintenance of cellular osmolarity (de Jesús-Pires et al., 2020). In contrast to the sugar maple, the genes involved in the response to stress in the white spruce are mainly up-regulated under the long-term conditions (Table 3). Two genes were identified that were up-regulated under both stress conditions, including a putative gene belonging to the DMR6-like oxygenase family (DLOs) (JZKD02S0813268.1), which has been described to be involved in downy mildew resistance in *Arabidopsis thaliana* and grapevine (Pirrello et al., 2022), and a potential plant EC metallothionein-like protein (MTs) (JZKD02S1386491.1). These are known to play a critical role in maintaining homeostasis and tolerance, and studies in *Arabidopsis thaliana* and wheat have highlighted their ability to detoxify cells exposed to heavy metals (Pan et al., 2018).

Several genes encoding transmembrane transporters in sugar maple were induced under long-term water stress conditions (Table 2). These include two putative MFS transporters, one putative amino acid transporter, and one auxin transporter WAT1-related protein

(ACSA_23663), which was up-regulated under both conditions. These transmembrane transporters facilitate the diffusion or active transport of substrates across the cell membrane and may play crucial roles in maintaining homeostasis, regulating water and nutrient uptake, and transporting signaling or secondary molecules (Dahuja et al., 2020; Niño-González et al., 2019; Ranocha et al., 2013; Wan et al., 2017). In white spruce, relevant induced genes are also associated with transport and osmotic regulation (Table 3), including an amino acid polyamine transporter that facilitates the movement of water and small molecules, and a putative proline gene known for its osmoprotective properties (Sancho-Knapik et al., 2017). Induction of expression of a putative S-adenosylmethionine decarboxylase (SAMDC) gene encoding a potential osmoprotectant is expected to prevent loss of intracellular water and protect macromolecules by stabilizing protein structures and neutralizing reactive oxygen species (Wi et al., 2014).

Finally, in contrast to white spruce, a higher proportion of up-regulated genes associated with photosynthesis was observed in sugar maple including two genes coding for a heme oxygenase (Figure 4.6; Table 2), known to play a role in cellular defense and stomatal regulation (Mahawar & Shekhawat, 2018), and one gene coding for a member of the nuclear-encoded PSII Subunit P (PsbP) family proteins. These proteins are important components of the oxygen-evolving complex in chloroplasts and play a crucial role in the function and protection of PSII (Hong et al., 2020). The induced molecular functions related to photosynthesis in sugar maple under both long-term and severe short-term water stress (Table 6) are surprising, especially given the reported morphological (Figure 4.2) and physiological measurements (Mulozi et al., 2023), but may be a potential sign of drought acclimation in this species.

4.5 Conclusion

Our study provides a comparative analysis of molecular processes regulated in response to two types of drought conditions: moderate, long-term rapid, severe short-term. We examined the induced responses in two phylogenetically distinct species, sugar maple and white spruce, thereby advancing our understanding of long- and short-term stress responses in angiosperms and gymnosperms. Our results highlight key common mechanisms of drought response across both long-term and short-term drought and reveal candidate target genes for molecular breeding aimed at improving drought tolerance. In particular, both species shared strategies such as the

up regulation of transmembrane transport processes, potentially improving water and nutrient allocation? under long-term stress. The study also highlighted distinct strategies in each species, particularly in molecular responses related to stress response and photosynthesis. Sugar maple showed similar responses under both stress conditions, but with markedly different regulatory patterns, while white spruce showed more pronounced contrasts between the two scenarios. Similarities in the transcriptionally responsive gene families under both severe short-term and moderate, long-term drought suggested common molecular regulatory mechanisms. However, the specific genes involved in the drought response differ between species, indicating species-specific functionality of key drought-tolerance genes and highlighting the divergence in molecular responses between an angiosperm and a gymnosperm species. Future research could include co-expression analysis and network reconstruction to identify interconnected genes during stress responses, providing a comparative perspective between these two very different tree species. Further investigations should explore how phenotypic plasticity, such as gene expression changes in these two tree species, contributes to acclimatation but also long-term evolutionary adaptations for drought tolerance, including studies targeting gene expression plasticity and complex phenotypic adaptations in tree growth under extreme drought conditions. Based on the patterns of gene expression observed herein, possible marker-based applications in breeding programs should be explored to enable the selection of genetically diverse more drought-tolerant planting stocks. Given the relative sensitivity of these species to both short-term and long-term drought stress and in light of increased frequency and intensity of drought episodes under mid-northern latitudes, establishing plantations on reforestation sites with biophysical characteristics amplifying drought stress should also be avoided. Finally, the study could be extended to additional angiosperm and conifer tree species to generalize and refine the findings regarding short- and long-term responses as relevant to plant adaptation.

4.6 Supplementary data

4.6.1 Supplementary figures

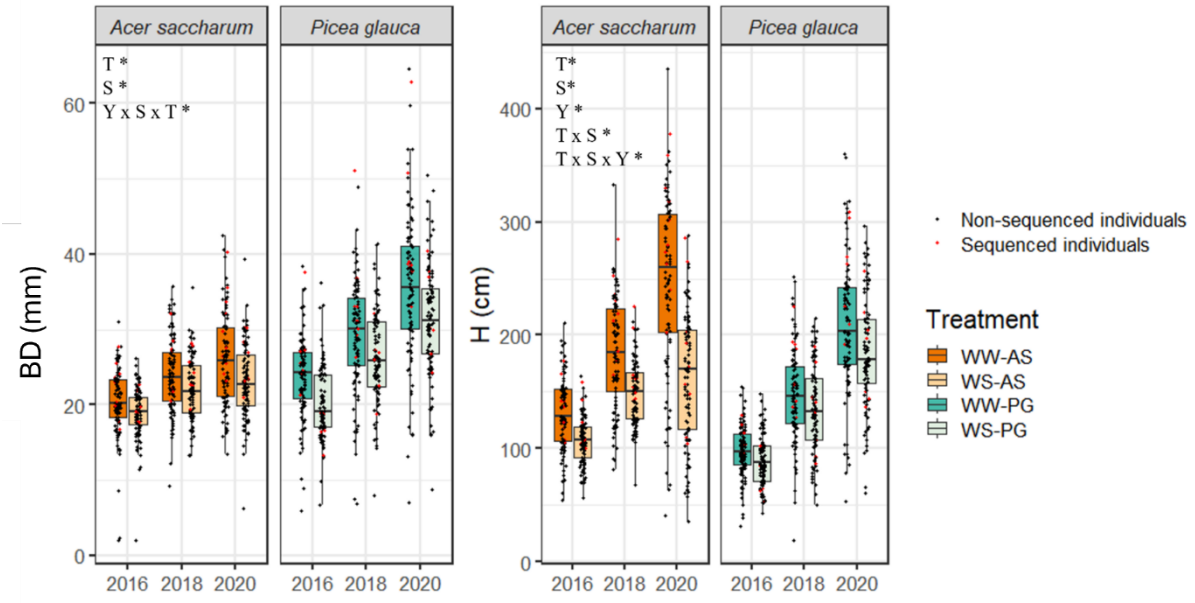

Figure S2.1. Morphological measurements of trees subjected to long-term water exclusion, both sequenced and non-sequenced. The diameter at breast height (BD, in mm) and height (H, in cm) measured in 2016, 2018, and 2020 are derived from all *Acer saccharum* (n = 197) and *Picea glauca* trees (n = 200) in monoculture stands in the IDENT experimental plot which have been subjected to either summer irrigation (well-watered, WW) or water exclusion (water stress, WS) since 2014. Results of multifactorial repeated measures ANOVA conducted on all individuals are indicated in the BD and H panels: T = water treatment, S = species, Y = year, X = interaction, * = significant difference ($p < 0.05$). Red dots represent individual trees randomly selected for RNA sequencing and the transcriptomic analysis (n = 20 for sugar maple and n = 16 for white spruce). Black dots represent other individual trees.

4.6.2 Supplementary tables

Table S3.1. Summary of plant material used for the *de novo* transcriptome assembly and transcriptomic analyses.

Experiment	Experimental conditions	Characteristics of trees	Sample size	Data source
Plant material used for *de novo* transcriptome assembly of sugar maple				
Long-term water exclusion	Experimental plot IDENT submitted to water exclusion and irrigation since 2014	9 to 10-year-old	10 samples of irrigated trees and 10 samples of water exclusion-treated trees	unpublished
Hardwood Genomics project	Several abiotic stress (drought, ozone, cold, heat)	seedlings	51 libraries composed of abiotic stress-exposed seedlings	NCBI Short Read Archive bioproject accession PRJNA273272
Plant material used for transcriptomic analyses				
Sugar maple				
Long-term water exclusion	Experimental plot IDENT submitted to water exclusion and irrigation since 2014	9 to 10-year-old	10 samples of irrigated trees and 10 samples of water exclusion-treated trees	unpublished
Short-term water stress	Greenhouse experiment designed with a water-stress treatment of 21 days	2-year-old	4 samples per condition (control and water stress) and time point (7, 14 and 21 days), (n = 24)	Mulozi et al. (2023)
White spruce				
Long-term water exclusion	Experimental plot IDENT submitted to water exclusion and irrigation since 2014	9 to 10-year-old	10 samples of irrigated trees and 10 samples of water exclusion-treated trees	unpublished
Short-term water stress	Greenhouse experiment designed with a water-stress treatment of 22 days	2-year-old, 3 clones (C8, C11, C95: 2 replicates of each clone per time point)	6 samples per condition (control and water stress) and time point (0, 14, 18 and 22 days), (n = 48)	Ribeyre et al. (2025)

Table S3.2. Complete functional annotation of sugar maple *de novo* transcriptome assembly based on OmicsBox analysis. See the separate Excel file, *Table_S3.2.txt.*

Table S3.3. Functional annotation of sugar maple *de novo* transcriptome assembly based on BLASTn et BLASTx analyses. See the separate Excel file, *Table_S3.3.xls.* The Excel file contains six sheets; Caption, Table S3.3.1 (BLASTn analysis performed against the sugar maple coding DNA sequence genome), Table S3.3.2 (BLASTx analysis performed against the public *Viridiplantae* database.), Table S3.3.3 (BLASTx analysis performed against the public Swissprot database), Table S3.3.4 (BLASTx analysis performed against the public PlantTFDB database), Table S3.3.5 (BLASTx analysis performed against the public *Arabidopsis thaliana* from PLAZA 5.0 database).

Table S3.4. Gene ontology (GO) annotation of differentially expressed transcripts (DETs) of sugar maple and white spruce and their corresponding unigenes. See the separate Excel file, *Table_S3.4.xls.* The Excel file contains four sheets; Caption, Table S3.4.1 (GO annotation of differentially expressed transcripts of sugar maple (*Acer saccharum*, AS) of both long- and short-term water stress experiments), Table S3.4.2 (GO annotation of differentially expressed transcripts of white spruce (*Picea glauca*, PG) of both long- and short-term water stress experiments), and Table S3.4.3 (Calcul of SP*i*, see Material and Methods).

Table S3.5. Statistical results of multifactorial repeated measures ANOVA and multifactorial ANOVA on morphological samples of sugar maple and white spruce. The post-hoc analysis used in multifactorial repeated measures ANOVA is a Bonferroni test by species. P-values are indicated in bold when the result is significant and as "NS" when results are not significant.

Multifactorial repeated measures ANOVA		
	Variables	P-values
Radial growth (BD, cm)	Treatment	**0.002**
	Species	**0.002**
	Year	**<0.00001**
	Treatment * Year	**0.017**
	Treatment * Species	**<0.00001**
Post hoc analysis (by species)		
Sugar maple	Treatment * Year	**0.039**
White spruce	Treatment * Year	NS
Primary growth (H, cm)	Treatment	**0.00015**
	Species	**0.013**
	Year	**<0.00001**
	Treatment * Year	**<0.00001**
	Treatment * Year * Species	**0.036**
Post hoc analysis (by species)		
	Treatment	**0.001**
	Year	**<0.00001**
Sugar maple	Treatment * Year	**0.00012**
	Treatment	**0.03**
	Year	**<0.00001**
White spruce	Treatment * Year	**0.035**
Multifactorial ANOVA		
Leaf area (cm^2)		
Sugar maple	Treatment	**0.0006**
White spruce	Treatment	NS
Specific leaf area (SLA, $m^2.kg^{-1}$)		
Sugar maple	Treatment	NS
White spruce	Treatment	NS

Table S3.6. Statistics annotation of data sets. This table provides information on the number of sequences from the *de novo* transcriptome assemblies of sugar maple and white spruce. It includes the number of differentially expressed transcripts (DETs) and the corresponding number of putative unigenes for both the long-term and short-term water stress data sets. The table reports the percentage of regulated DETs relative to the respective assemblies for the long-term and short-term water stress experiments. Additionally, it includes the percentage of gene ontology (GO) annotations (at levels 2, 3, and 4) obtained with OmicsBox for the datasets and transcriptome assemblies.

	Number of DETs	Number of putative unigenes	Percent of regulation	% GO annotation
Sugar maple				
De novo assembly (this study)	23,702	15,560	-	67.3%
Long-term experiment (number of DETs)	618	386	2.61	55.5%
Short-term experiment (number of DETs)	4,379	3,606	18.47	71.0%
White spruce				
De novo assembly (Ribeyre et al., 2025)	33,287	18,934	-	52.6%
Long-term experiment (number of DETs)	88	80	0.26	48.9%
Short-term experiment (number of DETs)	12,188	8,181	36.6	46.7%

Table S3.7. List of sugar maple differentially expressed transcripts (DETs) and their corresponding unigenes from long- and short-term water stress experiments. See the separate Excel file, *Table_S3.7.xls.* The Excel file contains three sheets; Caption, Table S3.7.1 (List of differentially expressed transcripts (DETs) and their corresponding unigenes of sugar maple from the long-term water exclusion experiment), Table S3.7.2 (List of differentially expressed transcripts (DETs) and their corresponding unigenes of sugar maple from the short-term water exclusion experiment).

Table S3.8. List of white spruce differentially expressed transcripts (DETs) and their corresponding unigenes from long- and short-term water stress experiments. See the separate Excel file, *Table_S3.8.xls.* The Excel file contains three sheets; Caption, Table S3.8.1 (List of differentially expressed transcripts (DETs) and their corresponding unigenes of white spruce from the long-term water exclusion experiment), and Table S3.8.2 (List of differentially expressed transcripts (DETs) and their corresponding unigenes of white spruce from the short-term water exclusion experiment).

Data availability: White spruce and sugar maple RNA-seq raw data sets from IDENT samples used for *de novo* transcriptome assembly and long-term transcriptomic analyses have been deposited at National Center for Biotechnology Information (NCBI) under the bioproject PRJNA1078812 (SRA accession numbers: SAMN40018912 to SAMN40018931). RNA-seq raw reads from Harmon et al. (2017) (Hardwood genomics) used to complete our *de novo* transcriptome assembly of sugar maple, were downloaded from the NCBI website (Short Read Archive, bioproject accession PRJNA273272). Supplementary Excel Tables and de *novo* transcriptome assemblies are available in Github repositories : the white spruce data can be found at (https://github.com/ZoeRibeyre/De-novo-transcriptome-assembly-and-discovery-of-drought-responsive-genes-in-white-spruce.git) and the sugar maple data are available at (https://github.com/ZoeRibeyre/Insights-into-drought-responses-sugar-maple-and-white-spruce.git).

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5) CONCLUSION GÉNÉRALE

L'ensemble de cette thèse s'est consacrée à caractériser les mécanismes physiologiques et moléculaires qui pourraient contribuer à l'acclimatation à la sécheresse des arbres, et plus particulièrement de l'érable à sucre et de l'épinette blanche. Le premier chapitre offre une synthèse des connaissances actuelles dans le domaine de l'épigénétique chez les arbres dans le contexte des changements globaux. Cette revue ouvre de nouvelles perspectives quant à la place de ces mécanismes dans le potentiel d'acclimatation des arbres. Les trois chapitres suivants ont examiné les réponses physiologiques et transcriptomiques induites chez l'érable à sucre et de l'épinette blanche lorsque ces espèces sont confrontées à différents types de sécheresse (répétées au sein d'une même saison de croissance, sévère à court terme, et modérée sur plusieurs années). La synthèse des résultats de l'ensemble des chapitres permet de mieux appréhender la capacité de ces deux espèces à faire face à la sécheresse. L'ensemble de l'étude a également permis d'identifier plusieurs gènes clés potentiels impliqués dans la réponse à la sécheresse des deux espèces. En guise de perspective, l'étude suggère de mieux intégrer la question de l'ontogénie et de variabilité génétique intraspécifique des arbres pour orienter les futures recherches. De plus, cette étude suggère que la caractérisation des effets de la sécheresse, particulièrement en milieux non contrôlés, doit prendre en compte les effets de synergie avec la chaleur, une perturbation concomitante à la sécheresse. La figure 5.1 illustre les concepts utilisés dans la thèse.

5.1 Synthèse des principaux résultats

5.1.1 L'épinette blanche

Notre étude a mis en avant que l'épinette blanche, bien que souvent décrite comme sensible à la sécheresse (Chen et al., 2017; Hogg et al., 2017; Peng et al., 2011), dispose aussi d'une capacité de tolérance et d'acclimatation à ce stress. Les résultats du chapitre 3 ont soulevé une forte sensibilité des plants (2-3 ans) d'épinette blanche à une sécheresse de 22 jours en serre. Cette sensibilité a notamment été mise en avant par une réduction prononcée du potentiel hydrique (mesuré et publié par Stival Sena et al., 2018), ainsi qu'à une intensification de la

régulation transcriptomique avec la durée d'exposition à la sécheresse. L'analyse fonctionnelle réalisée à l'échelle du transcriptome a révélé que sous ces conditions, l'épinette blanche accuse une baisse des processus de croissance et de photosynthèse, et mobilise des processus de défense dont des molécules antioxydantes, des HSP et des déhydrines. Ces résultats valident nos attentes quant à la stratégie adoptée par cette espèce de limiter sa perte en eau pendant une sécheresse. De plus, l'analyse a également mis en lumière le rôle potentiel du métabolisme des lipides dans la réponse à la sécheresse de l'épinette blanche. La caractérisation du métabolisme des lipides dans la réponse à la sécheresse chez les arbres est encore très marginale dans la littérature, ces résultats ouvrent donc la porte à de nouvelles perspectives de recherches.

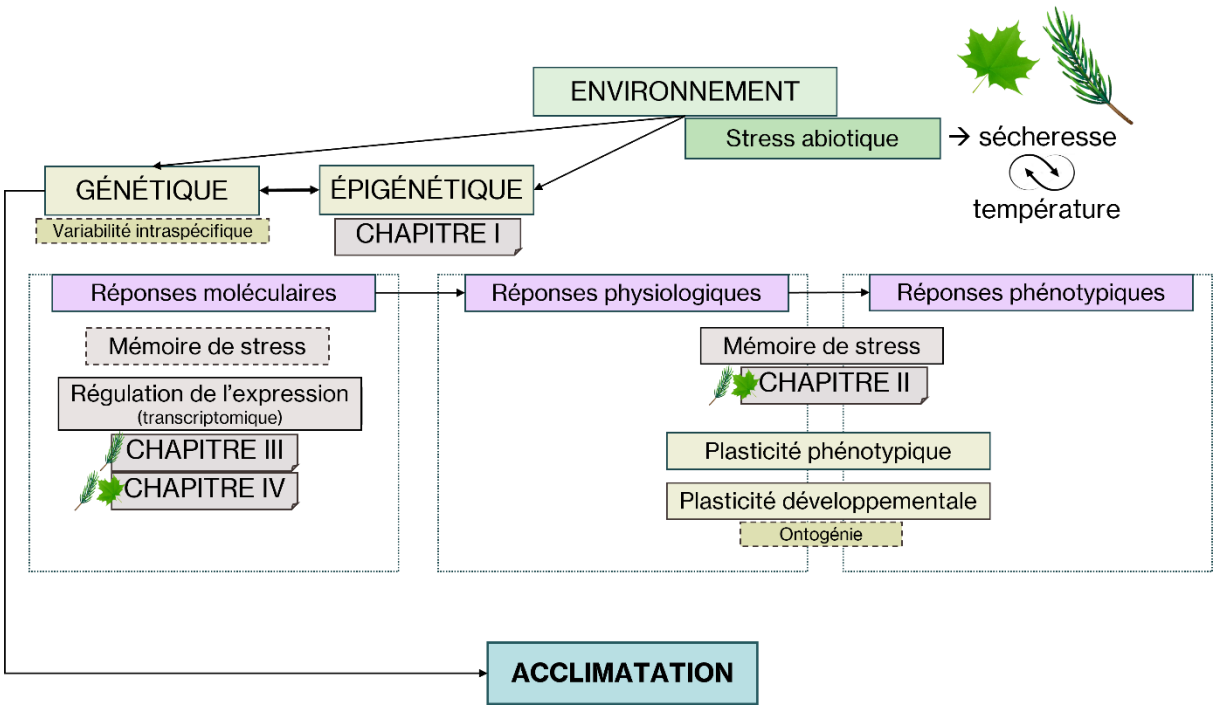

Figure 5.1. Schéma récapitulatif des travaux de la thèse. Les flèches indiquent la relation entre les différents processus étudiés. Les cadres en pointillés indiquent les perspectives et les variables à mieux considérer dans les futures recherches.

En revanche, de manière assez inattendue, les résultats du chapitre 2 ont mis en avant une bonne capacité des plants d'épinettes blanches du même âge (2-3 ans) à tolérer deux sécheresses consécutives (une sécheresse de 12 jours, suivi d'une sécheresse de 30 jours, entrecoupées par une période de récupération de 20 jours) en serre au cours d'une même saison de croissance. En effet, les mesures physiologiques n'ont pas révélé d'impacts des traitements sur les épinettes blanches stressées par rapport aux témoins. Bien que ces résultats invalident les hypothèses posées dans ce chapitre quant à l'induction d'un patron de mémoire des stress ou d'une accumulation de stress à la suite de deux sécheresses consécutives, ces derniers soulignent que l'épinette blanche est capable de démontrer une bonne résistance à la sécheresse. La différence de sensibilité observée entre les plants des chapitres 2 et 3, outre les différences expérimentales, pourrait être attribuée à une différence de génotype (Gazol et al., 2023; Schueler et al., 2021). En effet, les plants utilisés dans les deux études ne sont pas issus de la même provenance. Il a été démontré que l'épinette blanche dispose d'une grande variabilité génétique intraspécifique et que cette variabilité impacte la réponse à la sécheresse (De Lafontaine et al., 2010; Depardieu et al., 2020). Nous avons d'ailleurs observé des différences de régulation transcriptomique entre les clones étudiés dans le chapitre 3. Il est donc primordial de prendre en compte la variabilité intraspécifique pour évaluer le niveau de résistance et de sensibilité à la sécheresse d'une espèce.

5.1.2 L'érable à sucre

L'érable à sucre est décrit comme une espèce plastique (X. Guo et al., 2020, 2023) et avec une gestion assez conservatrice de son système hydraulique en cas de sécheresse (Roman et al., 2015; Yi et al., 2017). Les réponses physiologiques et moléculaires de l'érable à sucre en condition de sécheresse sont cependant encore assez peu caractérisées. Dans le chapitre 2, l'érable à sucre était fortement sensible aux traitements. Les résultats ont notamment mis en avant un impact prononcé de la première sécheresse, mais aussi des deux sécheresses consécutives. Cet impact a été mesuré par une diminution de la photosynthèse, de la teneur en eau foliaire et de la biomasse racinaire, ainsi que par une augmentation de la nécrose foliaire des plants stressés par rapport aux témoins. De plus, une réduction plus marquée de la photosynthèse chez les individus doublement stressés a été mise en évidence, validant l'hypothèse d'une accumulation de stress chez cette espèce. Ces résultats, en concordance avec d'autres travaux (Mulozi et al., 2023), indiquent donc une sensibilité à la sécheresse prononcée

des plants d'érable à sucre qui se caractérise par une dégradation des tissus foliaires (nécroses) et par une diminution de la photosynthèse, potentiellement causée par un excès de stress oxydatif. La méthodologie n'a pas permis de mettre en évidence un patron de mémoire de stress, mais les impacts causés par les différents traitements se sont révélés être très dépendants des individus, où certains présentaient une amélioration de la vigueur après les deux sécheresses successives. Nous émettons donc l'hypothèse que pour mieux appréhender un tel processus, il serait judicieux de mettre en place une méthodologie avec un suivi individuel au cours de plusieurs saison de croissance et de tester plusieurs génotypes.

5.1.3 Comparaison des deux espèces

Le chapitre 4 présente une méthodologie innovante en employant deux jeux de données par espèce, à savoir des données provenant d'une expérience d'exclusion hydrique en jardin expérimental mis en place pendant 6 ans (long terme), et des données issues de deux expériences en serre avec une sécheresse sévère de 3 semaines (court terme). Cette étude offre dans un premier temps une approche intraspécifique en caractérisant les réponses moléculaires à la sécheresse induites à long terme, peu connues, par rapport à des réponses induites à court terme chez les deux espèces. Cette approche a également permis d'identifier des gènes régulés à la hausse et exprimés communément en réponse aux deux traitements hydriques au sein de chaque espèce, dont certains ont déjà identifiés dans la littérature pour leur implication dans la réponse à la sécheresse. Nous avons notamment mis en avant plusieurs facteurs de transcription et des gènes impliqués dans le transport et la régulation osmotique. Dans un second temps, l'étude présente également une approche interspécifique entre l'épinette blanche et l'érable à sucre, qui décrit et discute les réponses communes et spécifiques observées en réponse aux deux types de sécheresse. Les résultats montrent une nette augmentation de la régulation du transport transmembranaire observée à long terme par rapport à court terme chez les deux espèces, laissant penser à une amélioration de la gestion des ressources disponibles. Malgré ces réponses communes, les deux espèces montrent des stratégies assez distinctes. En effet, les résultats montrent une régulation transcriptomique plus accentuée de l'érable à sucre que l'épinette blanche (386 gènes contre 80 gènes, respectivement) après avoir été exposés 6 ans aux mêmes conditions hydriques, ce qui pourrait indiquer une plus grande capacité d'ajustement moléculaire de la part de l'érable à sucre pour s'acclimater aux conditions d'une sécheresse prolongée. De plus, l'érable à sucre présente une similitude des réponses moléculaires dans les

deux conditions de stress (long terme *versus* court terme), avec toutefois une forte distinction des patrons de régulation des gènes. Cette espèce se démarque notamment par une régulation à la baisse prononcée des processus de défense dans l'expérience à long terme et une régulation à la hausse des processus en lien avec le fonctionnement photosynthétique dans les deux expériences. En revanche, l'épinette blanche présente un plus fort contraste de réponses entre les deux conditions.

Les effets du stress thermique ne faisaient pas partie de la question de recherche de cette étude, mais il est notoire, notamment dans le contexte des changements climatiques, que les stress liés à la sécheresse et à la chaleur seront de plus en plus indissociables (Hammond et al., 2022). Si notre étude a montré que l'épinette blanche dispose d'une certaine capacité de résistance à la sécheresse, elle a également souligné lors du chapitre 2 et en concordance avec de précédentes études, que cette espèce est sensible à la chaleur (D'Orangeville et al., 2018; Gagne et al., 2020; Lapenis et al., 2022). La vague de chaleur imprévue lors de notre étude a induit une forte mortalité des plants d'épinettes blanches (> 50%) avant la mise en place des traitements de sécheresse. Cette forte mortalité suggère que les épinettes blanches ont atteint leur seuil critique de résistance thermique (O'sullivan et al., 2017; Teskey et al., 2015) lors de cet événement. En revanche, la vague de chaleur n'a pas occasionné de mortalité ou de dommages phénotypiques visibles (nécroses ou flétrissement des feuilles) sur la grande majorité des plants d'érable à sucre. L'érable à sucre démontre donc une meilleure capacité à tolérer un évènement de chaleur intense et passager dans un contexte hydrique favorable que l'épinette blanche. Cette différence observée entre les deux espèces pourrait résulter d'une meilleure gestion de l'excès de chaleur chez les érables à sucre par un mécanisme de refroidissement des feuilles plus efficace (Drake et al., 2018; O'sullivan et al., 2017). Par ailleurs, l'effet prononcé de la première sécheresse sur les érables à sucre par rapport à la seconde sécheresse laisse penser que le stress de chaleur a augmenté leur vulnérabilité à ce premier traitement. Ces observations soulignent donc l'importance de considérer les effets de synergie entre la sécheresse et la chaleur en se penchant, par exemple, sur la caractérisation du seuil de résistance thermique et des mécanismes qui contribuent à la gestion des pics de chaleur des arbres, en synergie ou non avec la sécheresse.

5.1.4 Le rôle des mécanismes épigénétiques dans l'acclimatation au stress

Comme cela a été exposé dans le premier chapitre, les recherches sur les processus épigénétiques ont connu un réel essor chez les arbres ces dernières années, même si celles-ci restent encore limitées à quelques espèces. Plusieurs études ont notamment mis en avant l'influence de l'environnement sur les réponses épigénétiques, ainsi que le rôle de ces dernières dans la régulation des gènes chez les arbres (Bräutigam et al., 2013; Sow, Allona, et al., 2018). Il a également été mis en avant que la mémoire épigénétique, ou mémoire de stress environnementale, participe à l'établissement d'une plasticité adaptative qui facilite la réponse des arbres à de nouvelles perturbations (Bäurle & Trindade, 2020; Lämke & Bäurle, 2017). Le chapitre 2 a été mis en place dans le but de tester la possibilité de déclencher et mesurer une mémoire de stress chez l'érable à sucre et l'épinette blanche à l'échelle phénotypique pour poursuivre les investigations à l'échelle moléculaire avec des analyses épigénétiques. Cependant, la méthodologie employée n'a pas permis d'observer et de mesurer la mise en place d'un patron de mémoire de stress chez les deux espèces. Comme la phase de validation à l'échelle phénotypique n'a pas pu être remplie, nous avons décidé de ne pas poursuivre avec les analyses épigénétiques qui sont très coûteuses. Cependant, l'observation de ce processus chez des espèces d'arbres de différentes familles (p. ex. *Salicaceae*, *Pinaceae*) dans des conditions de stress divers (p. ex. des sécheresses ou des stress thermiques chaud et froid) (Carneros et al., 2017; A.-L. Le Gac et al., 2018; I. A. Yakovlev et al., 2016; I. A. Yakovlev & Fossdal, 2017), suggère que l'existence de ce processus doit s'étendre à d'autres espèces ligneuses. Les points méthodologiques à améliorer de ce chapitre sont discutés dans la section "limites de l'étude et perspectives de recherches". Ainsi, avant d'envisager l'utilisation des variations épigénétiques pour l'amélioration et la sélection des arbres, il est crucial de combler nos lacunes sur la compréhension de la relation entre les mécanismes épigénétiques et les traits qui favorisent leur acclimatation. Un des défis est l'adaptation de certains outils moléculaires utilisés en génétique à l'étude de l'épigénome. Chez des plantes modèles comme *Arabidopsis thaliana*, des lignées EpiRIL (lignées qui présentent peu ou pas de polymorphismes génétiques, mais avec des variations épigénétiques) (Hou & Wan, 2021; C. L. Richards et al., 2017) ont été utilisées pour mieux comprendre et évaluer la contribution spécifique des modifications épigénétiques par rapport aux mécanismes génétiques dans la réponse aux variations de l'environnement (Catoni & Cortijo, 2018). Un autre outil prometteur en développement est l''édition de l'épigénome (ou *epigenome editing*), une technique basée sur la technologie

CRISPER. Elle permet de modifier le paysage épigénétique dans le but de moduler l'expression génique sans toucher à la séquence d'ADN (Klupczyńska & Ratajczak, 2021; Nuñez et al., 2021). Cependant, l'amélioration des ressources génétiques des espèces non modèles est un cap indispensable à franchir pour pouvoir appliquer ces méthodes aux arbres (García-García et al., 2022; C. L. Richards et al., 2017).

5.2 Contribution de l'étude

Cette thèse apporte une contribution significative pour améliorer notre compréhension des mécanismes physiologiques et moléculaires induits par la sécheresse chez l'érable à sucre et l'épinette blanche. Bien que l'épinette blanche ait fait l'objet de plusieurs études transcriptomiques, ces recherches se sont principalement concentrées sur certaines familles de gènes (Depardieu et al., 2021; Hornoy et al., 2015; Stival Sena et al., 2018). En revanche, les études sur l'érable à sucre dans ce domaine n'en sont qu'à leurs prémices (McEvoy et al., 2022; Mulozi et al., 2023). Dans notre étude, nous avons privilégié la caractérisation des réponses induites par la sécheresse à l'échelle du transcriptome, afin d'apporter une vue d'ensemble et de souligner la complexité du réseau de réponses impliqué en contexte de stress. De plus, notre étude a permis de valider le rôle important de certains gènes, déjà mis en avant dans d'autres études pour leur implication dans l'adaptation à la sécheresse chez l'épinette blanche (Depardieu et al., 2020; Hornoy et al., 2015; Prunier et al., 2017; Stival Sena et al., 2018; Van Ghelder et al., 2019) (chapitre 3). Nous avons également révélé de nouveaux gènes clés potentiels dans la réponse à la sécheresse pour cette espèce (chapitres 3 et 4) et pour l'érable à sucre (chapitre 4). Ces gènes constituent des candidats prometteurs pour de futures études fonctionnelles. Par ailleurs, l'étude du chapitre 3 a mis en évidence un rôle potentiel du métabolisme des lipides dans la réponse au stress chez l'épinette blanche, une découverte jusqu'alors non explorée dans la littérature. Notre étude apporte aussi sa contribution au domaine encore peu exploré de l'épigénétique des arbres. Le chapitre 1 offre notamment une revue complète des connaissances du domaine et propose des orientations pour les futures recherches. De plus, le chapitre 2 en initiant l'exploration du processus de mémoire de stress chez l'érable à sucre et l'épinette blanche, a souligné les défis liés à sa mise en place et à son identification, ainsi qu'à l'importance d'établir un suivi longitudinal des individus. Enfin, l'étude contribue également à l'amélioration des ressources transcriptomiques des deux espèces par la publication de deux assemblages transcriptomiques *de novo*. L'approche utilisée a été d'échafauder nos assemblages *de novo*

avec des assemblages déjà disponibles des espèces (Harmon et al., 2017; Rigault et al., 2011) en ajoutant des échantillons de tissus foliaires de plants soumis à un stress hydrique en serre et d'arbres de 9-10 ans soumis à des conditions de sécheresse de plusieurs années en plantation, ce qui est encore très peu utilisé en transcriptomique forestière. Ces ajouts d'échantillons ont permis de compléter les assemblages déjà publiés et d'apporter une meilleure représentativité des gènes en réponse à la sécheresse. Par ailleurs, nos assemblages *de novo* indiquent une potentielle réduction de la redondance des séquences par rapport aux anciens assemblages résultant d'une plus faible fragmentation de nos assemblages *de novo*. Cette diminution de la redondance améliore la qualité des analyses en réduisant le risque d'alignements erronés. Cette avancée constitue une contribution significative à l'enrichissement des ressources génomiques disponibles pour ces deux espèces.

5.3 Limites de l'étude et perspectives de recherches

5.3.1 Différents stades de développement

Dans notre étude, nous avons analysé les réponses à la sécheresse de jeunes plants (de 2-3 ans dans les chapitres 2 et 3) et de jeunes arbres (de 9-10 ans dans le chapitre 4). Cette approche permet d'avoir une caractérisation plus approfondie de l'espèce en prenant en compte plusieurs stades de développement (Ochoa-Lopez et al., 2020). Cependant, cette démarche limite la comparaison directe et la généralisation de nos résultats, car le stade de développement est un paramètre qui peut fortement influencer le niveau de sensibilité et les réponses à la sécheresse d'une espèce. Plusieurs travaux ont notamment mis en avant une différence dans le niveau de plasticité, de résistance et d'acquisition et de gestion des réserves carbonées selon le stade de développement d'une espèce (Fernández de Simón et al., 2020; Lasky et al., 2015; Niinemets, 2010; Sendall et al., 2015). Nous avons particulièrement dû faire face à ce questionnement dans le chapitre 4, où nous avons utilisé deux jeux de données par espèce provenant d'expériences menées à long terme sur des arbres, et à court terme sur de jeunes plants. Les résultats obtenus sont donc à interpréter avec prudence, car par exemple, un même gène régulé chez un plant ou chez un arbre peut présenter différents champs d'action, notamment à cause de modifications post-traductionnelles qui peuvent varier en fonction du stade de développement (Guerra et al., 2015; Prall et al., 2019).

5.3.2 Un manque de mesures physiologiques et moléculaires de validation

Afin d'appuyer les résultats et d'améliorer notre compréhension des processus décrits à l'échelle transcriptomique, il aurait été souhaitable d'intégrer davantage de mesures physiologiques et/ou moléculaires (chapitres 3 et 4) et de faire des validations de l'expression de certains gènes clés identifiés par une méthode complémentaire de RT-qPCR (chapitre 4). Dans le chapitre 3, des mesures du potentiel hydrique et des RTq-PCR ont été effectuées dans l'étude de Stival Sena et al., (2018), dont sont issues les données brutes de séquençage de l'ARN que nous avons analysées. Les mesures par RT-qPCR ont notamment mis en avant l'expression différentielle de quatre déhydrines qui ont également été identifiées à partir de notre assemblage transcriptomique *de novo*. Par ailleurs, ce chapitre aurait pu être enrichi en examinant davantage le rôle du métabolisme des lipides dans la réponse à la sécheresse mis en avant de manière dans cette étude. Par exemple, la réalisation de dosages lipidiques ou de la mesure d'activités d'enzymes impliquées dans la néoglucogenèse ou dans le cycle du glyoxylate (Walker et al., 2021) aurait permis d'explorer plus en détail l'impact d'une sécheresse sur le métabolisme des lipides dans les aiguilles de l'épinette blanche. Les échantillons biologiques n'étaient plus disponibles lorsque nous avons récupéré les données brutes de séquençage de l'ARN, ne nous permettant donc pas de réaliser ces analyses. Cependant, il serait primordial de poursuivre les investigations sur le rôle des lipides dans la réponse à la sécheresse lors de futures études sur cette espèce et sur d'autres conifères.

Par ailleurs, des mesures physiologiques ou moléculaires supplémentaires des deux espèces auraient permis de mieux définir l'état de stress perçu et donc la sévérité du traitement de sécheresse. En effet, les deux espèces cibles ont été parfois comparées dans l'étude après avoir été soumises à des stress similaires ou identifiés comme tels. Mais, les deux espèces étant évolutivement très distantes et ayant des caractéristiques écologiques diverses, un même traitement n'occasionne pas nécessairement la même sévérité. Il serait donc primordial de mieux définir la sécheresse, c'est à dire à partir de quel niveau de réponse peut-on parler de stress sous sécheresse pour une espèce donnée.

5.3.3 Un manque d'informations sur la variabilité intraspécifique

Les espèces d'arbres avec une large aire de répartition, comme nos espèces cibles (Horsley et al., 2002; Nienstaedt & Zasada, 1990), présentent souvent une grande variabilité génétique ce

qui les rend plus à même de persister face à aux changements environnementaux (Bussotti et al., 2015; Mulozi et al., 2023). Pour mieux appréhender les limites de tolérance d'une espèce à la sécheresse et aider la conservation et la gestion des forêts, il est nécessaire de mieux comprendre les différences de réponses au sein et entre les populations et d'identifier les traits génétiques potentiels modulant la réponse à la sécheresse (Gazol et al., 2023). Une des limites de notre étude est le manque d'information sur la variabilité génétique intraspécifique des arbres étudiés, notamment pour les chapitres 2 et 4. En effet, seul le chapitre 3 a abordé cet aspect avec l'incorporation de trois génotypes de l'épinette blanche (les clones C8, C11 et C95). Bien que les résultats aient montré une variation notable de l'expression génique en réponse à la sécheresse entre les génotypes, le plan expérimental utilisé ne permettait pas de tester cet aspect de manière robuste au cours du temps. En effet, nous ne disposions pas de suffisamment de réplicas biologiques de chaque clone par point de mesure. Par ailleurs, dans le chapitre 2, nous voulions tester la capacité des deux espèces à déclencher une mémoire de stress, un processus sous le contrôle de mécanismes épigénétiques. Nos résultats pointent la difficulté à déclencher et mesurer un tel processus, notamment à cause des variations individuelles. Bien que cela représente un possible défi technique pour des espèces non modèles, il aurait été souhaitable de mener cette étude sur (1) des clones, afin de minimiser les réponses individuelles causées par la variation génétique, et (2) tester la capacité de différents génotypes à déclencher une mémoire de stress.

5.3.4 Évaluer la récupération à la sécheresse

L'évaluation de la récupération à la sécheresse ne faisait pas partie de nos objectifs. Cependant, il est indéniable que ce paramètre est essentiel pour mieux caractériser la capacité des arbres à faire face à ce stress (Gessler et al., 2020; Lloret et al., 2011) et pour appréhender leur capacité de résilience (Ingrisch & Bahn, 2018). Dans le chapitre 2, la difficulté de mesurer une mémoire de stress chez nos deux espèces pourrait, en plus des points abordés ci-haut, provenir d'une approche temporelle trop courte. À la lumière de notre étude, et de la revue de littérature du chapitre 1, nous estimons que l'évaluation de la mémoire de stress chez des arbres serait plus efficace en établissant des protocoles expérimentaux sur plusieurs années. Il aurait donc été judicieux de mesurer la capacité de récupération des plants soumis aux différents traitements avec un suivi de leur vigueur pendant la saison de croissance suivante. Cette approche avait été envisagée lors de la mise en place du protocole, mais a été abandonnée faute de temps et

d’espace. Par ailleurs, les plans expérimentaux des études menées dans le chapitre 3 (mis en place par Stival Sena et al., 2018) et dans le chapitre 4 (dispositif IDENT mis en place depuis 2014), ne permettaient pas d’intégrer l’évaluation de la récupération à la sécheresse. Cependant, pour de futures études, un tel suivi serait envisageable dans le jardin expérimental IDENT en ajoutant un traitement de réhydratation de certaines parcelles soumises à l’exclusion hydrique depuis 2014. Dans cette optique, la capacité de récupération à l’exclusion hydrique longue pourrait être mesurée en poursuivant le suivi annuel de la croissance primaire et radiale mis en place depuis 2016. De plus, au sein de ce jardin expérimental, la présence de plusieurs espèces ligneuses soumises à des conditions environnementales identiques depuis plusieurs années serait propice pour mieux appréhender les différences de capacité de récupération et de résilience à la sécheresse qui ont été soulevées dans plusieurs travaux entre les angiospermes et les gymnospermes (DeSoto et al., 2020; X. Wu et al., 2022).

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