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Do Various Conifers Respond Differently to Water Stress? A Comparative Study of White Pine, Concolor and Balsam Fir

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Abstract

Aim of study: Two-year-old containerized balsam and concolor fir and one-year-old Eastern white pine transplants were grown under variable watering regimes with the goal of identifying plant morphological and some physiological traits under water stress.

Area of study: This experiment was conducted in a greenhouse at the Tree Research Center on the Michigan State University campus, East Lansing, Michigan.

Material and methods: Relative root collar diameter (RRCD), height growth (RHG), and root length (RRL) were measured as growth parameters. Stem water potential (Ψ), stomatal conductance (*gs*), net photosynthetic rate (*Anet*), intrinsic water use efficiency (*iWUE=Anet/gs*), foliar potassium (K^+), and calcium (Ca^{+2}) concentration were measured as physiological traits.

Main results: Well-watered transplants had significantly higher RRCD, RHG, and RRL in fir species. Balsam fir and white pine transplants had a higher Ψ than concolor fir under severe stress. Fir species had higher *Anet*, *gs*, and a lower *iWUE* than white pine. White pine had a lower foliar K^+ concentration, while balsam fir had the highest foliar Ca^{+2} concentration. Balsam fir had higher growth and *Anet*, *gs*, and *iWUE* under water stress due to their ability to maintain higher water uptake despite a reduced soil water content.

Highlights: It is concluded that white pine has better drought tolerance because of the ability to withstand water stress through the mechanism of reduced photosynthetic activities and growth, minimize water loss, and increase water uptake.

Keywords: Drought Tolerant, Irrigation, Morphology, Physiology, Water Stress

Bazı Kozalaklı Türlerin Su Stresine Tepkileri Farklı Mıdır? Beyaz Çam, Balsam ve Concolor Göknarının Karşılaştırmalı Bir Çalışması

Öz

Çalışmanın amacı: İki yaşındaki tüplü balsam ve gümüşi göknarı ile bir yaşındaki beyaz çam fidanlarının su stresine altındaki morfolojik ve bazı fizyolojik etkilerinin belirlenmesi amacıyla farklı sulama rejimlerinde yetiştirildi.

Çalışma alanı: Bu çalışma Michigan State Üniversitesi kampüsündeki Ağaç Araştırma Merkezi serasında gerçekleştirilmiştir.

Materyal ve yöntem: Büyüme parametreleri olarak bağıl kök boğazı çapı (RRCD), bağıl boy büyümesi (RHG) ve bağıl kök uzunluğu (RRL) ölçülmüştür. Fizyolojik özellik olarak ksilem su potansiyeli (Ψ), net fotosentez (*Anet*), stoma iletkenliği (*gs*), içsel su kullanım verimliliği (*iWUE=Anet/gs*), yaprak potasyum (K^+) ve kalsiyum (Ca^{+2}) konsantrasyonları ölçüldü.

Temel sonuçlar: Göknar türlerinden iyi sulanan fidanlarında daha yüksek RRCD, RHG ve RRL değerleri tespit edildi. Şiddetli stres altında balsam göknarı ve beyaz çam fidanları gümüşi göknar fidanlarına kıyasla daha yüksek Ψ ölçülmüştür. Göknar türleri beyaz çam fidanlarından daha yüksek *Anet*, *gs* ve daha düşük bir *iWUE*'ye sahiptir. Düşük yaprak K^+ konsantrasyonu beyaz çam fidanlarında gözlenirken en yüksek yaprak Ca^{+2} konsantrasyonu balsam göknarında ölçülmüştür. Balsam göknarı topraktaki su içeriğinin azalmasına rağmen daha yüksek su alımını sürdürme yeteneklerinden dolayı, su stresi altında beyaz çamdan daha iyi bir büyüme ve daha yüksek *Anet*, *gs* ve *iWUE* değerlerine sahiptir.

Araştırma vurguları: Azalan fotosentetik aktivite ve büyüme mekanizması, su kaybını en aza indirme ve su alımını artırma yeteneği yoluyla su stresine karşı koyma beyaz çamı iki göknar türüne göre daha yüksek kuraklık toleransına sahip olduğu sonucuna varılmıştır.

Anahtar Kelimeler: Kuraklık Toleransı, Sulama, Morfoloji, Fizyoloji, Su Stresi

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Introduction

Water is an essential compound for all living creatures, especially plants, to continue their life. Plants mainly depend on the water source from germination to death for their growth, development, and all the physiological processes throughout their life cycle (Koç, 2021a; b). Water stress is a critical abiotic stressor limiting plant growth and productivity among the environmental stresses (Shao et al., 2009; Koç, 2021a; b; Nehemy et al., 2021). The frequency and intensity of plant water deficits are expected to increase due to the ongoing escalation in atmospheric CO_2 concentrations and linked warmer temperatures in the temperate forest (Meehl et al., 2007) due to rising fossil fuel usage (Koç, 2021c). Pine and fir species have sufficient precipitation to maintain soil moisture in their natural habitat supporting tree growth and survival (Gilliam, 2016), but the future is uncertain.

However, pine and fir species are commercially grown in different environmental conditions, which may increase challenges such as low water accessibility and increased temperatures. Under these conditions, plants respond differently to various environmental stresses (Bhattacharjee & Saha, 2014; Koc, 2018). Species have varying abilities to acclimate and survive under disparate hydraulic and water conditions (McDowell et al., 2008; Allen et al., 2010). Even closely related pine species such as Scots pine (*Pinus sylvestris* L.) (Martínez-Vilalta & Piñol 2002) and black pine (*Pinus nigra* Arn. subsp. *salzmannii* (Dunol) Franco) (Sánchez-Salguero et al., 2015) respond differently to water stress.

Plants can develop numerous morphological, physiological, and chemical adaptive traits to alleviate water stress effects and ensure survival and growth under water stress conditions (Ilyas et al., 2021; Seleiman et al., 2021; Zia et al., 2021). The water stress response in the plant is primarily dependent on differences in hydraulic characteristics and patterns of water use (Allen et al., 2010). To reduce water loss by transpiration, robust stomatal control is needed to minimize plant stem water potential changes as the soil water content is reduced. However, this, in turn, would restrict plant carbon gain in photosynthesis and CO_2 intake (Brodribb et al., 2007). Moreover, plants possess mechanisms such as signal transduction in guard cells to control stomatal aperture. For instance, plants synthesize abscisic acid (ABA) in response to water stress, triggering stomatal pores' closing and inhibiting stomatal opening.

Plants with developed network systems in their organs, such as guard cells in leaf epidermises, can signal within a single cell to respond to water stress (Mäser et al., 2003). In the signaling process of stomatal closure, potassium (K^+) in plant tissues not only plays a vital role in the physiology of plants (Paoli et al., 2005) but also controls foliar water content (Babita et al., 2010) and, indirectly, the stomatal functions (Fernández et al., 2006). K^+ is vital for plants on drier sites or during drought conditions for a plant's adaptation to water stress (Cakmak, 2005) and increased resistance to drought conditions (Egilla et al., 2005). Calcium (Ca^{+2}) impacts physiological functions and plant responses to environmental changes through hormonal signals increasing the ability to adapt to drought conditions (Hepler & Wayne, 1985).

The adverse effects of climate change on conifer species such as pine and fir species include increasingly facing drought in their natural habitat and plantation sites. It is known that soil water deficiency induces physiological stress and affects tree species differently depending on specific functional traits such as leaf phenology and phenotypic plasticity (Camarero et al., 2015). For example, pine species tend to close their stomata to avoid water loss via transpiration and embolism in the xylem, which reduces carbon assimilation (Hubbard et al., 2001). The reduction in carbon assimilation, in turn, diminishes growth and productivity (Tyree & Sperry, 1988).

Among conifer species, white pine (*Pinus strobus* (L.) (Michigan)), concolor fir (*Abies concolor* 'Cibola' (New Mexico)) and balsam fir (*Abies balsamea* (L.) Mill. 'Cooks' (New York)) are preferred for this study because of economic importance, renewable resources, desirable color for ornamental value such as Christmas tree production and landscaping. White pine is native to eastern North America, fast-growing softwood, and has been widely used for reforestation, landscaping, and Christmas tree production (Wendel & Smith, 1990). Balsam fir is native to most eastern and central Canada and the northeast United States (Frank, 1990), a slow-growing tree, and has been widely preferred for a Christmas tree due to its strong scent and dark green needles (Cregg, 2016). Concolor fir is native to the western United States, including Colorado, Arizona, Utah, Idaho, Nevada, and New Mexico, and is widely distributed in the southern Rocky Mountains (Laacke, 1990). Concolor fir trees receive less rainfall than white pine and balsam fir in their native range and adapt to drier environments. In their natural habitat, white pine receives 510-2030 mm precipitation annually (Wendel & Smith, 1990) while balsam fir and concolor fir receive 760-1100- and 520-890-mm precipitation, respectively (Frank, 1990; Laacke, 1990).

It has been suggested that species such as white pine and concolor fir could be drought-tolerant (Wennerberg, 2004a; 2004b; Wood, 2006). However, balsam fir performance in unfavorable environmental conditions is not well established. Understanding how these species respond to low water availability is crucial due to global climate change. These species are very economically important for various intensive production systems, and there are indications that they will continue to be planted in large-scale afforestation programs. By improving the knowledge of underlying mechanisms and responses to water stress, managers will better be prepared for changing precipitation patterns and soil water conditions. In this study, we hypothesized that because they tend to produce longer taproots during the early growth period, white pine would better withstand water stress than concolor and balsam fir. The specific objective of this study was to compare the morphological and some physiological responses of species under water stress conditions.

Material and Methods

Study Area

This experiment was conducted in a greenhouse running east to west at the Tree Research Center (TRC) on the Michigan State University campus, East Lansing, Michigan, in 2015. The coordinates of the TRC are 42°65’N and 84°42’W. The greenhouse was covered with a double layer of clear plastic. The maximum daytime and minimum nighttime temperature were 27.04 and 18.52 °C in the greenhouse throughout the experimental period (June 15 - August 15).

Plant Materials and Containerization Substrates

Two-year-old (plug+1) containerized concolor fir and balsam fir, and one-year-old bare-root white pine transplants were obtained from a commercial nursery (Vans Pine nursery, West Olive, MI) beginning of May. Upon reception, tree species were transplanted into black cylindrical 11.2 liters (3-gallons) plastic containers with Fafard 52 potting mix (Conrad Fafard, Inc. Agawam, MA). The potting mix contains processed pine bark (%55), Canadian sphagnum peat moss (30%), a mixture of (15%) perlite, vermiculite, dolomitic limestone, and wetting agents. For uniformity, the roots which are longer than 15 cm were pruned. After transplanting, trees were well-watered to establish them in the containers before the treatments were started on July 15.

Each container received 60g of granular fertilizer applied as a top dress at the beginning of the season. The fertilizer, Osmocote Plus (N:P:K - 15:9:12) fertilizer, is a 5-6 month controlled-release formulation containing 15% total nitrogen, 9% of available phosphate, and 12% of soluble potash (Everris NA Inc, Dublin, OH, USA). In addition to these macronutrients, the fertilizer also contains several micronutrients including 1% magnesium, 2.3% sulfur, 0.02% boron, 0.05% copper, 0.45% iron, 0.06% manganese, 0.02% molybdenum, and 0.05% zinc.

Irrigation Treatment

Before water stress treatment started, five containerized seedlings were used to determine plant water requirements according to the following procedure for each species. A total of 15 containerized transplants (3 species x 5 containerized transplants) were well-watered and allowed to drain gravitationally for 2 hours, and then their weight was measured. After 2 days, the final weights of the containers were recorded. This procedure was repeated 3 times. The difference between each containerized plant's initial and final weights was assumed to correspond to the weight of the water utilized by the tree plus the evaporation from the container's substrate.

Irrigation treatments were then established at 25, 50, and 100% of the initial water requirement as determined above. These values were increased by approximately 10-15% to meet the increasing water demands by transplant later in the test period. The three irrigation volumes corresponding with weekly watering volumes of 1) low (severe-stress) (750 ml), medium (mild-stress) (1500 ml), and high (well-watered) (3000 ml) for balsam and concolor fir; 2) low (severe-stress) (900 ml), medium (mild-stress) (1800 ml), and high (well-watered) (3600 ml) for the pine seedlings. The irrigation regime was applied manually throughout the test period.

Growth Parameters

Stem height, root length, and root collar diameter growth were measured at the beginning and the end of the test period. Initial root length was measured before transplants were placed in containers. Relative height growth (RHG), relative root collar diameter growth (RRCD), and relative root length (RRL) were calculated as the change between the end of the test period and the initial measurement divided by the initial values.

Stem Water Potential

Stem water potential (Ψ) was measured using the pressure chamber method with a plant water status console model 3115 (Soil Moisture Equipment Corp, Santa Barbara, CA) according to Turner (1988). Five cuttings were taken from the new year growth of terminal shoot length ranging from 5-10 cm for each treatment. The samples were placed in the pressure chamber, and the chamber was then pressurized. Measurement was taken when the cut surface became wet or shiny, indicating xylem water coming back to the surface. Measurements were taken at midday between 11:30 AM – 2:30 PM.

Gas Exchange Measurements

Photosynthesis measurements were conducted on three randomly selected trees from each treatment using a Li-Cor (LI6400XT, Lincoln, NE, USA) conifer chamber with a RGB light source (6400-18A). The instrument was calibrated before each measurement, and the reference CO_2, airflow rate, and the quantum flux were maintained at 400 µmol mol^{-1}, 500 µmol mol^{-1}, and 1800 µmol s^{-1}, respectively. The current years' mature sub-terminal shoots were used o for this measurement. All gas exchange parameters were expressed on the projected needle area basis. The procedure was explained by Koç (2021a). ImageJ software was used for image analysis on scanned images (Rashband, 2016). The measured needle area for each specimen was entered as a section in the Li-Cor system for correction of gas exchanges parameters previously measured in the greenhouse for the specimen.

Several physiological parameters, such as net photosynthetic rate (*Anet*) and stomatal conductance (*gs*), were measured. Intrinsic water use efficiency (*iWUE*) was calculated as *iWUE* = *Anet*/*gs*. The ratio of carbon gain to water loss is defined as *iWUE* (Sinclair et al., 1984).

Foliar Potassium (K^+) and Calcium (Ca^{+2}) Concentration

Foliar tissue samples from all plants in each container were collected to analyze foliar K^+ and Ca^{+2} concentrations. Samples were sealed in plastic bags and stored at 4 ºC before the analysis. The fascicles were placed in paper bags and oven-dried (at 65 ± 5 ºC for 72 hours). Needle samples were ground into a fine powder until they passed through a 40-mesh (0.42mm) sieve. Then around 0.3 g of powder was placed in 100-mL digestion, mixing with H_2SO_4 (4.5 mL) and H_2O_2 (1.5 mL) to pre-digest overnight. The next day, the tubes were placed in a digestion block (model AIM600) and heated 340 ± 10 ºC (increasing 5 ºC every hour until 340 ºC), where they stayed constant for an hour. The digested samples were diluted with distilled water and placed on a centrifuge to mix the solution. K^+ and Ca^{+2} analysis was performed on an aliquot of digested tissue on an atomic absorption spectrometer (Aanalyst 400, Perkin Elmer, Waltham, MA, USA).

Data Analysis

The experimental setup was a factorial design with 3 species (white pine, concolor fir, balsam fir) and 3 irrigation levels [low, medium, high]. Each treatment had 5 seedlings for the fir species and 4 seedlings for the pine species totaling 168 individual trees. A level of significance of α=0.05 was used for inferring any statistical significance. Statistical analyses were performed using SAS software 9.4 (SAS Institute Inc., Cary, NC, USA). All variables were tested for normality using PROC TRANSREG and histogram. Data that were not normal were transformed as appropriate, such as RRCD, RHG, and *gs* were normalized using a log transformation when RRL and *iWUE* were normalized using a square root transformation. PROC MIXED was used to conduct analyses of variance (ANOVA) for all variables. Species, irrigation, and time effects were analyzed using repeated measures within PROC MIXED. Mean separation using Tukey's adjustment was used to compare with all significant responses. We also determined both simple linear regressions for allometric variables and correlation coefficients for *Anet* and *gs* in species. In addition, an average linkage, K-means cluster method was used to compute cluster grouping of three conifer species based on *gs* and *Anet*. K-means cluster analysis was conducted using SYSTAT 13 software (Systat Software Inc., Chicago, Illinois, USA).

Results

Relative Root Collar Diameter (RRCD), Height (RHG), and Root Length (RRL) Growth

Balsam fir seedlings had the highest RRCD, followed by concolor fir and white pine (Table 1). Irrigation treatment was significant ($P<0.05$), and well-watered seedlings had higher RRCD than medium and low irrigated transplants. There was no significant effect on the interaction of species and irrigation ($P>0.05$) (Table 2).

Balsam fir transplants had the highest RHG, followed by concolor fir and white pine (Table 1). Low and medium irrigated transplants had lower RHG values, while well-watered transplants showed higher values (Table 1). Under the interaction of species and irrigation, increased irrigation only increased the RHG for balsam fir transplants.

White pine seedlings had the lowest RRL compared to concolor and balsam fir (Table 1). There were no significant differences between fir species in terms of RRL.

Table 1. Mean and standard errors of morphological traits under the interaction of species (balsam fir, concolor fir, and white pine) and irrigation levels.

		Species			
	Irrigation	Balsam fir	Concolor fir	White pine	Mean
RRCD (mm/mm)	Low	0.61±0.02 b	0.51±0.02 b	0.17±0.02 c	0.43±0.01 C
	Medium	0.66±0.02 b	0.53±0.02 b	0.28±0.02 b	0.49±0.01 B
	Well-watered	0.78±0.02 a	0.70±0.02 a	0.36±0.02 a	0.61±0.01 A
	Mean	0.68±0.01 A	0.58±0.01 B	0.27±0.01 C	
RHG (cm/cm)	Low	0.33±0.01 b	0.31±0.01 a	0.20±0.01 a	0.28±0.01 B
	Medium	0.37±0.01 ab	0.29±0.01 a	0.21±0.01 a	0.29±0.01 AB
	Well-watered	0.39±0.01 a	0.30±0.01 a	0.21±0.01 a	0.30±0.01 A
	Mean	0.36±0.01 A	0.30±0.01 B	0.21±0.01 C	
RRL (cm/cm)	Low	0.81±0.01 a	0.80±0.01 a	0.73±0.01 b	0.79±0.00 AB
	Medium	0.81±0.01 a	0.83±0.01 a	0.76±0.01 a	0.80±0.00 A
	Well-watered	0.80±0.01 a	0.76±0.01 b	0.74±0.01 ab	0.77±0.00 B
	Mean	0.81±0.00 A	0.81±0.00 A	0.74±0.00 B	

Note: [a]Means within a column followed by the same capitalized letter are not significantly different at 0.05 level for species. [b] Means within row followed by the same capitalized letter are not significantly different at 0.05 level for irrigation. [c] Means and standard errors in tables followed by the same lowercase letter are not significantly different at 0.05 level for irrigation within each species.

Table 2. Degrees of freedom (df), F values for the repeated measures of analysis of variance (ANOVA) for relative root collar diameter (RRCD), relative height growth (RHG), relative root length (RRL), stem water potential (Ψ), net photosynthesis (*Anet*), stomatal conductance (*gs*) and intrinsic water use efficiency (*iWUE*) among three species and three irrigation levels.

Source of variation	df	F-values RRCD	RHG	RRL	Ψ	*Anet*	*gs*	*iWUE*
Between subjects								
Species (S)	2	273.24***	219.81***	98.67***	4.35*	232.12***	135.10***	6.41**
Irrigation (I)	2	53.59***	3.65*	0.36	35.55***	4.12*	7.44**	6.22**
Within subjects								
SxI	4	1.44	2.83*	2.30	0.69	3.36*	6.40**	8.63***

Note: Significant levels for repeated measures are given as corrected probabilities: * P < 0.05; ** P < 0.01; *** P <0.001

Physiological Parameters

Stem Water Potential

Species and irrigation had a significant effect on Ψ (P<0.05) (Table 2). For the independent variable species, balsam fir transplants had statistically higher Ψ when concolor fir had the lowest Ψ. There were no significant differences between white pine and concolor fir transplants. Well-watered treatment transplants had higher Ψ and followed by medium and low watered treatment had the lowest values. Under the interaction of species and irrigation, the well-watered transplants that received high irrigation levels maintained higher Ψ when compared to medium and low irrigation for each species (Figure 1). Balsam fir had higher Ψ compared to white pine and concolor fir under well-watered treatment (Figure. 1). However, under low watered treatment, white pine had higher Ψ than balsam fir, where balsam fir was affected more from water stress, and there were no statistical differences between these two species (Figure 1). There were no statistical differences between species under medium stressed treatment (Figure 1).

Net Photosynthetic Rate (Anet)

Species and the interaction of species and irrigation had a significant effect on *Anet* (P<0.05) (Table 3). White pine seedlings had the lowest *Anet* when compared to the two fir species. The interaction between species and irrigation was significant (P=0.012) when *Anet* increased with increasing irrigation (Well-watered > Medium = Low).

Low and medium-watered white pine transplants had the lowest *Anet* compared to all other interactions of species and irrigation (Figure 2A). Balsam and concolor species transplants under each combination of species and irrigation had significantly higher *Anet* than white pine transplants (Figure 2A).

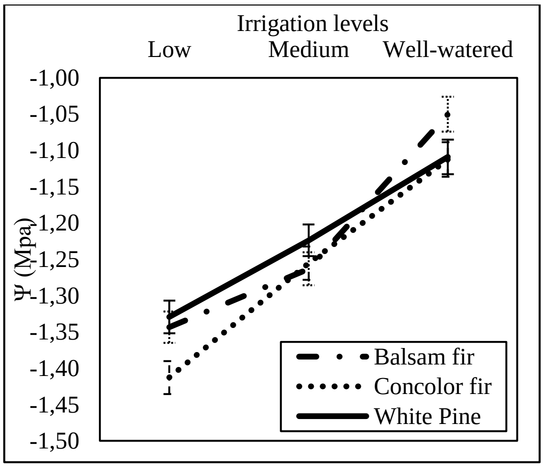


Figure 1. Mean and standard errors of white pine, balsam, and concolor fir transplants of midday stem water potential (Ψ) (Mpa) under three irrigation levels. Irrigation levels: Low= 750 ml, Medium= 1500 ml, Well-watered= 3000 for balsam and concolor fir; for white pine Low= 900 ml, Medium= 1800 ml, Well-watered= 3600 ml of water requirement.

Stomatal Conductance (gs)

Species, irrigation, and their interaction were significant for *gs* (P<0.05) (Table 2). For the independent variable species, white pine seedlings had a lower *gs* when compared to balsam and concolor fir. For the independent variable irrigation, *gs* increased with increasing irrigation (Well-watered > Medium = Low) across all treatments. Under the interaction of species and irrigation treatments, two fir species had higher *gs* than white pine transplants (Figure. 2B). Low and medium watered white pine transplants had the lowest *gs* than well-watered treatment.

Intrinsic Water Use Efficiency (*iWUE* (%))

iWUE was significantly affected by species, irrigation, and their interaction ($P<0.05$) (Table 3). For the independent variable species, white pine transplants had a higher *iWUE* than balsam and concolor fir, and there were no significant differences between the two fir species. For the independent variable irrigation, well-watered transplants had higher *iWUE* compared to low and medium irrigation treatments. The interaction of species and irrigation was significant ($P<0.0001$), showing that well-watered white pine transplants had higher values compared to all other interactions (Figure. 2C).

The significant relationship between *Anet* and *gs* was highest on white pine ($R^2 = 0.53$) (Figure. 3C) followed by balsam fir ($R^2 = 0.30$) (Figure. 3A) where the lowest relationship on concolor fir ($R^2 = 0.23$) (Figure. 3B). In general, both well-watered seedlings had higher *gs* and *Anet*, however, increased *gs* more rapidly increased *Anet* linearly in white pine compared to fir species. Cluster analysis classification technique was used to group low-watered and well-watered treatments within each species in terms of *gs* and *Anet*. Three distinct cluster groups can be identified within species (Figure. 3D). Both fir species behaved the same without significant changes with irrigation treatments compared to white pine seedlings. However, white pine transplants clustered within two groups that well-watered transplants had higher *gs* and *Anet* than low-watered transplants.

Potassium (K^+) and Calcium (Ca^{+2}) Concentration on Needle Tissues

Potassium concentration differed among species and irrigation treatments ($P<0.05$). White pine transplants had a lower K^+ concentration than balsam and concolor fir when examining species as a single factor (Figure. 4A). Severe-stressed transplants had the highest K^+ concentration (2.87 mg/L) compared to mild-stressed (2.81 mg/L) and well-watered transplants (2.31 mg/L) when examining irrigation as a single factor (data not shown).

Calcium concentrations were only measured twice (day17 and day 32) during the experiment period. Species, irrigation, and their interaction were significant ($P<0.05$). Balsam fir transplants had the highest Ca^{+2} concentration (2.75 mg/L), followed by white pine transplants (2.24 mg/L), and concolor fir transplants had the lowest values (1.71 mg/L) when examining species as a single factor (data not shown). For the independent variable irrigation, well-watered seedlings had the highest Ca^{+2} concentration (3.01 mg/L) than medium watered (1.95 mg/L) and low watered (1.74 mg/L) seedlings with no significant difference between medium and low watered treatments (data not shown).

Well-watered balsam fir seedlings had the highest Ca^{+2} concentration, while low-watered concolor seedlings had the lowest values under the interaction of species and irrigation (Figure. 4B). Increasing irrigation increased Ca^{+2} concentration for concolor and white pine seedlings while increasing irrigation reduced Ca^{+2} concentration (medium) first and then increased Ca^{+2} concentration (well-watered) for balsam fir (Figure. 4B).

Discussion

Effect on RRCD, RHG, and RRL Response

In this study, species and irrigation significantly affected tree growth and development under water deficiency. Pine species tend to increase root growth and/ or relative allocation to roots (Teskey et al., 1987), while two fir species tend to increase their height growth under water stress conditions (Gower et al., 1992) which was similar to our results. Balsam fir transplants had the greatest increase in RRCD and RHG followed by concolor fir and white pine seedlings. While, as expected, increased irrigation resulted in a subsequent increase in growth parameters, the species did have different response mechanisms to growth under water stress conditions. In general, height growth occurs in the early growing season, while diameter expansion occurs later in the growing season, where water availability has a greater impact (Nikiema et al., 2012). Decreasing water availability reduces cell elongation, negatively affecting

RRCD and RHG while inducing root production (Klooster et al., 2010; Nzokou & Cregg, 2010). Roots not only may grow deeper into the soil to reach available water but may also act as sensors for shoots regarding water shortage conditions (Hamanishi & Campbell, 2011). Increasing root length allows plants to access deeper soil water and is vital for transplants under water stress (Cregg & Zhang, 2001). However, plant stock type selection can influence root growth in drier sites due to different growth capabilities (Grossnickle, 2012) that containerized-grown seedlings may have a greater root growth during the first field growing season (Becker et al., 1987). In general, containerized seedlings experience less planting stress than bare-root seedlings due to stock type differences (Nilsson & Örlander, 1995).

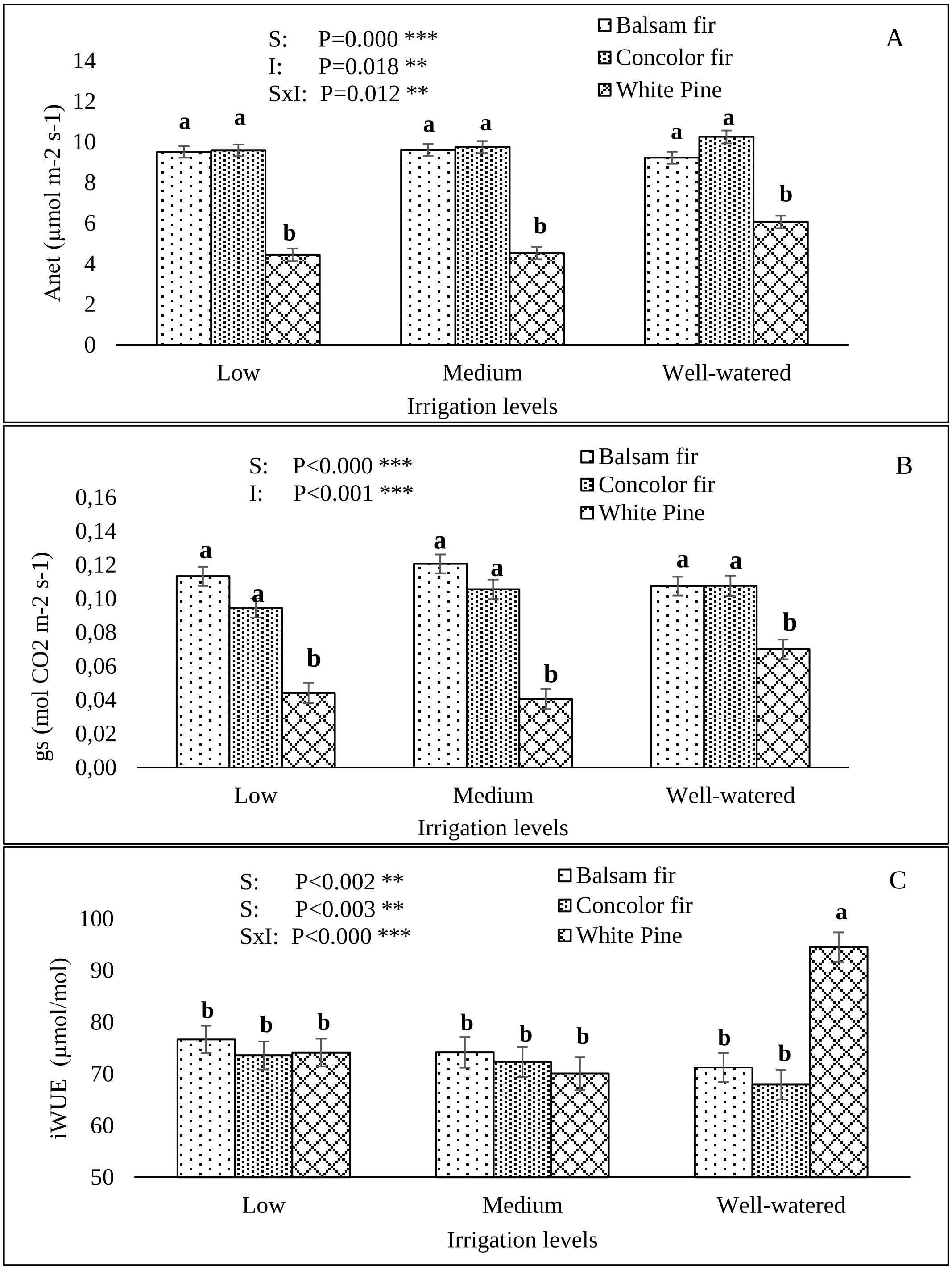


Figure 2. Mean, standard errors, and p-values of *Anet* (A), *gs* (B), and *iWUE = Anet/gs* (C) under the combination of three species and irrigation levels. $^{**}P < 0.01$; $^{***}P < 0.001$.

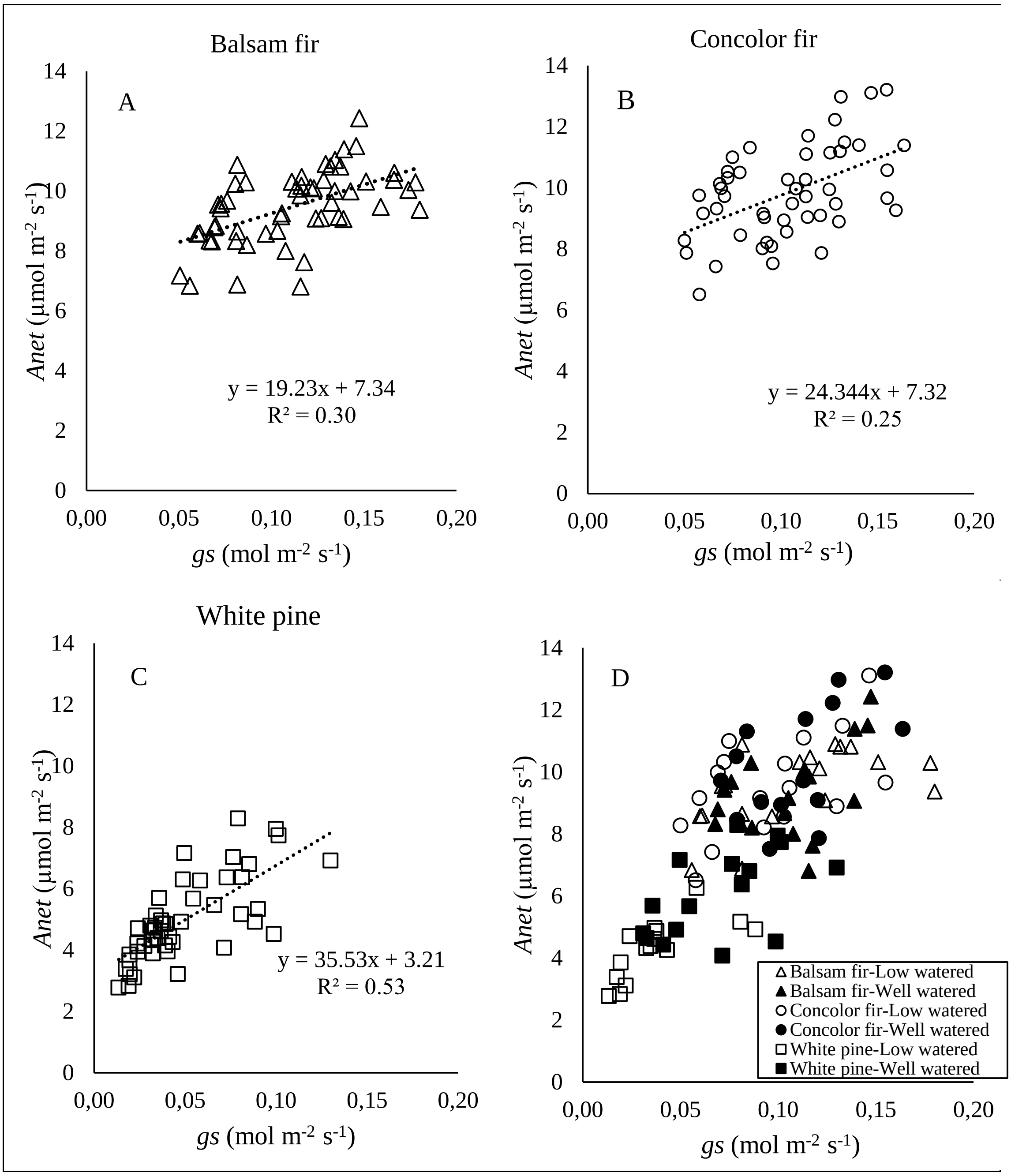


Figure 3. Relationship between *Anet* and *gs* within balsam fir (A), concolor fir (B), white pine (C) species, and K-means cluster analysis of the *Anet* and *gs* for these species under two irrigation levels (D) (low and well-watered).

Note: Cluster analysis of *Anet* and gs separated species in three groups [White pine low-watered (-.-.-), White pine well-watered (-) and two fir species (…..) under low and well-watered treatments (Figure. 3D).

Effect on Plant Physiology

Stem water potential was used as one of the main indices of water stress in this study. Vapor pressure deficits are elevated in the middle of the day, resulting in decreased Ψ in plants (Williams & Araujo, 2002). The needle-to-air vapor pressure deficit, needle/plant water status, and ABA accumulation were physiological factors for stomatal opening and closing (Farquhar & Sharkey, 1982). To reduce the adverse effects of water deficit, plants tend to close their stomata. However, there are inherent differences between species in terms of water stress responses. This study found that concolor fir seedlings had a lower midday Ψ than the other two species under high water stress, while there were no differences between species under medium stress. Another study found similar differences between Douglas-fir and lodgepole pine in terms of patterns of midday Ψ (Andrews et al., 2012). Balsam fir showed the highest Ψ compared to other species when there were no differences between white pine and concolor fir seedlings under well-watered treatment, which showed differences in their response to water stress. Under severe and mild water stress, a more conservative strategy of stomatal conductance regulated by water loss was observed for white pine seedlings in this study. Similar results were observed for Douglas-fir (Domec et al., 2008). However, containerized seedlings may improve root growth compared to bare-root seedlings, reducing their resistance to water uptake (Grossnickle & Blake, 1987). This may support a greater *Anet* in containerized seedlings due to a favorable plant water status.

In general, with decreasing water availability, xylem pressure potential and *Anet* and *gs* decreased (Koç 2021a; b). We observed higher *Anet* and *gs* values in two fir species than white pine transplants in this study. The differences in leaf morphology and the drought-adaptation mechanisms (morphological, physiological, biochemical) of tree species may be caused these differences. However, the relationship between *gs* and *Anet* was higher in white pine transplants than fir species seedlings (Figure. 3. (A, B, C)). The cluster analysis results were also in line with linear regressions results that fir species transplants clustered in a group while white pine transplants separated into two groups. Due to root morphology and length, adaptation mechanisms of species, needle size, and shape, fir species had a different cluster than white pine transplants in terms of *Anet* and *gs*. Well-watered white pine transplants had a different cluster than low-watered ones, while no differences within irrigation treatments for balsam and concolor fir.

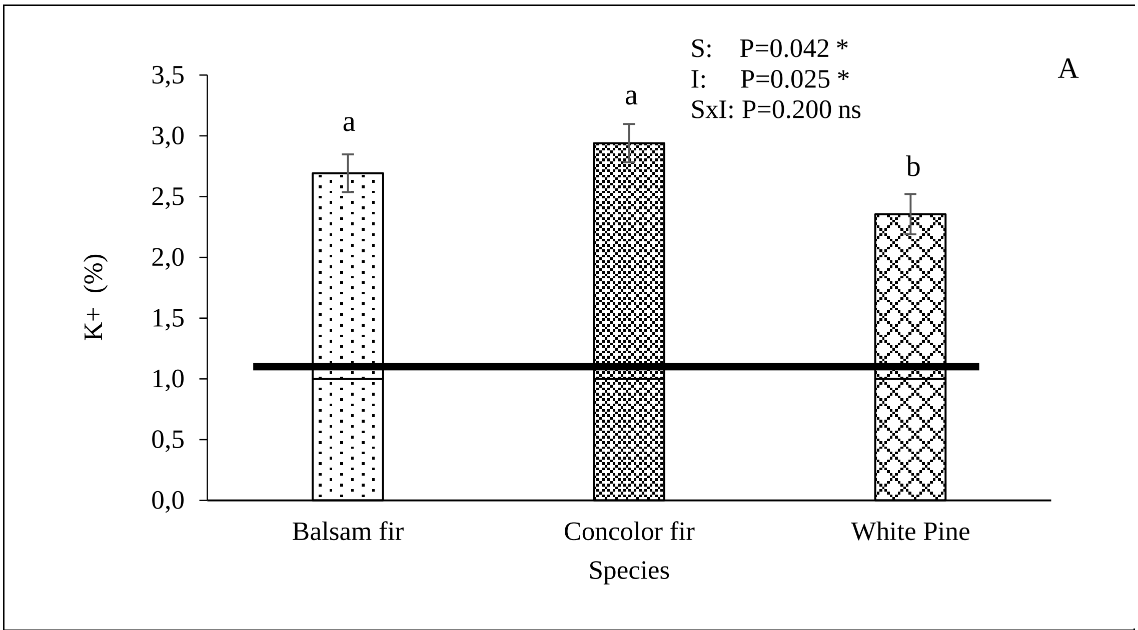
In conifers, stomatal closure is driven by a combination of reduced Ψ and increased hormones such as ABA. Water stress triggers leaf cells' responses to cause a decline in guard cell turgor, thereby reducing or closing stomatal apertures (Buckley, 2005; Brodribb & McAdam, 2013;). In this experiment, fir species transplants had a higher *gs* compared to white pine seedlings. In the combination of species and irrigation, there were no significant differences in *gs* within each species except white pine, where increasing irrigation increased the *gs* values. Similar results were observed for *Anet*.

In contrast to the fir species, white pine species close their stomata at an earlier stage of drought, thus reducing photosynthetic activities (Ilyas et al., 2021; Seleiman et al., 2021; Zia et al. 2021). Similar gs results were observed between Scots pine, Douglas-fir, and black pine (Zweifel et al., 2009). Similarly, the reduction of photosynthetic activities was reported in other studies (Ciais et al., 2005; Granier et al., 2007; Reichstein et al., 2007) where transpiration within each species also showed similar trends like *gs* and *Anet* (Li et al., 2003).

iWUE had an opposite trend to the other gas exchange parameters, where white pine transplants had a higher *iWUE* than the two fir species used in this experiment. Even though species did not differ with increasing irrigation levels, increasing irrigation regimes decreased the *iWUE* for balsam and concolor fir seedlings. Plants can achieve higher *iWUE* through either high *Anet* values (Sinclair et al., 1984), low *gs*, or both. Trees use water more efficiently to increase growth under water stress (Wright et al., 1993; Koç 2021a; b). Other studies have also shown that trees with higher *iWUE* values show higher productivity or growth than trees with lower *iWUE* under water stress conditions (Jones 1993; Koç 2021a; b). Higher *iWUE* often results from decreased *gs* and/or reduced leaf area (Zhang et al., 1997), which act as constraints for *Anet* and growth (Brendel et al., 2002).

This study found that fir species had a higher K^+ concentration in their foliar tissues than white pine transplants. Decreasing irrigation increased the K^+ concentration in general. Similar results have been reported for Norway spruce and the northern red oak (Le Thiec et al., 1995). Additionally, Nilsen (1995) pointed out that increasing drought strain decreased the K^+ concentration in Norway spruce.

Ca^{+2} concentration significantly decreased with decreasing irrigation levels for each species in this study. This shows Ca^{+2} is involved in the regulatory mechanisms in trees' responses to water stress due to an evoke increases in Ca^{+2} in guard cells by ABA (Bartels & Sunkar, 2005), aligning with previous studies (Ma et al., 2005; Shao et al., 2008; Xu et al., 2013). Concolor and white pine showed the highest Ca^{+2} concentration that refers to the stomatal closure of these two species compared to balsam fir under the interaction of species and irrigation. This may make these two species more susceptible to water stress compared to balsam fir.



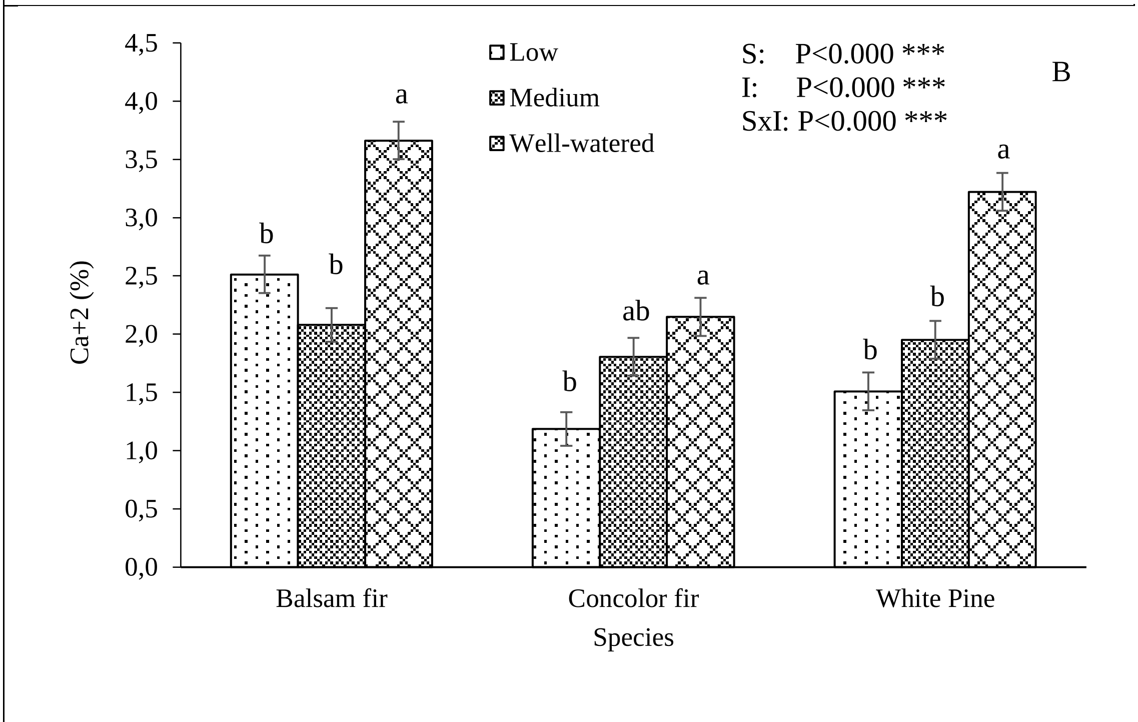


Figure 4. Mean (±s.e) of white pine, balsam, and concolor fir transplants of needle (**A)** potassium (K^+) within species, and (**B**) calcium (Ca^{+2}) concentration (%) under three irrigation levels within each species (alpha=0.05). Sufficient concentration for K^+ in conifer species is approximately 1 % (Cregg, 2005), shown in this graph (**A**) with a bold line. ns =not significant;*P < 0.05; ***P <0.001.

Conclusions

The study aimed to investigate various morphological and physiological parameters for three temperate climate conifer species under water stress conditions. Balsam fir seedlings responded to water stress with greater RRCD and RHG growth, followed by concolor fir and white pine. White pine had a lower RRL compared to the fir species rejecting our hypothesis. Due to higher Ψ and greater root development, the low drought-tolerant balsam fir seedlings took advantage of increasing plant growth. Under severe stress, white pine and balsam fir seedlings showed the more positive Ψ values, where concolor showed the more negative Ψ values and are more susceptible to water stress.

Additionally, white pine can use adaptation mechanisms to tolerate water stress, such as reducing Ψ via closure of stomata, reduction of growth and cell enlargement, and limitation of gas exchanges. Due to these adaptations, the fir species showed higher gas exchange parameters; *Anet*, *gs*, and a lower *iWUE* than white pine. However, white pine seedlings quickly respond to water stress by closing their stomata, thus leading to a lower *Anet*. This may play a vital role in having a higher *iWUE* in terms of being a better drought-tolerant species than the two fir species.

In addition to having higher Ca^{+2} values, balsam fir has a higher K^{+1} value together with concolor firs than white pine. It is well-known, K^{+} and Ca^{+2} play a vital role in signaling under water stress conditions where increased K^{+} concentration opens stomata (Ilyas et al., 2021). This mechanism can explain to some degree drought tolerance in specific species (white pine and concolor fir species) and why these species may be more susceptible to stomatal signaling than weak drought-tolerant species.

We conclude that the three species tested showed differences in terms of tree morphology and gas exchange parameters. White pine and balsam fir seedlings showed some similarities in stem water potential under severe water stress conditions. However, the physiological responses to water stress in conifer species are more complicated than just morphological responses. This implies a more direct link between genetic differences of species and responses to waters stress. Additional research looking at the genetic differences of conifer species is needed to explain the various morphological and physiological responses to water stress.

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Ethics Committee Approval

N/A

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Author Contributions

Conceptualization: İ.K., P.N.; Investigation: İ.K.; Material and Methodology: İ.K.; Supervision: P.N.; Visualization: İ.K., P.N.; Writing-Original Draft: İ.K.; Writing-review & Editing: İ.K., P.N.; Other: All authors have read and agreed to the published version of manuscript.

Conflict of Interest

The authors have no conflicts of interest to declare.

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