

Growth and photosynthetic traits differ between shoots originated from axillary buds or from adventitious buds in *Populus balsamifera* L. cuttings

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Abstract

Bud development influences shoot branching and the plasticity and adaptability of plants. To explore the differences of post-embryonic development of different types of buds, shoots originated from adventitious buds and axillary buds of cuttings in two populations of balsam poplar (*Populus balsamifera* L.) were investigated for differences in leaf morphology, photosynthetic and growth characteristics, and the effects of a carbonic anhydrase (CA) inhibitor on CA activity, photosynthesis and mesophyll conductance (g_m). The results showed that axillary buds produced ovate first few leaves and longer shoots while adventitious buds produced lanceolate first few leaves with higher specific leaf area (SLA). There were no significant differences in leaf area-based photosynthetic rate (A_n), maximum carboxylation rate (V_{cmax}), and maximum electron transport rate (J_{max}) between shoots originated from the two bud types. Based on the principal component analysis, shoots of adventitious bud origin grouped on daytime respiration and SLA, while cuttings from axillary buds clustered toward the opposite direction of quantum yield and light saturation point. Shoots originated from different types of buds had different growth rates and biomass, but the direction of the differences varied with the population of the mother tree. The two populations differed in A_n , g_m , and relationships between CA, A_n , and g_m . There were differences in post-embryonic growth traits of shoots from axillary buds and those from adventitious buds, which may be an adaptive strategy for regeneration under different light conditions.

JEL CLASSIFICATION

Ecophysiology, Stress and Adaptation

1 | INTRODUCTION

There are different types of buds in plants, i.e. terminal buds, axillary buds, and adventitious buds. Buds of different types can greatly influence plant plasticity in their responses to environmental changes (Ejaz et al. 2021; Yao & Finlayson 2015). The formation and flushing of different types of buds, especially the branching model of axillary buds, shape the plant architecture, which in turn affects growth, yield, and competitiveness (Bertheloot et al. 2020). The

phenology, dormancy, and burst of a bud are determined by the interaction between plant physiology and the environment and play critical roles in synchronizing the growth and development of plants with changes in environmental conditions, influencing plant survival, particularly under harsh climate conditions (Lundell et al. 2020; Singh et al. 2018).

Climate change can affect the relative competitiveness of different tree species in various ways, such as the setting, development, and flushing of different types of buds and their responses to

environmental conditions (McKown et al. 2018). Climate change may disrupt the synchronization of bud phenology with seasonal changes in temperature and photoperiod, increasing the risk of injuries by unfavorable environmental conditions, such as freezing temperature (Villouta et al. 2021; Yordanov et al. 2014). Usually, the development of axillary buds in most tree species is inhibited by the terminal bud, which is called apical dominance (Bertheloot et al. 2020). However, terminal buds and axillary buds have the same development model and potential (Chabikwa et al. 2019; Rinne et al. 2015). Vegetative buds, such as terminal buds and axillary buds, are more prone to browsing by herbivores because their meristems are rich in nutrients but low in defense chemicals (Sobuj et al. 2020). Although terminal and axillary buds are usually protected by scales, they are vulnerable to damages, such as during a crown fire (Nolan et al. 2020). Damages to buds can modify tree architecture and change their competitiveness in changing environmental conditions (Chabikwa et al. 2019; Villouta et al. 2021).

Adventitious buds may play an important role in tree response to climate change (Chao et al. 2017). The meristem of an adventitious bud is believed to be derived from a single cell located in the epidermal layer or vascular cambium (Nabeshima et al. 2017), different from the origin of the meristem of a terminal bud or an axillary bud; thus, shoots developed from adventitious buds may have different phenology than their parent plant (Vanden Broeck et al. 2018). This phenotypic variability may improve the response of trees to climate change and heterogeneous environments (McKown et al. 2014). While there has been extensive research about the terminal and axillary buds, there is a paucity of information on adventitious buds in the literature.

As a means of vegetative propagation, cutting is extensively used in agriculture, horticulture, and forestry (Li et al. 2018). Cuttings are also used as a research tool for investigating the bud phenology of deciduous plants or as substitutes for large trees in phenological studies (Camargo Alvarez et al. 2018). Balsam poplar (*Populus balsamifera* L.) is a widespread boreal species distributed throughout temperate boreal regions of North America (Suarez-Gonzalez et al. 2018). Balsam poplar has robust tolerance over a wide range of environmental conditions and plays a key role in local ecosystems (Prunier et al. 2019). Shoots formed on cuttings can originate from adventitious buds, axillary buds or both. Adventitious buds occur in the internodes of many poplar tree species in vitro (Douglas 1984) and serve as a potential bud bank to enable plants to overcome the meristem's limitation and thus better tolerate severe damages (Klimešová et al. 2014). The expenditure of resources (e.g. carbohydrates and nutrients) differs between the production of an axillary bud and that of an adventitious bud (Mishra et al. 2021).

However, it is unknown whether plants derived from different types of buds are physiologically different. In this study, we observed differences in growth characteristics and some physiological traits between leaves of shoots derived from adventitious buds and those derived from axillary buds in two populations of balsam poplar. Those differences could potentially affect the performance of the regenerates.

2 | MATERIAL AND METHODS

2.1 | Plant materials and growth conditions

Twigs of balsam poplar (*Populus balsamifera* L.) were cut from 12 trees of two populations growing at Thunder Bay, Canada: Lakehead University arboretum (48.428°N, 89.261°W; Population 1, P1) and Confederation College campus (48.402°N, 89.263°W, P2). Trees of similar sizes were selected at least 50 m apart from each other. Twigs of about 1 cm diameter and 100 cm length were sampled and cut into 10 cm sections containing two axillary buds. There was a total of 192 cuttings per population. The sections were treated with a rooting hormone (Plat Prod Stim Root #3, Plant Products Co. Ltd.) and planted in styroblock containers filled with a mixture of peat moss and vermiculite (1/1, v/v). The containers were placed in a polyethylene tent in the greenhouse to maintain high humidity and misted continuously to induce rooting. Rooted cuttings were transplanted into 3.5 L plastic pots filled with peat moss and vermiculite (1:1, v:v). The plants were watered as needed to keep the growing medium moist and fertilized twice a week with 75 mg L⁻¹ of a fertilizer solution (All-Purpose, 24-8-16 N-P-K fertilizer, Plant Products Co. Ltd.).

The greenhouse conditions were 23°C/16°C day/night temperatures, 16-h photoperiod, and 50% RH. The flux density of photosynthetically active radiation at the canopy was 500 μmol m⁻² s⁻¹ on sunny days. The environmental conditions were monitored and controlled using an Argus Titan System (Argus Control Systems Ltd.).

Two kinds of shoots occurred on the cuttings: those originated from an axillary bud and those resulting from an adventitious bud. Many *Populus* species have the capacity to produce shoots from axillary buds and preformed adventitious buds (Figure 1; Douglas 1984) and the first few leaves on those different kinds of shoots are generally morphologically different (Figures S1 and S2; Critchfield 1960). Many *Populus* species can produce adventitious buds in stem internodes (Douglas 1984). The different types of shoots were identified by the corresponding bud type: the first few leaves with narrowly elliptic to lance-ovate shape were derived from adventitious buds, and those with ovate to widely ovate shape were derived from axillary buds (Figures S1 and S2).

2.2 | Gas exchange measurements and carbonic anhydrase inhibitor treatment

When the plants developed about 10 fully expanded leaves, four plants were randomly selected from each combination of population and bud type for gas exchange measurements on the fourth fully expanded leaf (test leaf) from the top (Figure S4). The leaf immediately below the test leaf (i.e. fifth leaf) was used as the feeding leaf for importing anhydrase inhibitor according to Lin's petiole-feeding method (Lin et al. 2011). First, a light response curve, Laisk script measurements, an A/C_i curve (CO₂ response curve), and simultaneous chlorophyll fluorescence measurement were conducted using a PP-Systems CIRAS-3 Portable Photosynthesis System and a PLC3 Universal Leaf Cuvette with auto environment control and a built-in

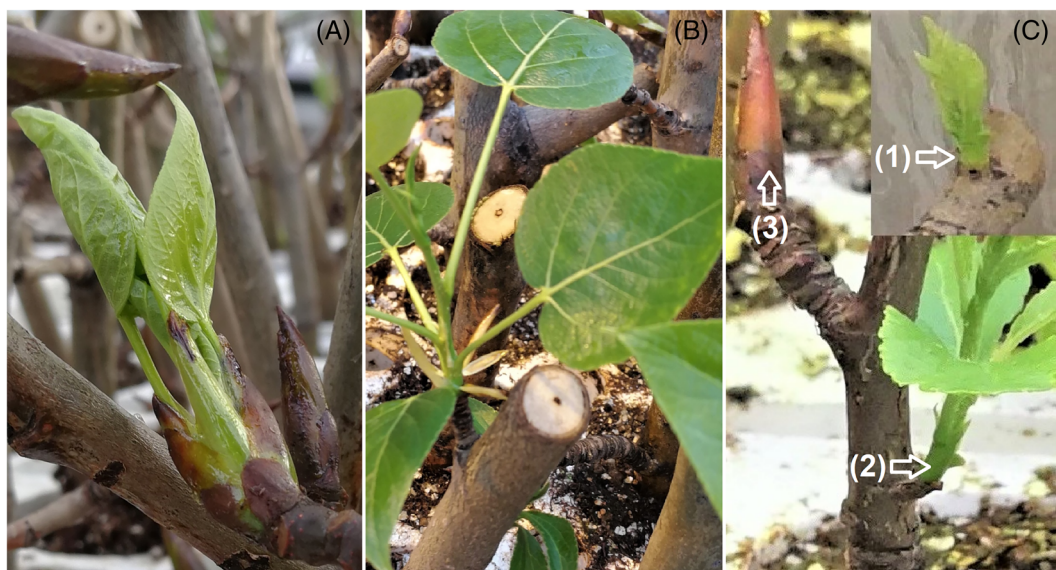


FIGURE 1 Bud break of balsam poplar cuttings. Green leaf emerged from an axillary bud (A) and early leaves on a shoot from an axillary bud (B). Adventitious bud break from bark (C, (1)), shoot with early leaves from adventitious bud (C, (2)) and the axillary buds will eventually drop off (C, (3)). More pictures are provided in the Appendix S1

CFM-3 Chlorophyll Fluorescence Module (PP Systems International, Inc.). Then, the feed leaf was cut off and a syringe was connected to its petiole immediately to feed the plant with 5 mL carbonic anhydrase inhibitor (Acetazolamide) solution (Figure S4; Fromm et al. 2016). The inhibitor solution contained 1 mM acetazolamide and 10 mM NH_4OH (pH = 9.4) (Momayyezi & Guy 2017). Pre-treatment tests showed that there was no significant difference in net photosynthetic rate in the test leaf before and after petiole-feeding treatment with a control solution (5 mL 10 mM NH_4OH without acetazolamide; Table S2). The feed leaf was placed into a freezer of -35°C immediately after being cut off for measuring carbonic anhydrase activity and photosynthetic pigments. Within 1.5 h after the inhibitor was completely absorbed, A/C_i curve and chlorophyll fluorescence measurements on the same test leaf were repeated. Following the measurements, the test leaf was cut off and stored at -35°C to evaluate the inhibition effect on carbonic anhydrase activity.

The Laisk script measurements were conducted at 50, 100, 150, and 200 $\mu\text{mol mol}^{-1} \text{CO}_2$ concentration (C_a) and 75, 150, and 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR. The A/C_i curve was measured at 400, 300, 200, 150, 100, 50, 400, 600, 800, 1000, and 1200 $\mu\text{mol mol}^{-1} \text{CO}_2$ and 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR. The light response curve was performed at 400 $\mu\text{mol mol}^{-1} \text{CO}_2$ and 50, 100, 150, 200, 300, 400, 600, 800, 1000, 1200, 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR.

The A/C_i data were analyzed using the Plantecophys fitaci function of the R package to produce the maximum carboxylation rate of ribulose-1,5-bisphosphate (RuBP; V_{cmax} , $\mu\text{mol m}^{-2} \text{s}^{-1}$), and the maximum electron transport rate of photosynthetic (J_{max} , $\mu\text{mol m}^{-2} \text{s}^{-1}$; Duursma 2015). The light response curve data were analyzed using the rectangular hyperbola model to obtain the apparent quantum yield (Φ), light compensation point (LCP), and light saturation point (LSP).

The “constant J method” was used to determine the mesophyll conductance (g_m) from the rate of daytime respiration (R_d) and CO_2

TABLE 1 ANOVA p -values for the effects of population and bud type on photosynthetic traits, pigment content, and carbonic anhydrase activity of cuttings from axillary buds or adventitious buds of two balsam poplar populations

Factors	Population	Bud type	Population* bud type
V_{cmax}	0.128	0.769	0.314
J_{max}	0.016	0.476	0.701
A_n	0.032	0.465	0.132
g_m	<0.001	0.056	<0.001
C_i^*	0.372	0.051	0.007
R_d	0.016	<0.001	0.153
Φ	0.025	0.001	0.45
P_N	0.435	0.137	0.655
LCP	<0.001	0.012	0.023
LSP	0.002	<0.001	0.967
CA	0.014	0.203	0.002
ACNs	<0.001	0.005	<0.001
Chl	0.009	0.4	0.681
Biomass	<0.001	0.038	0.002
Shoot length	<0.001	0.039	0.361
SLA	<0.001	0.007	0.103

Note: “**” indicates an interaction between population and bud type, significant p -values ≤ 0.05 are bolded.

Abbreviations: ACNs, leaf anthocyanins concentration; A_n , net photosynthetic rate at 400 $\mu\text{mol mol}^{-1} \text{CO}_2$ and 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR; CA, carbonic anhydrase activity; chl, leaf chlorophyll concentration; C_i^* , CO_2 compensation point at intercellular; g_m , mesophyll conductance; J_{max} , maximum electron transport rate of photosynthetic; LCP, light compensation point; LSP, light saturation point; P_N , the max photosynthetic rate at 400 $\mu\text{mol mol}^{-1} \text{CO}_2$ and 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR; R_d , daytime respiration rate; SLA, specific leaf area; V_{cmax} , maximum carboxylation rate of ribulose-1,5-bisphosphate (RuBP); Φ , quantum yield.

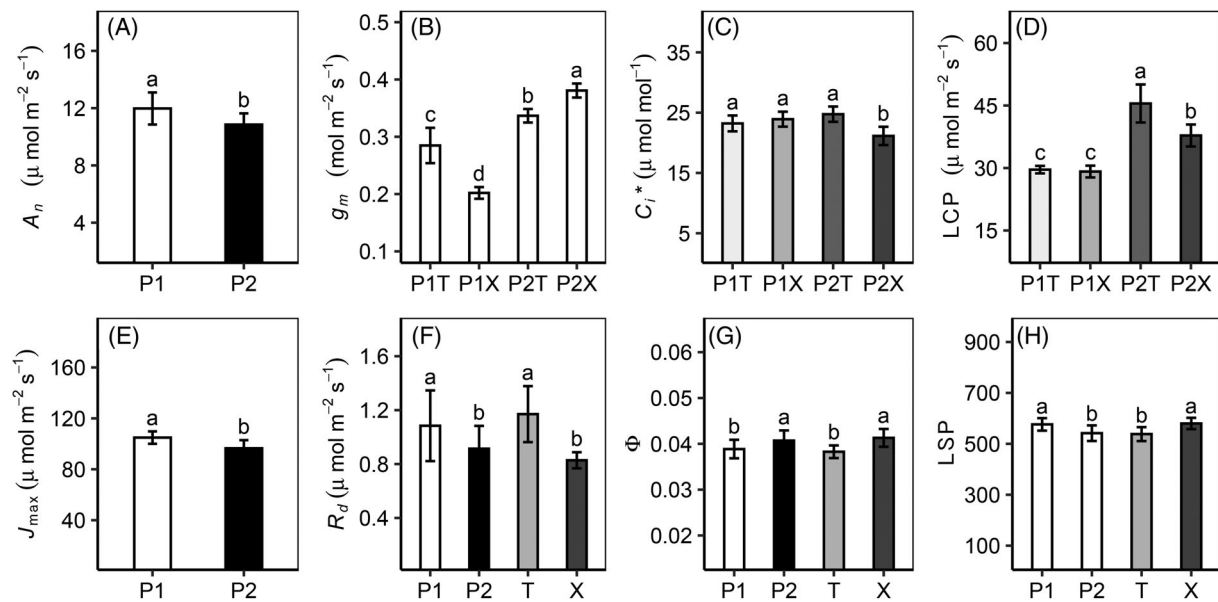


FIGURE 2 Mean values \pm SE ($n = 8$ for panels A and E–H; $n = 4$ for panels B–D) of photosynthetic traits of shoots originated from axillary buds (X) or adventitious buds (T) in two balsam poplar populations (P1 and P2). Means with different letters are significantly different from each other ($p \leq 0.05$). For abbreviations, see Table 1

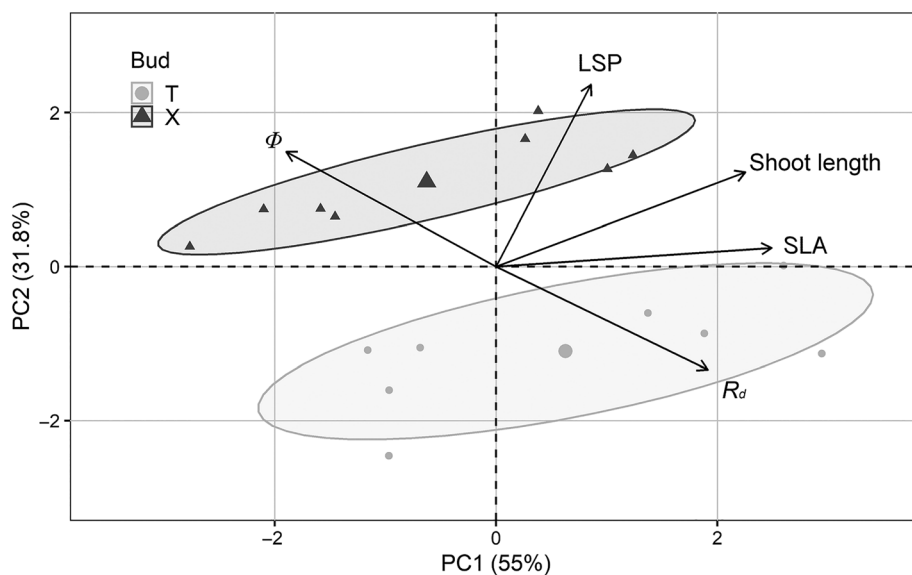


FIGURE 3 Principal component analysis (PCA) of cuttings from axillary buds or adventitious buds of balsam poplar by ϕ (quantum yield), LSP (light saturation point), shoot length, SLA (specific leaf area), and R_d (daytime respiration)

compensation point (intercellular, C_i^*) estimated from the Laisk measurements (Momayyezi & Guy 2017) and electron transport rate (J) from chlorophyll fluorescence measurements (Harley et al. 1992). The chlorophyll fluorescence measurement was taken using the built-in CFM-3 model of the PP Systems CIRAS-3 system.

2.3 | Assay of chlorophyll, anthocyanin content, and carbonic anhydrase activity

Leaf lamina (0.2 g) from the sample leaf was placed in a vial containing 1 mL of 25% acetone water solution, and another 0.2 g leaf lamina

from the same leaf was placed in a vial containing 1 mL water solution of 3 M HCl and 1 M MeOH. After 24 h incubation in darkness, the vials were centrifuged at 840g for 10 min and absorbances were measured with a spectrophotometer (Bio-rad Smart Spec Plus Spectrophotometer) at 530, 647, 653, and 663 nm. The total chlorophyll concentration of the acetone extract was measured at A_{647} and A_{663} according to Alan' method (Wellburn 1994). Anthocyanin concentration was determined from the MeOH extract at A_{530} and A_{653} as $A_{530} - 0.24 \times A_{653}$ (Gould et al. 2000).

Carbonic anhydrase activity was estimated based on the bromothymol blue colorimetric test described by Wilbur and Anderson (1948). Leaf blade (0.2 g) was ground in 1 mL of 40 mM potassium

phosphate buffer (pH = 8.3) using a mortar and pestle on ice. The homogenate was centrifuged for 10 min at 5000g and 4°C, 20 μ L of the supernatant was added to 1 mL of the buffer solution containing 20 mg L⁻¹ bromothymol blue as a pH indicator. One milliliter of CO₂-saturated water at 4°C was then added and the time (as *T*) that it took for the pH of the reaction system to change from 8.3 to 6.3 was recorded. Twenty microliter buffer solution only was used as control and the time of pH change from 8.3 to 6.3 after adding 1 mL CO₂-saturated water was recorded as *T*₀. The carbonic anhydrase activity was calculated as CA (EU) = 10 × (*T*₀/*T* - 1). Our ANOVA tests showed no significant difference in carbonic anhydrase activity between the test leaf and the feed leaf of the same plant (Table S2).

2.4 | Measurement of growth parameters

Twenty discs (the midvein was avoided) were randomly obtained from the fourth and fifth fully expanded leaves of four additional plants with a 2 cm diameter punch, dried and weighed to determine the specific leaf area (SLA). The plants were harvested after height measurement, the root system was washed with running water, and oven-dried at 80°C for 48 h to determine the biomass.

2.5 | Statistical analysis

The effects of population, bud type, and their interactions were tested using two-way ANOVA. The Shapiro test and residual plots were conducted to check the normality and homogeneity of the data. Power transform for variables that did not meet the ANOVA assumptions and the transformed data all met the assumptions. The population was treated as a random factor, while bud type was treated as a fixed factor

in ANOVA. In the 3-way ANOVA for the petiole-feeding experiment, inhibitor and bud type were treated as fixed factors, while the population was again treated as a random factor. Tukey-HSD posthoc pairwise comparisons of means were conducted when the ANOVA showed a significant interaction ($p \leq 0.05$). Pearson correlation analysis and linear regression were performed to examine the relationships between the *A_n*, *g_m*, and carbonic anhydrase activity. All the statistical analyses were carried out using the R package. To investigate intercorrelations among different variables, a principal component analysis (PCA) was performed using the PCA function from the FactoMineR package.

3 | RESULTS

3.1 | Leaf shape and photosynthetic traits

The first few leaves on shoots derived from different types of buds and different populations had different shapes: lanceolate and widely

TABLE 2 ANOVA *p*-values for the effects of population, bud type, and carbonic anhydrase inhibitor (acetazolamide) on *A_n*, *g_m*, and CA activity in balsam poplar cuttings

Factors	<i>A_n</i>	<i>g_m</i>	CA
Population	0.189	<0.001	<0.001
Bud	<0.001	0.003	0.35
Inhibitor	<0.001	<0.001	<0.001
Population * bud type	0.8	0.001	<0.001
Population * inhibitor	0.017	<0.001	0.205
Bud:Inhibitor	0.763	0.3	0.137
Population * bud * inhibitor	0.027	<0.001	0.006

Probabilities ≤ 0.05 are boldface

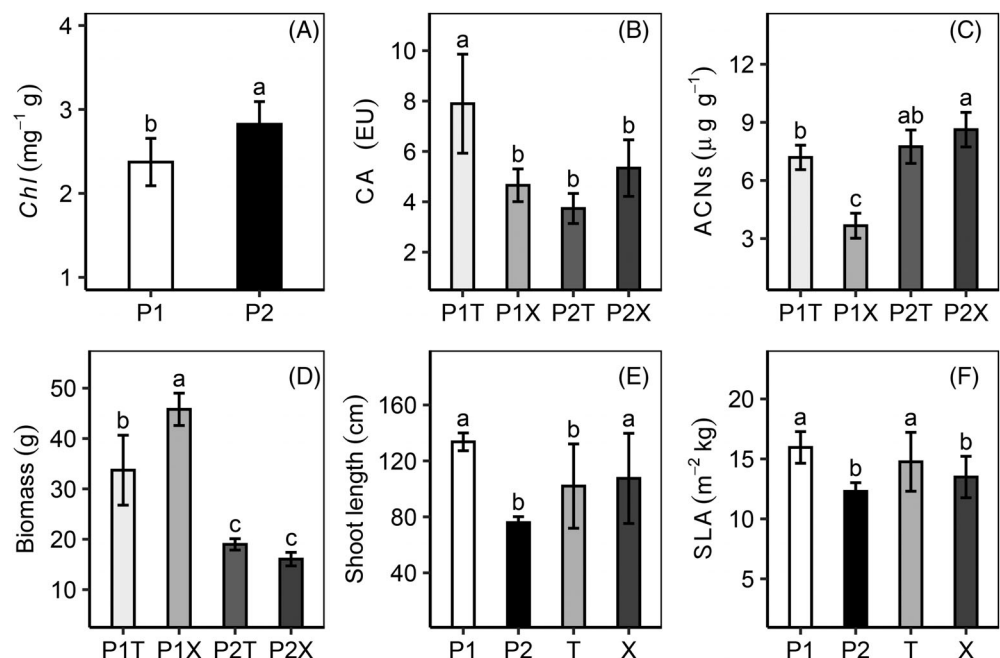


FIGURE 4 Mean values \pm SE ($n = 8$ for panels A, E, F; $n = 4$ for panels B–D) of leaf chlorophyll concentration, carbonic anhydrase activity, and growth traits of shoots from axillary buds or adventitious buds in the two balsam poplar populations

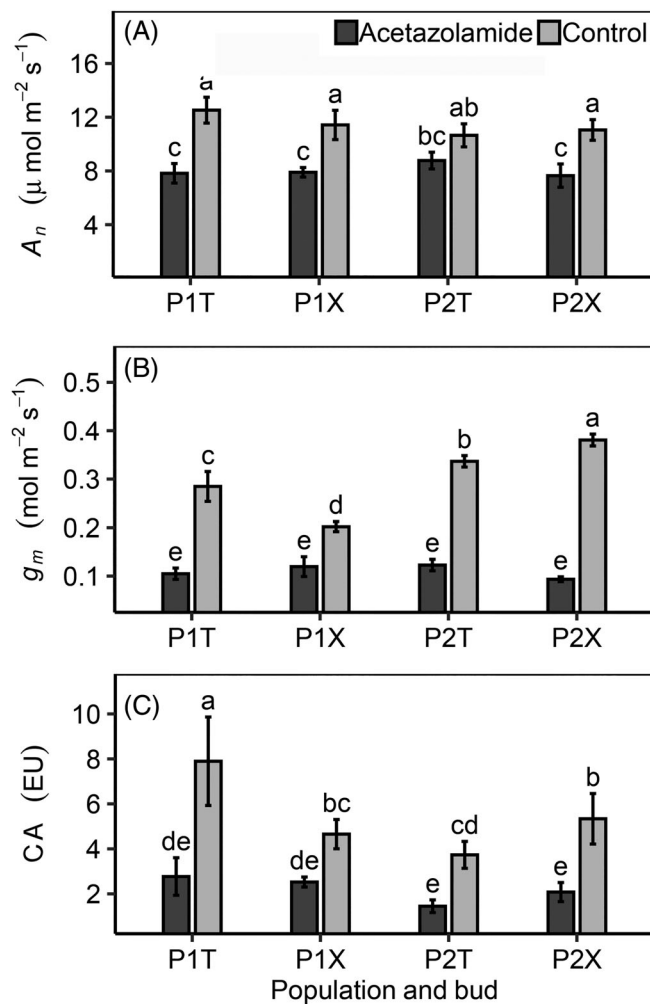


FIGURE 5 FI Effects of carbonic anhydrase inhibitor (acetazolamide) on mean values (\pm SE, $n = 4$) of A_n , g_m , and carbonic anhydrase activity (CA) in balsam poplar cuttings. “Control” stands for the values measured on the test leaf before anhydrase inhibitor treatment, while “acetazolamide” represents the measures on the same test leaf after petiole-feeding of 5 mL anhydrase inhibitor solution containing 1 mM acetazolamide and 10 mM NH_4OH

ovate for adventitious and axillary shoots of Population 1 (P1 from Lakehead University arboretum), respectively (Figure S1A); narrowly elliptic and ovate for adventitious and axillary shoots of P2 (from Confederation College campus), respectively (Figure S1B,C).

There was no significant difference in V_{cmax} , J_{max} , A_n , and P_N between shoots derived from adventitious buds and those originated from axillary buds (Table 1). However, A_n and J_{max} of population 1 were significantly higher than those of population 2 (Figure 2A,E). Population and bud type interactively affected g_m , C_i^* , and LCP (Table 1): g_m of shoots originated from adventitious buds was significantly greater than those from axillary buds in population 1 but the trend was the opposite in population 2. In contrast, C_i^* and LCP of shoots originated from adventitious buds were significantly greater than those from axillary buds in population 2, while there were no significant differences between bud types in population 1 (Figure 2B–D). R_d of shoots that originated from adventitious buds was

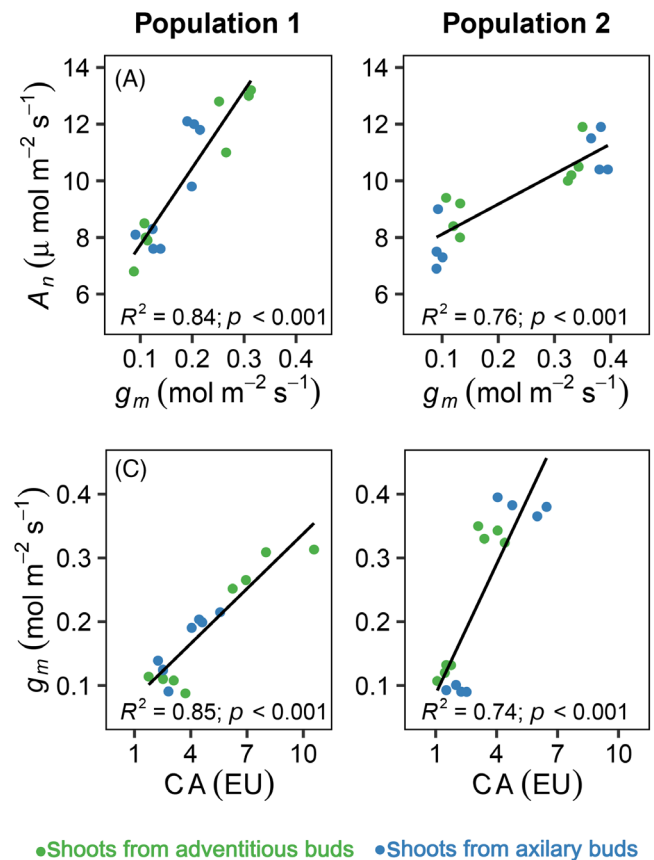


FIGURE 6 Relationship between net photosynthetic rate (A_n , $\mu\text{mol m}^{-2} \text{s}^{-1}$), mesophyll conductance (g_m , $\mu\text{mol m}^{-2} \text{s}^{-1}$), and carbonic anhydrase activity (CA, EU) in two balsam poplar populations. The values of shoots from adventitious buds were shown with green points and from axillary buds were with blue points

significantly higher than that from axillary buds in both populations (Figure 2F), while Φ showed the opposite trend (Figure 2G). The LSP of shoots from adventitious buds was lower than that from axillary buds and was also significantly different between populations (Figure 2H). Principal component analysis revealed that leaves of shoots derived from adventitious buds grouped on daytime respiration and SLA, while cuttings from axillary buds clustered toward the opposite direction of quantum yield and light saturation point (Figure 3).

3.2 | Leaf chlorophyll concentration and growth

Leaf chlorophyll concentration in populations 1 was significantly lower than that in populations 2 (Figure 4A) but was not significantly different between bud types (Table 1). CA and ACNs of shoots from adventitious buds were significantly greater than those from axillary buds in population 1 but not significantly different between bud types in population 2 (Table 1, Figure 4B,C). The shoots from adventitious buds had significantly greater total biomass than those from axillary buds in population 1 but bud type had no significant effect on biomass in population 2 (Table 1, Figure 4D). Shoots from adventitious buds were

shorter but had greater SLA than those from axillary buds, while population 1 had significantly longer shoots and greater SLA than population 2 (Table 1, Figure 4E,F).

3.3 | Effects of carbonic anhydrase inhibitors on A_n , g_m , and CA

There were significant interactive effects among population, bud type, and carbonic anhydrase inhibitor on A_n , g_m , and CA (Table 2). The treatment of petiole-feeding inhibitor significantly reduced A_n , g_m , and CA but the degree of the reduction varied with population and bud types (Figure 5). A_n was positively correlated to g_m , which in turn was positively correlated to CA, but the slopes of the regressions differed between the two populations and the relationships were tighter in population 1 than in population 2 (Figure 6).

4 | DISCUSSION

4.1 | Adventitious buds produced different leaves and shoots than axillary buds

The branching pattern of bud post-embryonic development depends on the type and activity of the meristem and determines the morphological plasticity and reproductive efficiency of higher plants (Silvestro et al. 2020). Axillary buds are derived from axillary meristems and usually develop into dormant buds with scales at leaf axil sites, while adventitious buds occur in tissue produced by cambium and phloem cells (Douglas 1984; vanden Broeck et al. 2018). Our observations show that the first few leaves that originated from adventitious buds in balsam poplar were lanceolate, but the first few leaves from axillary buds were ovate, and the former had a higher SLA but adventitious buds produced longer shoots than did axillary buds.

Variations of repetitive modules, such as the basic shoot units made of a leaf and bud, affect the diversity and environmental plasticity of plant growth (Ejaz et al. 2021). There were obvious variations in photosynthetic characteristics of leaves on shoots from different origins in balsam poplar. However, there were no significant differences in the two parameters related to photosynthetic capacity between the two bud types, confirming that photosynthesis alone may not be a sufficient basis for predicting growth and that respiration and nitrogen assimilation should also be taken into account (Tcherkez et al. 2017). We found that the R_d and SLA of balsam poplar leaves on shoots originated from adventitious buds were significantly higher than those from axillary buds. Furthermore, PCA revealed that shoots from adventitious buds grouped on R_d and SLA. R_d is a manifestation of light-induced metabolic recombination in leaves and represents a highly dynamic metabolic pathway (Tcherkez et al. 2017). Although the data are not sufficient, Tcherkez and Atkin (2021) regard R_d as an important parameter of photosynthesis and a core factor in leaf carbon use efficiency and nitrogen assimilation. The physiological significance of SLA in combination with respiration is the cost of light interception by leaves (Poorter

et al. 2009). SLA is sensitive to light, and higher SLA is conducive to shaded habitat (Liu et al. 2016). It is suggested that having adventitious buds may represent a competitive advantage and improve regeneration capacity under shaded conditions.

Forty-nine and twenty-nine percent of the light energy absorbed by non-stressed C3 plants are used for carbon sequestration and photorespiration, respectively (Skillman 2008). The remainder (22%) may represent diversion to other metabolisms, including R_d , nitrogen assimilation, and starch synthesis. It is found that a decrease in Φ may be more closely related to R_d than to photorespiration (Quero et al. 2019). Our results show that there was a negative correlation between Φ and R_d in balsam poplar cuttings, indicating that there might be an interaction between them (Tcherkez & Atkin 2021). The shoots originated from axillary buds were grouped toward quantum yield, and were significantly longer than those from adventitious buds, suggesting that the ability to produce shoots from axillary buds may represent a competitive advantage for balsam poplar (Li et al. 2020).

It is important to note that the maintenance of axillary buds requires more resources and that axillary buds are vulnerable to herbivore browsing and wildfire damages (Sobuj et al. 2020). The maintenance of adventitious buds, in contrast, consumes less energy (Chabikwa et al. 2019). Under the protection of the bark, the vigor of adventitious buds to produce shoots represents the resilience of a tree species to regenerate after suffering damages (Nolan et al. 2020). It is reported that adventitious roots and primary roots perform similarly in supporting black spruce seedlings but the seedlings invest heavily in adventitious roots (Harley et al. 1992), indicating different development patterns of adventitious organs aboveground and underground. Bud ontogenesis is the result of the interaction of hormones, carbohydrates, and environmental conditions (Lundell et al. 2020). Therefore, the differences in the ability of axillary buds and adventitious buds to produce shoots may reflect a survival strategy and adaptability of a tree species (Landhäusser et al. 2012).

4.2 | Population variation of balsam poplar

It is generally believed that phenotypic variation of tree species has a genetic background and is the result of local adaptation in a heterogeneous environment (McKown et al. 2018). Moreover, a tree species with a larger intraspecific variation generally has a wider range of plasticity and adaptability (Curasi et al. 2019). Based on the two-node cutting system in this study, we found that 4.7% of the cuttings in population 1 produced callus at the incision which then differentiated shoots, while this phenomenon was not observed in the cuttings of population 2. In population 2, 20.8% of the cuttings had double shoots, one originated from an adventitious bud and the other from an axillary bud, but the phenomenon was rarely observed in population 1. The differences may reflect differences in hormones and carbohydrate supply between the populations (Chabikwa et al. 2019), which may also be one of the reasons for the variation in post-embryonic development of different types of buds.

In addition, the cuttings of population 1 had higher photosynthetic capacity and biomass, while cuttings of population 2 had higher g_m , chlorophyll concentration, and LCP. The differences in

physiological characteristics of cuttings from different bud types suggest that shoots from axillary buds in population 1 (p1X) may have greater growth ability and better potential to be used for the regeneration of short-rotation energy forests (Chmura et al. 2021).

4.3 | Effects of CA on photosynthesis and g_m

The g_m , a key limitation to photosynthesis, describes the diffusion of CO₂ from the intercellular space to carboxylation sites in the mesophyll (Carricú et al. 2021; Han et al. 2019; Sun et al. 2019). CA catalyzes the conversion between the gas phase and liquid phase of CO₂ in the mesophyll, which is considered to impact g_m (Carricú et al. 2019; Momayyezi et al. 2020). Acetazolamide (an inhibitor of carbonic anhydrase) that is fed via a leaf-petiole has been found to reduce g_m in balsam poplar (Momayyezi & Guy 2017). We have found similar results in this study, although the determination coefficients from the correlation analysis of changing CA, A_n , and g_m with inhibitor differed between the two populations, suggesting that the photosynthesis of population 1 may be more sensitive to the changes of g_m and CA than population 2. The ability to adjust g_m in response to changes in irradiance in plants is a component of the adaptation to the shade-sun transitions (Fini et al. 2016). It is suggested that balsam poplar may also participate in the adaptation to changing light by developing axillary buds or adventitious buds.

5 | CONCLUSION

Different types of buds in balsam poplar cuttings produced different-shaped leaves and post-embryonic shoots with different growth characteristics: axillary buds produced ovate first few leaves and longer shoots, while adventitious buds produced lanceolate first few leaves with greater specific leaf area. There was no significant difference in photosynthetic capacity between leaves produced by the different types of buds. However, leaves from axillary buds showed greater photosynthetic quantum yield but lower daytime respiration rates than those from adventitious buds. The two populations of balsam poplar showed differences in photosynthetic rates, mesophyll conductance, and relationships between carbonic anhydrase activity, photosynthetic rate, and mesophyll conductance. Different post-embryonic growth traits were found between axillary buds and adventitious buds of balsam poplar, which may be important for the species to different light conditions (Wang & Dang 2021).

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AUTHOR CONTRIBUTIONS

Lei Wang carried out the experiment, data collection and analysis as well as prepared and revised the manuscript. Qing-Lai Dang advised Wang on the experimental design, data interpretation, manuscript preparation and revisions. Both authors read and approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

The dataset generated for this study is available at Lakehead University Dataverse. See specifications under the reference Wang and Dang (2021).

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