



Gas exchanges and chlorophyll content of endemic Caspian locust (*Gleditsia capsica*) seedlings under flooding and flooding-recovery conditions

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ABSTRACT

Aims: Caspian locust, native to the Hyrcanian forests of Iran, is one of the pioneer species distributed in the moist soils of these forests. So far, the response of its seedlings to permanent and temporary flooded beds has yet to be reported. This study was conducted to analyze the physiological responses of Caspian locust seedlings to flooding and flooding-recovery conditions.

Materials & Methods: Flooding conditions were examined for 90 days in the greenhouse of Tarbiat Modares University. The study was carried out in a factorial experiment as a completely randomized design with five treatments and four replications. Treatments included: (1) continuous flooding for 90 days (F90), (2) flooding for 60 days followed by a 30-day recovery (F60+R30), (3) flooding for 45 days followed by a 45-day recovery (F45+R45), (4) flooding for 30 days followed by a 60-day recovery (F30+R60) and (5) Control.

Findings: Results showed that flooding for 90 days induced a significant decrease in net photosynthesis (-91%), stomatal conductance (-77%), transpiration (-81%), Chl a (-63%), Chl b (-67%) and Chl_{Tot} (-64%) compared to the control ($p < 0.05$). When flooding was removed for 30-60 days, plants were able to recover gas exchange activities from 30 to 90% and Chl content by 55-90%.

Conclusion: Based on our results, seedlings of *G. capsica* can survive and grow throughout a medium period of soil waterlogging. Therefore, the Caspian locust is a promising species for reforestation programs in the riverine areas and temporarily flooded wetlands.

Keywords: Net Photosynthesis; Soil Waterlogging; Stomatal Conductance; Permanent Flooding; Temporary Flooding.

CITATION LINKS

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Introduction

Riparian areas in flood plains of the Hyrcanian forests of Iran are periodically disturbed by flood events. So, its duration, frequency, and intensity strongly affect survival, growth, reproduction, community structure, and coastal vegetation pattern ^[1]. Investigating the ability of plant species to survive and grow in flooding and waterlogging conditions is one of the essential factors for evaluating the success of establishing plant species on the banks of rivers and wetlands. Therefore, before introducing species for afforestation, it is crucial to know how they respond to these conditions. Under these stresses, insufficient oxygen in the Rhizosphere environment causes changes in various processes of plants, including energy consumption, gene expression, cellular metabolism, growth, and development, which causes physiological changes in the leaves of plants, including the decrease in the rate of photosynthesis and stomatal closure as well as leaf water potential ^[1].

In fact, in flooded and wet environments, oxygen reduction in the root Rhizosphere causes limited aerobic respiration of plant roots ^[2, 3]. Therefore, investigating plant physiological responses is important under flooding and waterlogging conditions ^[4, 5, 6, 7, 8] and after those (recovery stage). In fact, during the recovery stage, plants resistant to flooding and waterlogging conditions open their stomata and gradually increase photosynthesis and transpiration to reach the stable stage ^[5].

Several studies have evaluated the effects of flooding stress and recovery on the tolerance and adaptation of woody plants to restore wetland ecosystems exposed to seasonal flooding. To determine the tolerance of *Ormosia arborea* seedlings to flooding stress, Junglos et al. (2018) ^[9] designed an experiment with seedlings immersed for 0, 15, 30, 45, and 60 days. The findings

showed a decrease in the quantum efficiency of PSII and gas exchanges during more extended periods of flooding. The metabolism was restarted during the recovery. Parad et al. (2016) ^[5] evaluated the effect of flood stress on *Quercus castaneifolia* seedlings. The findings indicate a decrease in the Gas exchanges of the seedlings during 120 days of continuous flooding, and after removing the flooding stress, they were able to recover their physiological activity (49-80% compared to the control)

The Caspian locust (*Gleditsia caspica* Desf.) belonging to the Fabaceae family is an endemic deciduous species found in the endangered flood-prone plains of Hyrcanian forests in the north of Iran ^[11]. It is also a pioneer tree species of secondary succession with 3–12 m tall and a wide geographical distribution in this region ^[10]. Since this species is highly adaptable to different soil conditions, it grows well in abandoned open lands and has intense regeneration in areas with anthropic activities ^[11]. In addition, it can be found mainly only in areas exposed to transient periods of soil waterlogging.

Caspian locust, a kind of fuelwood, has hardwood used as structural poles in rural uses. It can also be used in folk medicine. The thorns treat carbuncles, scabies, various cancers, heart and vascular diseases, and suppurate skin diseases, while the mature pods are mainly helpful in treating productive cough, asthma, apoplexy, and headache ^[7]. The bark and green and mature pods of this tree are used as livestock fodder. The seed is a valuable source of protein, and fat is used for animal feeding. Due to the N-fixation potential, it is assumed as one of the important pioneer species in silvopastoral systems ^[12].

The indiscriminate cutting of Caspian Locust for rural purposes, livestock breeding, and the conversion of forest lands into agricultural fields has caused it to appear today

in the form of individual stands and small groups on the margins of agricultural farms and cattle farms. Because of its high ability to adapt successfully in extreme conditions and the high value of genetic diversity, the protection of Caspian locust and their habitats is vital ^[11].

So far, no study has been related to the morphological response of Caspian locusts in flooded and recovery conditions. However, more information on this species needs to be related to salinity ^[14] and drought ^[15] stresses. The study aims to investigate the effect of flooding periods along with recovery or drainage periods (after flooding) on some physiological traits (i.e., gas exchanges and chlorophyll content) in Caspian locust seedlings under greenhouse conditions.

Materials & Methods

Seedling production and flooding stress
mature, and healthy seeds of Caspian locust (*Gleditsia capsica* Desf.) collected from Kash-

pel forest park in Noor, Mazandaran, in the north of Iran (36° 26' 40" N, 52° 3' 20" E) (Figure 1). They were carried out in a germinator at the laboratory of the Faculty of Natural Resources and Marine Sciences, Noor. The seeds were exposed to H₂SO₄ for 60 minutes due to dormancy breaking (Nourmohammadi et al. (2019) ^[11]). After seed germination had been completed (6 days), the seedlings were transplanted into polyethylene plastic bags (10 × 20 cm) containing a mixture of forest soil, sand, and vermiculite (1:1:1).

The seedlings grew in the greenhouse until the start of waterlogging treatments due to natural light levels and photoperiod (one year). Then, 120 healthy seedlings with a uniform size (collar diameter 6±0.9 mm, stem height 72±3 cm) were selected. Each was transplanted into a 15 × 30 cm pot containing forest soil, sand, and loamy sand (in 3:1:1 proportion).

The experiment was conducted in the greenhouse of the Tarbiat Modares University, Mazandaran, Noor (36° 34' 54" N, 52° 2' 40"

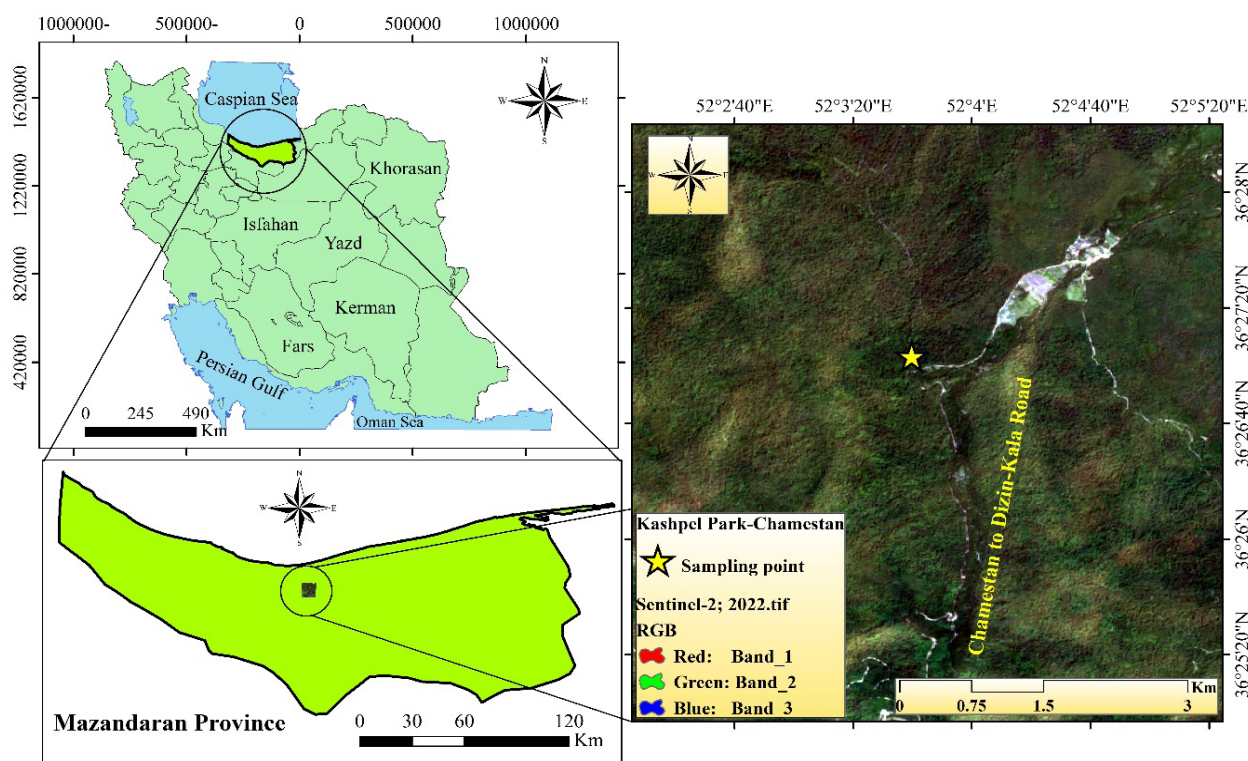


Figure 1) Caspian locust seed collection location in Kashpel forest park, north of Iran.

E) (Figure 2). The Seedlings were subjected to five treatments for 90 days, following a fully randomized design with four replicates. Treatments included: 1) continuous flooding without a recovery period (F90), 2) flooding for 60 days followed by recovery for 30 days (F60+R30), 3) flooding for 45 days followed by recovery for 45 days (F45+R45), 4) flooding for 30 days followed by recovery for 60 days (F30+R60), 5) control. The water level was maintained 5 cm above the soil surface in all flooded treatments. In all recovery phases, plants were allowed to grow during a subsequent period of flooding days in well-drained conditions.

At the end of the experiment (day 90), physiological variables such as net photosynthesis, transpiration rate, and stomatal conductance were measured by a portable infrared gas analyzer (Model LCpro +, ADC BioScien-

tific Ltd., Hertfordshire, UK). For this purpose, between 9 and 11 in the morning on a sunny day with a light intensity equal to $1400\text{--}1200 \text{ micromoles photon.m}^{-2}\text{/s}^{-1}$. At the same time, air temperature and relative humidity were also monitored and used to estimate the air vapor pressure deficit (VPD air), which varied between 1.50 and 2.90kPa (mean of 2.00kPa). 3-6 leaves from each replication were selected from the most mature and completely expanded leaves at the 3rd to 6th node from the shoot apex [16]. A measure of water use efficiency was calculated by dividing photosynthesis by transpiration [17]. Lichtenthaler and Wellburn's (1983) [18] method was used to measure chlorophyll (Chl) contents, including Chl a, Chl b, and Chl Total (Chl_{Tot}). For this purpose, 2-3 mature and healthy leaves from each treatment were randomly selected, and after separating the

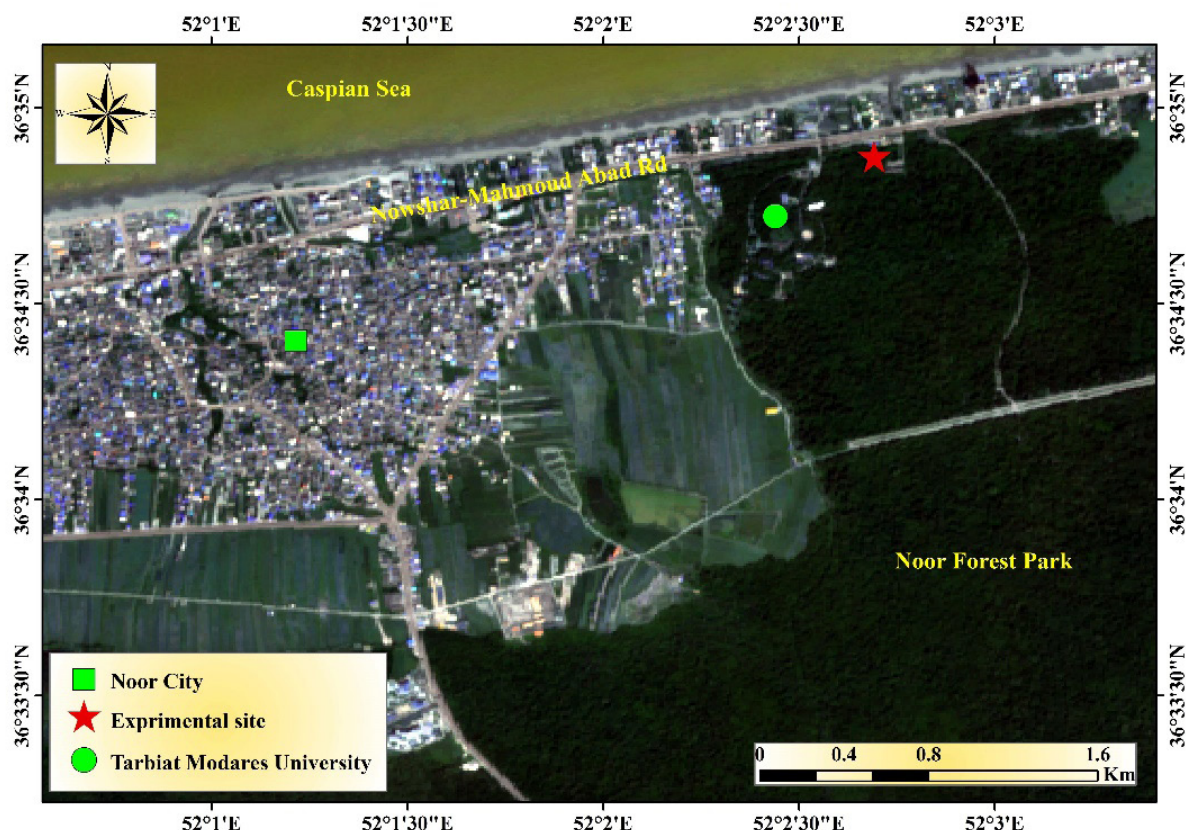


Figure 2) Map of the experimental site, Tarbiat Modares University, Mazandaran, Noor ($36^{\circ} 34' 54'' \text{ N}$, $52^{\circ} 2' 40'' \text{ E}$) (Source: Google Earth Engine, 2022).

veins, the remaining parts were crushed and frayed. Then, 0.1 g of the sample was carefully weighed and placed in a 10 ml centrifuge tube. Then, 9 ml of 80% acetone solution and ethanol with a 1:1 ratio were added, and the solutions were centrifuged at 8000 rpm. Spectral measurements were performed at 663 nm and 646 nm wavelengths [19].

Statistical analysis

Physiological parameters were performed with SPSS statistical package (version 17.0, SPSS Inc., Chicago, USA). In this way, after obtaining normal conditions (Kolmogorov - Smirnov test) and homogeneity of variance (Levene's test), one-way ANOVA, and Duncan were conducted for comparison between treatments and mean, respectively [20].

Findings

Gas exchanges

After 90 days of flooding, a comparison of mean values indicated that with the increase in flooding stress intensity during the experiment, the net photosynthetic rate decreased significantly, so that the highest and lowest amount of photosynthesis, amongst the different levels of flooding treatment, were relative to the control treatment ($7.18 \mu\text{mol.m}^{-2}.\text{s}^{-1}$) and permanent flooding ($1.64 \mu\text{mol.m}^{-2}.\text{s}^{-1}$), respectively.

After removing the stress and placing the seedlings in drainage conditions, photosynthesis gradually increased. Thus, on the 30th (F30+R), 45th (F45+R), and 60th (F60+R) days, the net photosynthesis rate compared to the F90 treatment (control), were increased by 6.6, 5.16, and $3.51 \mu\text{mol.m}^{-2}.\text{s}^{-1}$, respectively, but it was 8-51% less than that in control (Figure 2a). Stomatal conductance in permanent submergence (F90) was very low, but in F30+R60, F45+R45, and F30+R60 treatments, it was increased by 31, 70 and 90% compared to the control, respectively (Figure 1b).

In addition, leaf transpiration was decreased

under submergence stress by photosynthesis, so the lowest occurred in the permanent flooding ($0.26 \mu\text{mol of water.m}^{-2}.\text{s}^{-1}$). On the day of 60, after the water subsides away from the F60+R30 treatment, the size of the transpiration rate. In the F30+R60 treatment, where the seedlings had 60 days to recover, the transpiration rate increased to $1.95 (\mu\text{mol of water.m}^{-2}.\text{s}^{-1})$. It recovered close to control plants, which was not statistically different from the control ($p < 0.05$) (Figure 2c). Water use efficiency was not significantly changed between treatments (except the F90 treatment), but about 50% decreased compared to the F90 treatment (Figure 2d).

Chlorophyll content

After 90 days from the beginning of the flooding stress, the contents of Chl b, Chl a, and Chl_{Tot} of the treatments under flooding and flooding-recovery stress were significantly lower than the control seedlings (Figure 3). By increasing the flooding period for 90 days (F90), the concentrations of Chl a, Chl b, and Chl_{Tot} in the Caspian locust seedlings decreased by about 63, 67, and 64%, respectively. However, during the recovery stage, for example, after 60 days of the recovery phase (F30+R60), when flooded pots were drained, Chl b concentration was increased gradually and recovered to levels similar to the control by the end of the experiment (Figure 3, a,b,c).

The recovery period led to an increase in Chl b, Chl a, and Chl_{Tot} by 146, 172 and 152% in F30+R60, and 121, 126 and 122% in F45+R45 and 78, 66 and 75% in F60+R30 compared to F90, respectively. The concentration of Chl a/b did not change at different levels of flooding and non-flooding stress (Figure 3, d). In general, after 45 days of the recovery stage, Chl content increased between 75% and 81% compared to the control (Figure 3).

Based on the above results, flooding stress had caused a significant decrease in the

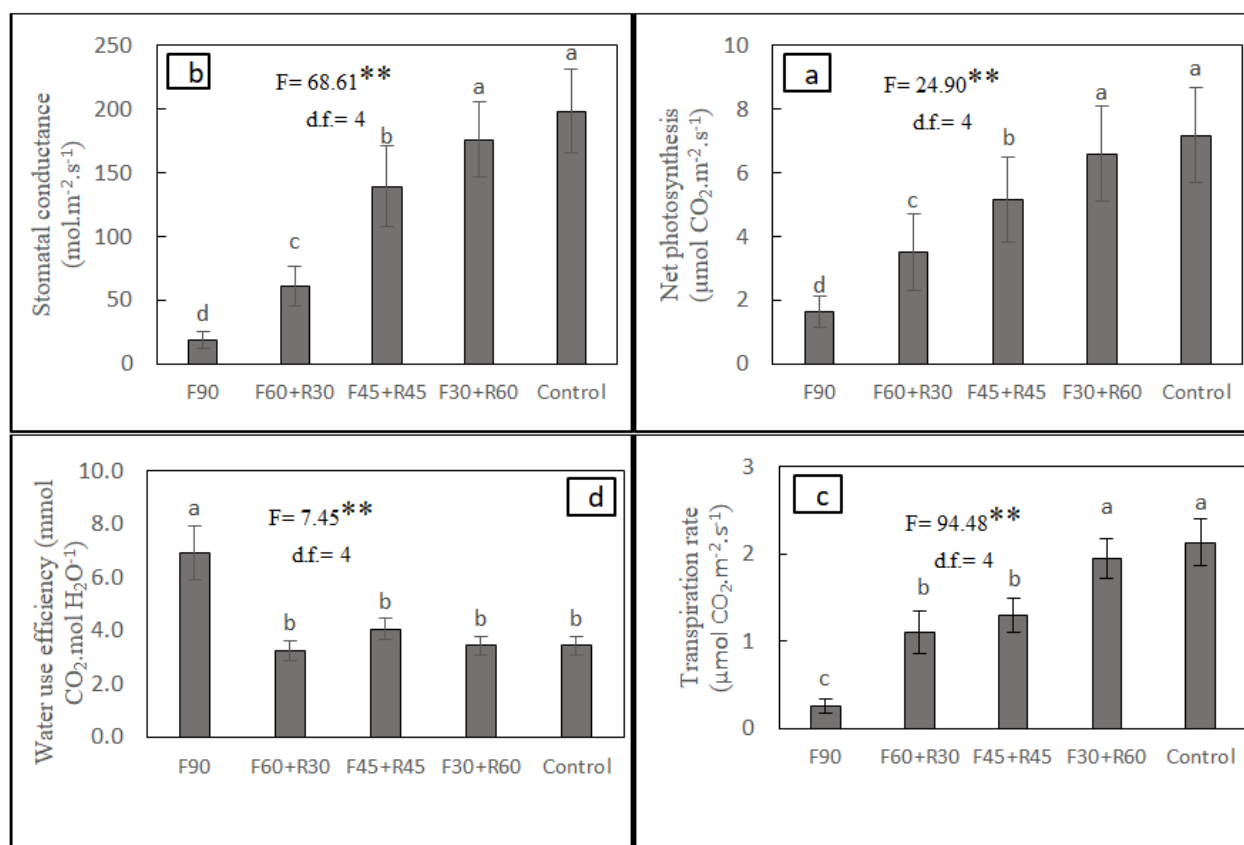


Figure 3) Net photosynthesis rate (a). Stomatal conductance (b), transpiration rate (c), and water use efficiency in seedlings of Caspian locust subjected to control and different flooding periods for 90 days. According to Dun-can's test, other letters indicate a significant difference ($p < 0.05$) in the tested treatments.

amount of gas exchange and Chl content. In contrast, when the seedlings were recovered for 30-60 days, Caspian locust seedlings could recover the gas exchange by 30-90% and the Chl content by 55-90%, respectively.

Discussion

Under Flooding conditions, plants can reduce stress injury and maintain plant growth through their resistance mechanisms. The severity of the adverse effects caused by flooding usually varies with the plant's age and vegetative stage and the flood's characteristics (such as period, depth, time, and frequency) [21, 22, 23, 24].

In this research, Caspian locust seedlings were subjected to waterlogging stress during the growing season for three months, resulting in seedlings being resistant to waterlogging stress. It is worth noting that de-

spite this acceptable tolerance level in this species, adventitious roots and hypertrophic pores, both essential strategies of tolerant species against flooding, were not observed. Therefore, by increasing the duration of flooding stress to more than three months, the growth and survival of lilacs will face problems. In this regard, Glenz et al. (2006) [25] also reported that two species of *G. Triacanthos* and *G. aquatic* are moderately resistant to waterlogging.

Reducing gas exchanges is one of the strategies for tolerance to flooding conditions in stressed seedlings. In the present study, all physiological parameters in two-year-old Caspian locust seedlings were significantly reduced under flooding treatments. Similarly, negative effects of flooding stress on photosynthesis, stomatal conductance, and transpiration have been reported in some

woody species (Gomes and Kozłowski, 1988^[26] on *Eucalyptus camaldulensis*; Miao et al., 2017^[27] on *Populus deltoides*; Junglos, 2019^[9] on *Ormosia arborea*). Also, we can refer to similar studies related to the Fabaceae family: Xin et al. (2012)^[28] on *Indigofera spicata*, Maxwell et al. (2016)^[21] on *Acacia stenophylla* and A. Cunn, Rosa, et al. (2018)^[28] on *Copaifera langsdorffii*; Vidal et al. (2019)^[28] on *Copaifera lucens*; Linne et al. (2021)^[31] on *Dipteryx alata*, all of which show a significant decrease in plant physiology activities under flooding stress.

Monitoring the physiological response and assessing the tolerance of plants to flooding stress after stopping flooding and entering the drainage period, which is associated with improving gas exchanges, is important in wetland forests^[31]. This reveals that stomata reopen when the flooding stress conditions are removed in plants resistant to flooding, and the gas exchange rate is increased^[5, 27, 31]. In the present study, photosynthesis, transpiration, and stomatal conductance rates decreased during flooding periods. Still, they recovered rapidly following drainage: on F30+R, F45+R, and F60+R days, the net photosynthesis rate compared to the F90 treatment (control) increased by 6.6, 5.16, and 3.51 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, respectively, but it was 8-51% less than that in control (Figure 2a). Stomatal conductance was increased by 31, 70, and 90% compared to the control, respectively (Figure 1b). In addition, by photosynthesis, leaf transpiration was decreased under submergence stress (0.26 μmol of water $\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Water use efficiency was about 50% decreased compared to the F90 treatment (Figure 2d). This shows that the decrease in gas exchanges is temporary when the plant is exposed to flooding.

The opening capacity of stomata depends on the type of species and the duration of flooding. For example, Parad et al. (2015)^[5] showed that 42 days of drainage was nec-

essary for *Fraxinus excelsior* seedlings to bring their gas exchanges to a stable level and close to the control treatment. Likewise, Junglos et al. (2018)^[9], working on *Ormosia arborea*, found that the photosynthetic rates, stomatal conductance, and carboxylation efficiency were decreased in flooding stress. Still, metabolism was restarted during the recovery or drainage period. Similarly, in the present study, the gas exchanges in Caspian locust seedlings approached the control level 45 and 60 days after drainage. In other words, this can imply the ability to properly recover seedlings after drainage and their satisfactory growth and establishment in flooded areas and riverbanks.

In the present research, consistent with the findings of Vu and Yelenosky (1991)^[33], Dreyer (1994)^[34], Gravatt and Kirby (1998)^[35], the content of Chl b, Chl a and Chl_{Tot} in the leaves of seedlings decreased with the increase of the flooding period. By increasing the flooding period, the concentrations of Chl a, Chl b, and Chl Tot in the seedlings decreased by about 63, 67, and 64%, respectively. The reduction of photosynthetic pigments (Chl a, Chl b, Chl_{Tot}, and carotenoid) is a type of protective mechanism to maintain the photosynthetic structures of flooded plants to reduce sunlight absorption and prevent photo-oxidation^[36, 18].

Due to the importance of the immediate recovery of photosynthesis in maintaining the survival and growth of submerged plants in wetland forests after flooding^[1], limited data are available regarding recovery following the removal of flood stress. The majority of past studies on species growth tree species and their physiological responses to flooding are limited to their response to continuous flooding^[5].

In addition, rapid recovery of Chl content after complete submergence is a vital attribute of flood-tolerant species such as *Tabernaemontana juruana*^[37], *Alnus japonica*

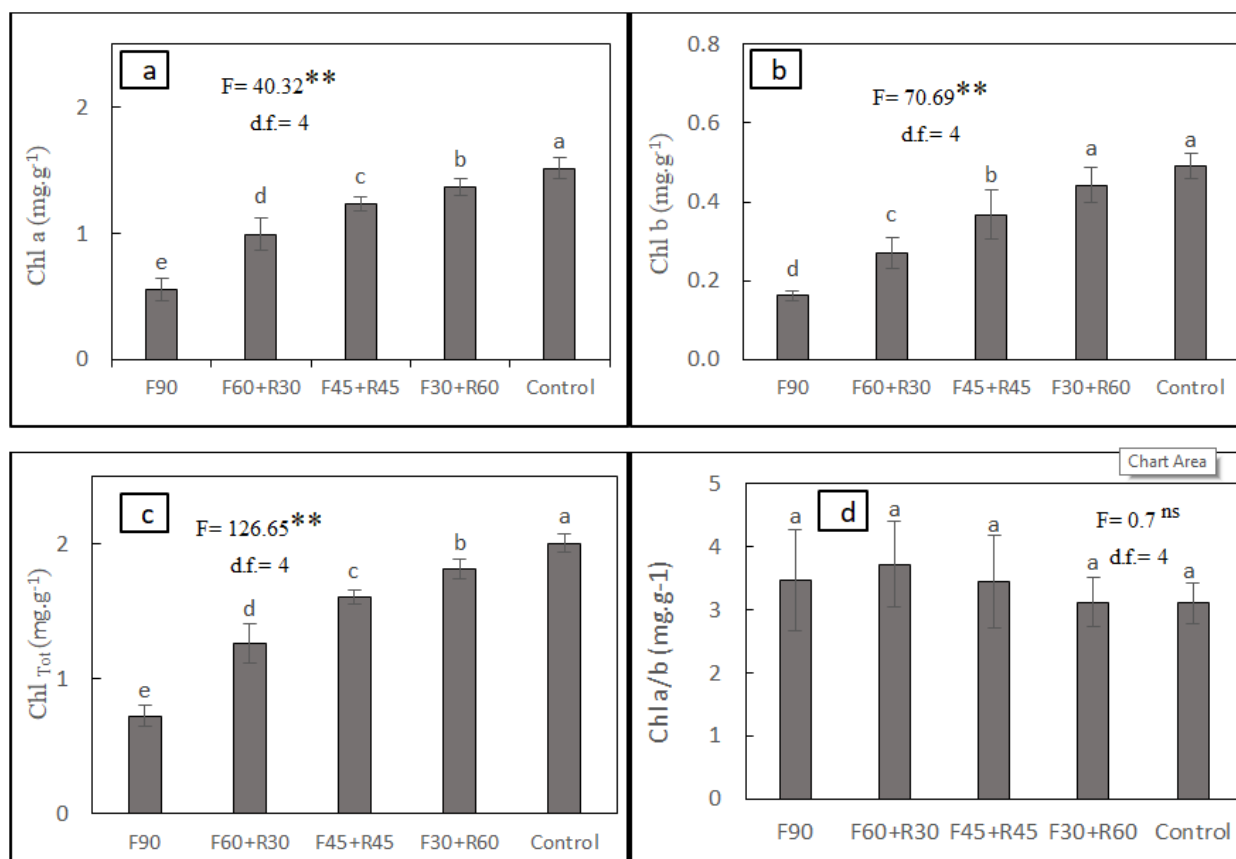


Figure 4) Chl a, Chl b, Chl_{Tot} and Chl a/b concentration of Caspian locust under control and soil flooding conditions for 90 days. Values are means (n = 4) with standard deviation.

(Thunb.) Steud^[38] and *Melaleuca alternifolia*^[39]. Pezeshki (2009)^[40] suggested that rapid recovery of Chl content performance was crucial for the growth of woody plants after a period of flooding. In the present study, after 45 days of recovery, Chl content increased from 75 to 81% compared to the control. In addition, our study showed that after 60 days of immersion, the Chl content recovered to the same level as the controls, which again indicates the recovery capacity of the Caspian locust. Therefore, the obtained results confirm our hypothesis (reduction of photosynthesis completely during submergence and its rapid recovery after soil drainage).

Conclusion

Based on the results of this study, in permanent flooding (for 90 days), the number of gas exchange variables and Chl content de-

creased significantly. However, by stopping the flooding stress and applying the drainage treatment, the Caspian locust seedling could recover the gas exchange by 30-90% and the Chl content by 55-90% in 30-60 days. This research revealed that flooded seedlings fully recover their physiological characteristics by increasing the recovery period up to 60 days. Therefore, in the plains and midlands of the north of Iran, which are exposed to periodic floods, planting Caspian locust seedlings can help to preserve, protect and restore the ecosystem.

Declarations

Ethics Approval: We confirm that this manuscript has not been published elsewhere and is not under consideration by another journal.

Consent to Participate: All authors have participated in the manuscript and agree with submission to ECOPERSIA.

Consent to Publication: Authors allow publication if the research is accepted.

Conflict of interest: The authors declare no conflict of interest.

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Credit author statement

K. Nourmohammadi: Material preparation, data collection, analysis, and first draft preparation.

M. Tabari: Conceptualization, supervision, proofreading, Writing - original draft, review & editing.

S.E. Sadati: Review & editing.

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