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Growth and Physiological Responses of Common Yew Seedling to Drought Stress

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ABSTRACT

Aims: The common yew (*Taxus baccata* L.) is an endangered species in Iran. Considering the prospect of climate change and global warming in the coming years, research on the tolerance of its seedlings to drought stress can be helpful.

Materials & Methods: This research was conducted on four-year-old common yew potted seedlings. For this purpose, the effect of drought stress (100% and 30% of field capacity (FC)) on the growth and physiological traits of common yew seedlings was carried out in a completely randomized design with three replications for six months.

Findings: The results showed that survival, shoot growth, and root diameter growth of seedlings did not change under water deficit, but a significant adverse effect in most of the physiological variables (except for stomatal conductance) was found under stress (30% of FC); so, the activities of photosynthesis, transpiration, mesophyll conductance and water use efficiency decreased by 68.3%, 23.9%, 69.6% and 57.9%, respectively; On the contrary, intercellular CO_2 increased by 4%.

Conclusion: Due to their slow growth, water scarcity did not affect yew seedlings' growth traits in the first year of the growing season. Since common yew seedlings need several years of care in the nursery to prepare for the transfer to the natural field, it is recommended that they be managed in well-watered conditions to better respond to physiological traits and more favorable growth in the coming growing years.

Keywords: Growth; Photosynthesis; *Taxus baccata* L; Water Deficit Stress; Water Use Efficiency.

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Introduction

According to the report of the World Climate Change Conference [1], the pattern of global warming has been started for years and will continue in the future. In recent years, climate change in the form of drought has affected all world regions. So, based on the results of climate change studies in the Mediterranean Sea basin, the decrease in temperature and precipitation or the shortage of water has always been associated with an increase in the risk of forest fires in the region. So, due to the adverse effects of temperature increase on the water cycle, drought stress will be significant in the future [2].

Drought stress is considered the most critical and limiting abiotic stress for plant growth, and it has been stated that other stresses are affected by this type of stress [3,4,5]. Drought stress also, depending on the intensity and duration of drought, causes changes in the growth and absorption of elements in the roots and disrupts their transfer to aerial organs [6,7,8]. Water deficit in plants occurs when the amount of water the plant loses through transpiration is more than the water absorbed by the roots [9, 10, 11]. The first reaction of plants to drought stress is to close the stomata to prevent transpiration and water loss. In this situation, stomatal closure and reduction in gas exchanges and plant growth occur by the abscisic acid hormone [12]. The growth and development of plants are the result of various vital activities, including water availability. If the required water is not supplied due to the reduction of the turgescence pressure in the growing cells, the growth of the plant is disturbed [13, 14, 15, 16], and in the case of severe water shortage, the survival and establishment of the plant are exposed to danger (17,21).

Common yew (*Taxus baccata* L.) belongs to the Taxaceae family and is an evergreen and non-resinous plant [22]. This species is shade-loving and mixed with other forest species in the understory of humid forests in the Mediterranean region and some parts of Asia. In the forests of northern Iran, common yew is found at altitudes of 900-1800 m above sea level, from Astara to Aliabad. It is also present in the Zarrin Gol Ramian (Golestan Province), which consists of several almost pure stands [23]. Common yew grows in most soils but grows better in sedimentary soils and only grows well in solid and dry soils. The tolerance of common yew to air pollution is high, which is why it is generally used in parks and green spaces in cities [24]. The yew tree contains secondary metabolites such as taxol, considered the world's most effective known anticancer medicine [25].

Several researches have been conducted on the effect of drought or water scarcity on conifer species. In this regard, the research conducted on *Larix decidua* Mill [26], *Pinus ponderosa* [27], hybrid poplar genotypes [28], European larch and Norway spruce Douglas-fir, silver fir [29] can be mentioned. This is even though no specific study has been reported so far regarding the response of common yew to drought stress. Of course, in recent years, regarding other environmental stresses, including radian and/or shade, there have been several types of research on seedlings of common yew, which can be referred to as research conducted by [30, 35]. Common yew is one of the endangered species in Iran. Considering the prospect of climate change and global warming in the coming years [36], research on the tolerance of its seedlings to drought stress can be helpful. For this reason, the present research targets this essential with common yew seedlings. We hypothesize that drought stress does not influence common yew seedlings' growth characteristics but alters their physiological activities.

Materials & Methods

Research Design

This research was conducted on potted

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Figure 1) A view of examined common yew seedlings: well-watered seedlings (left) and drought-stressed seedlings (right).

seedlings of a four-year-old common yew (*Taxus baccata* L.). It is worth mentioning that, first, the collected seeds of common yew trees were sown in the nursery bed of Nowshahr Ecology Research Station, and in the fourth year, they were replanted in 3 kg plastic pots. The experiment was conducted in a completely randomized design with 144 seedlings in two levels of drought stress (30% and 100% of field capacity (as a control) for six months. Determination of soil field capacity was done according to the weight method [37]. Before applying drought stress, growth traits (shoot height and root collar diameter) were measured, and at the end of the period, these indices were measured again. The amount of shoot growth and root diameter growth was determined by subtracting the measurement of two periods.

Measurements

A graduated ruler was used to measure the height with an accuracy of mm, and a digital caliper with an accuracy of 0.1 mm was used to measure the collar diameter. Also, at the end of the drought stress period, the survival of seedlings was checked, and their values were determined by calculating the ratio of the number of live seedlings to the number of seedlings before the stress (as a percentage). At the end of the period, physiological variables, including net photosynthesis rate (A), transpiration rate (E), stomatal conductance (Gs), and intracellular CO_2 concentration (Ci) using a portable gas exchange device LI-6400 (LiCor Inc., Lincoln, USA) were measured. For this purpose, three seedlings were selected from each replication, and four fully developed and healthy leaves were selected from the upper part of each seedling. Measurements were made between 9 am and 12 am on a sunny day with a light intensity of 1400 $\mu mol.m^{-2}$ per second. Water use efficiency was calculated from photosynthesis to transpiration and mesophyll conductance from photosynthesis to intracellular CO_2 concentration [38].

Statistical Analysis

Kolmogorov-Smirnov's and Levene's tests

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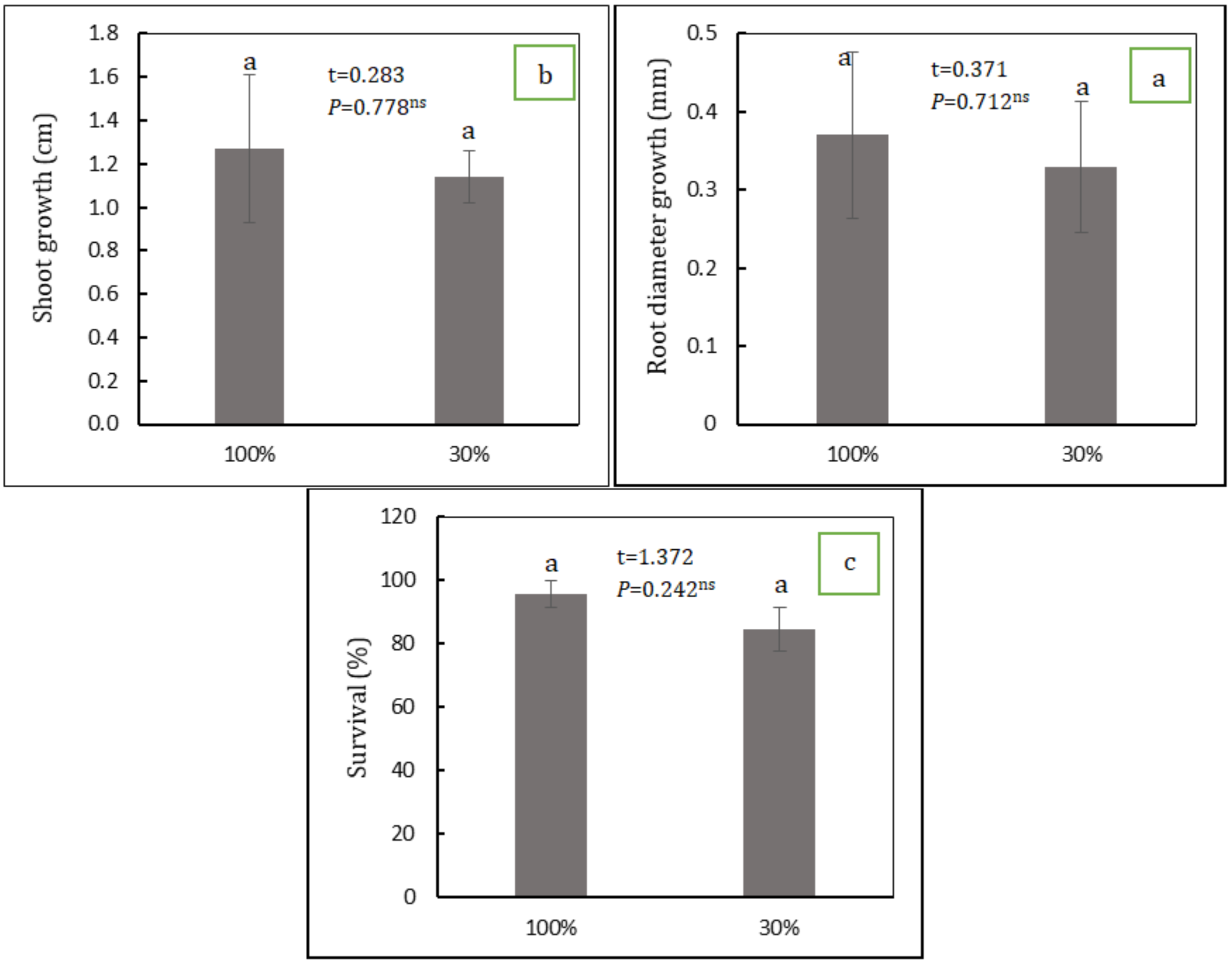

Figure 2) Comparison of growth traits of common yew seedlings under drought stress.
Shoot growth (a), root diameter growth (b), and survival (c), using the t-test at a significant level of 5%.

were used to evaluate the normality and homogeneity of the results, respectively. The effects of drought were analyzed using an independent samples t-test at a significance of p = 0.05. All statistical analyses were carried out using SPSS (version 22.0).

Findings

Growth Traits & Gas Exchanges

The results (Figure 2) showed that drought stress did not significantly affect growth traits (shoot growth, root diameter growth, and survival). The range of shoot growth, root diameter growth, and survival were 11.4-12.7 mm, 0.33-0.37 mm, and 84.7-95.8%, respectively (Figure 2, a-c). The effect of drought stress on gas exchange indices (except stomatal conductance), such as photosynthesis rate, transpiration, mesophyll conductance, water use efficiency, and intercellular CO_2, was statistically significant. At drought stress conditions or field capacity (FC) of 30%, the rates of photosynthesis, transpiration, mesophyll conductance, and water use efficiency decreased by 68.3, 23.9, 69.6, 57.9%, respectively (Figure 3, a-e), and the amount of intercellular CO_2 increased by 4% (Figure 3, f).

Discussion

The present study found that drought stress had a negligible effect on the survival of common yew seedlings and caused an 11.1 percent decrease in survival. Of course,

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this difference in the survival rate between seedlings under drought stress (FC 30%) and well-irrigated seedlings (FC 100%) was not statistically significant. In the literature review, the adverse effects of drought stress on the survival rate of some forest species such as *Quercus brantii* [39], *Eucalyptus aggregate* and *E. gunnii* [40], *Quercus castaneifolia* and *Q. persica* [41], *Eucalyptus camaldulensis* and *E. globulus* [42], have been noted.

In the present research, although the size of shoot length and root collar diameter of common yew seedlings showed a slight decrease with increasing irrigation period (drought severity), this difference between the two irrigation levels was not statistically significant. The research conducted with *Calotropis Procera* and *Suaeda aegyptiaca* [43] and *Calotropis procera* [44] indicated a decrease in the growth variables of seedlings under drought stress. Also, the negative effect of drought stress on the growth variables has been reported in *Cerasus mahaleb* [45], *Olea europaea* [46], *Populus nigra* [47], *Fraxinus excelsior* [48], *Conocarpus erectus*, *Acacia modesta*, *Salix tetrasperma* [49] and *Quercus variabilis*, *Robinia pseudoacacia* [50]. This research found that the physiological activities of common yew seedlings were strongly affected by drought stress, so the amounts of photosynthesis and transpiration decreased significantly. In general, the first response of plants to drought stress is to close the stomata and prevent water loss (by transpiration), reducing the absorption flow of carbon, carbon dioxide, and photosynthesis [51]. According to the literature review, water deficit causes a decrease in water potential and loss of turgescence, closing of stomata, and damage to the cell membrane along with protein degradation so that the rate of photosynthesis and transpiration decreases (52,53). The study of photosynthesis changes under drought stress can help to identify the influential factors in the tolerance of plants to this stress [54,55,56].

The reduction of photosynthesis in stressed seedlings can also be due to the defect in the effective absorption of CO_2 through improper opening and closing of leaf stomata [57]. The effect of drought stress and/water deficit stress on the reduction of photosynthetic activities has also been reported previously on *Picea asperata* [58], *Vitis vinifera* [59], *Acacia crassicarpa* and *Eucalyptus pellita* [60], *Jasminum sambac* [61], *Helianthus annuus* [62], *Larix kaempferi* and *Prunus sargentii* [63], *Cinnamomum camphora* [64]. Also, based on the findings of Rooki et al. (65,66), the amount of transpiration decreased with the increase of drought severity in *Cupressus arizonica* and *C. sempervirens* var. *fastigiat*e, *Fagus sylvatica,* which is in line with the results of the present study.

Drought stress reduces the size of stomatal pores [67]. Stomata are among the essential factors in plant water loss, and in general, stomatal conductance is one of the critical indicators for assessing water stress in plants. The plants prevent water losses by closing the stomata during drought stress. Closing the stomata is controlled by various factors, so one of the most important factors is the hormone abscisic acid (ABA). This hormone regulates plant growth and is stimulated under drought stress, increasing its amount. The stomata close after the increase of this hormone in plant tissues and cells to maintain or continue the plant's resistance to drought. The opening of the stomata results from increasing the pressure potential of the protective cells of the stomata to the surrounding cells. In an experiment, it was found that in drought conditions, the stomatal conductance decreased significantly, and the genotypes (SLMO46 and Okapi) with the highest osmotic regulation ability (0.355 and 0.350 $cm.s^{-1}$, respectively) had the least amount of

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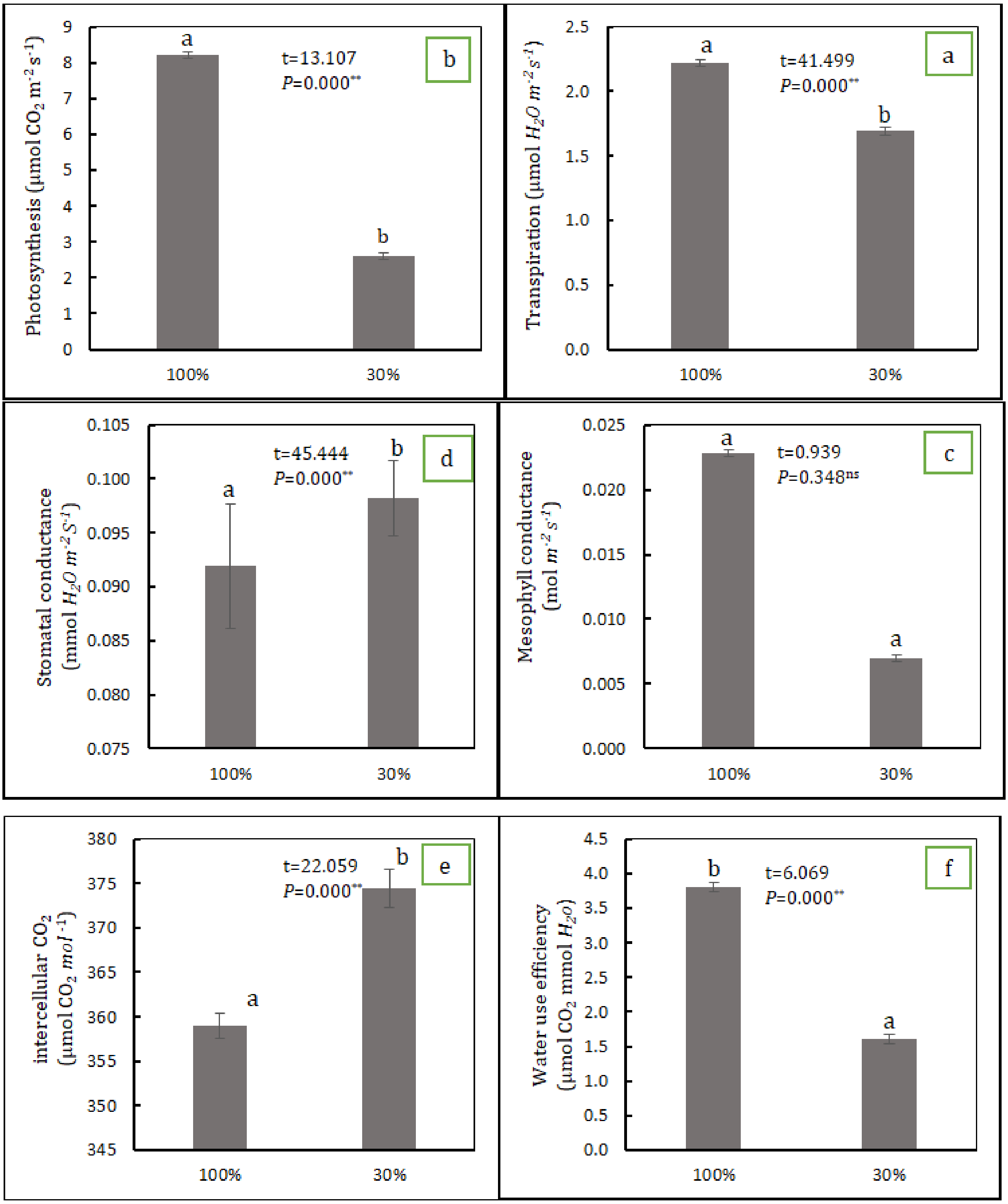

Figure 3) Comparison of gas exchange indices of common yew seedlings under drought stress. Photosynthesis rate (a), transpiration (b), stomatal conductance (c), mesophyll conductance (d), Water use efficiency (e), and intercellular Co_2 (f), using a t-test at a significant level of 5%.

stomatal conductance [68]. This reveals that these genotypes close their stomata when faced with drought stress to resist adverse environmental conditions. In the findings published with *Picea abies* [69], *Calotropis procera* [70], *Zizyphus spinous* [71] and *Pyrus*

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boisseriana [72], *Pinus nigra* [73], the reduction of photosynthesis and transpiration was attributed to the reduction of stomatal conductance. In the present study, stomatal conductance did not change significantly with the reduction of photosynthesis and transpiration.

Mesophyll conductance is a set of internal leaf mechanisms that lead to CO_2 processing. The lower rate of photosynthesis and CO_2 processing in the presence of high amounts of intracellular CO_2 means a low level of mesophyll conductance and the inability of mesophyll cells to use CO_2 [74]. Similar to the results conducted on *Quercus brantii* and *Q. libani* [75], in the present study, water deficit stress caused a significant decrease in mesophyll conductance. In some studies, increasing CO_2 concentration inside the stomata has been associated with a decrease in photosynthesis [76], which is in line with the results of the present study. On the other hand, in some studies, the stability of CO_2 concentration in the stomata has been reported in water-deficit conditions [77]. Drought stress, in addition to reducing stomatal conductance, prevents CO_2 processing available to the plant by affecting the internal mechanisms of the leaf [78]. The increase or stability of intra-stomatal CO_2 with the reduction of photosynthesis can be related to the plant's inability to process CO_2 or non-stomatal factors limiting photosynthesis [79].

Improving photosynthetic conditions and water use efficiency are essential variables when choosing suitable species for projects of afforestation/reforestation [80]. The current research results showed that the water use efficiency in yew seedlings decreased under drought stress. In the study of [81], with the increase of evaporation and transpiration, the amount of water use efficiency decreased in the two tested species, and under drought stress, it was higher in *Eucalyptus leucxylon* than *in E. flocktoniae*. *E. leucxylon* can produce more dry mass in drought-stress conditions. Similarly, a decrease in water use efficiency due to increased drought stress has also been reported for *E. globulus* and *E. amanuensis* [82]. This implies the relative closing of the stomata and the increase in the ratio of carbon dioxide entry to water exit from the stomata. When the water available is not enough for the plant, increasing the water use efficiency is considered an alternative strategy to improve growth performance under water deficit stress [83]. A study conducted on three species of oak (*Quercus petraea*, *Q. pubescens*, and *Q. ilex*) showed that the water use efficiency was higher in *Q. ilex*, a drought-adapted species [84]. Another study [75] also showed that *Q. brantii* has lower water use efficiency than *Q. libani*. Therefore, in drought-resistant species, with the increase of drought stress (due to the increase in the density of stomata and the reduction of the dimension of the stomata), the amount of transpiration decreases, and the efficiency of CO_2 fixation increases, which is an influential factor in improving water use efficiency [84].

Conclusion

Similar to our hypothesis, it was proved that drought stress had no significant effect on the growth characteristics of yew seedlings, but it had a negative effect on the physiological activities of the seedlings. So, with the decrease in soil moisture, the amount of photosynthesis, transpiration, mesophyll conductance, and water use efficiency significantly decreased. In other words, the lack of influence of the growth traits of drought-stressed common yew seedlings in the first growing year was likely due to its slow growth. Since the seedlings need several years of care in the nursery before being transferred to the plantation site, it is recommended that they be grown

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without water deficit stress for a better response of physiological traits and more favorable growth in the next growing years.

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