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Morphophysiological Parameters of Growth and Development of Seedlings of *Gleditsia triacanthos* L. in the Early Stages of Ontogenesis under Conditions of Changing Lighting Intensity

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Abstract

Background: *Gleditsia triacanthos* L. is a valuable taxon for agroforestry. This species is promising for the creation of protective plantations and has a wide range of economically valuable traits, in particular, drought tolerance. In this regard, the aim of the work was to evaluate morphophysiological parameters of growth and development of *G. triacanthos* seedlings at early stages of ontogenesis under conditions of changing light intensity.

Methods: To achieve this goal, seeds of *G. triacanthos* were sown with subsequent selection of individuals for the study possessing homogeneity of genetic structure. Analysis of close relatedness of individuals of *G. triacanthos* showed low intraspecific diversity by Nei (0.27 to 0.62). To evaluate the development of seedlings of *G. triacanthos* at early stages of ontogenesis, morphophysiological methods were used.

Result: Under unfavorable conditions due to low light, the growth and development of the embryonic root and seedling formation was slow. However, all subphases of *G. triacanthos* seed organogenesis were passed in 98% of individuals, thus forming seedlings under standard criteria. Accumulation of total chlorophyll content in unfavorable conditions was 3 times less compared to individuals in favorable conditions. At the same time, there was a tendency to increase the sum of chlorophylls in the leaves of *G. triacanthos* in each experimental group at 30 days ($p<0.05$). As a result, it was revealed that light intensity is a limiting parameter for accelerated growth and development of *G. triacanthos* seedlings.

Key words: *Gleditsia triacanthos* L., Illumination, Morphophysiological parameters, Ontogenesis.

Introduction

The “response-factor” system is one of the key traits for selecting woody shrub plants for the breeding process and solving agroforestry tasks. Southern territories of Russia are characterized by difficult climatic conditions for the growth of woody plants (Manaenkov *et al.,* 2020; Kulik *et al.,* 2023; Belyakov *et al.,* 2024). Search and selection of new promising species of woody and shrubby plants is necessary to increase species diversity (Tyshchenko *et al.,* 2023), having high forest and biological productivity and resistance to a wide range of negative environmental factors (Simpson, 2019).

One of such promising genera for creation of protective plantations of various functional purposes is *G. triacanthos* (Dizaji *et al.,* 2016). The most widespread species is *G. triacanthos*, which grows in southern chernozems, dark chestnut and light chestnut soils with solonetz complexes (Sergio *et al.,* 2020; Semenyutina *et al.,* 2021). Also *G. triacanthos*, has a wide range of economically valuable traits (Redwan, 2019; Melnik and Khuzhakhmetova, 2022).

In connection with the above-mentioned it is urgent to search and select *G. triacanthos* individuals with high productivity and resistance to unfavorable habitat factors at early stages of ontogenesis for further breeding and planting in dry-steppe zones (Belyaev *et al.,* 2022; Carta *et al.,* 2022). The main selection criteria are morphological and physiological-biochemical parameters, particularly the pigments of the photosynthetic apparatus in the early stages

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of ontogenesis and genetic structure. The genetic structure of woody plants can be determined by using DNA markers represented by ISSRs (Ramzan *et al.,* 2021; Saitwal *et al.,* 2022; Abd-Dada *et al.,* 2023). These markers identify the presence of loci that may be associated with a particular economically valuable trait in woody plants (Novikova *et al.,* 2012). Since the *G. triacanthos* genome has not been sequenced (Belyaev *et al.,* 2023), ISSR markers, which are non-specific multilocus markers (Luo *et al.,* 2018; Tantasawat *et al.,* 2021), are best suited to detect these loci.

The aim of the work was to evaluate morphophysiological parameters of growth and development of *G. triacanthos* seedlings at early stages of ontogenesis under conditions of changing light intensity.

MATERIALS AND METHODS

G. triacanthos seeds were collected from individuals of good vital mid-age ontogenetic condition located on the basis of cluster dendrology collections (#34:34:000000:122 - woody plant nursery of the Federal Scientific Center of Agroecology of the Russian Academy of Sciences). The research was conducted on the basis of the Federal Research Center for Agroecology of the Russian Academy of Sciences in the laboratories of molecular breeding, as well as bioecology of woody plants from March 2022 to February 2024. Collected seeds of *G. triacanthos* seeds were scarified before germination by immersion in concentrated sulfuric acid for 120 minutes, followed by rinsing in water for a day to destroy seed coating (Lorca *et al.* 2019; Balakina *et al.* 2021).

Swollen seeds of *G. Triacanthos* were placed in a seed germination tray for 36 h in a climate chamber at 26°C. After emergence, the seeds were placed in the soil. Next, two groups were formed which included 20 plants each.

To study the effect of illumination on the early stages of ontogenesis of seedlings of *G. triacanthos*. *G. triacanthos*, the conditions were modeled by changing the length of daylight hours. In the control group the optimal photoperiodism regime was set (illumination 50...260 lx), in the experimental group the duration of the light day was reduced to 7 hours (illumination 2...16 lx). Analyzed parameters were taken after 7, 15 and 30 days and material for genetic analysis was collected and stored at -20°C.

Evaluation of morphological parameters of ontogenesis at early stages of *G. triacanthos* was carried out by measuring plant height (cm) and leaf length (cm). The measurement was performed for all leaves of each individual and the mean value was indicated, which were used in statistical processing. Metabolites were quantified in leaves using a Dualex Scientific+ plant analyzer (Force-A, France) after 7, 15 and 30 days. For the counted plant, the analyses were carried out in three biological repetitions and the mean value was indicated, which were used in statistical processing. Measurement of chlorophylls (μg/cm^2 raw weight), flavonoids (μg/cm^2 raw weight), anthocyanins (μg/cm^2 raw weight) and nitrogen balance index (NBI) in leaf epidermis of woody plants.

Genomic DNA was extracted from 50 mg of leaf plates using a modified CTAB method with the addition of 0.2% β-mercaptoethanol (Shestibratov *et al.,* 2021). The concentration and quality (A260/A280) of DNA in the samples were assessed using a Qubit 4.0. fluorimeter (Thermo Fisher Scientific Inc., USA) and a SpectrostarNano spectrophotometer (BMG Labtech, Germany), respectively. For ISSR detection we used primers showing high amplifiability, synthesized by Evrogen CJSC, Russia (Table 1).

The ISSR-PCR-based amplification was conducted using kit qPCRmix-HS (cat: #PK145L) (CJSC Evrogen, Russia). The ISSR-PCR-based amplification was performed using kit qPCRmix-HS (cat: #PK145L, CJSC Evrogen, Russia) according to the manufacturer's instructions. The Applied Biosystems QuantStudio 5 (Thermo Fisher Scientific, USA) was used to amplify genomic DNA with ISSR markers. The conditions of ISSR-PCR amplifications included initial denaturation at 95°C for 10 min followed by 40 cycles at 95°C for 30 sec, annealing temperature for the various primers at 55°C for 30 sec (extension) and final extension at 72°C for 4 min.

The 2% agarose gel electrophoresis with TAE buffer was used to separate the results of the ISSR-PCR reactions (Al-Khayri *et al.,* 2022). The size of DNA bands on the gel was determined using a 100+bp DNA ladder (cat: #NL002, CJSC Evrogen, Russia). Ethidium bromide (Helicon, Russia) was used to stain the agarose gel, which was then visualized using a UV illuminator (VilberLourmat, France). The frequencies of polymorphisms and the number of bands produced by each primer were calculated individually.

The frequency of polymorphisms and the number of bands produced by each primer were calculated individually. ISSR loci were scored as present "1" or absent "0", each considered as independent. Data were compiled into a 0/1 binary data matrix using MS Excel. The generated binary data were used to calculate the polymorphism of ISSR loci in the POPGENE version 1.31 (Louati *et al.,* 2019). Quantitative data were processed using Statistica 12.0 software (StatSoftInc., USA) with calculation of the indices accepted to characterize nonparametric samples in biomedical research: median, 1st quartile, 3rd quartile (Me [Q1 ÷ Q3]). The Kraskell-Wallis test for multiple groups and the Mann-Whitney test for two independent samples ($p<0.05$) were applied to prove the reliability of differences.

Table 1: Name and sequence of the ISSR samples.

Name	Sequence 5′-3′	Tm, °C
UBC808	AGAGAGAGAGAGAGAGC	50.6
UBC811	GAGAGAGAGAGAGAGAC	54.2
UBC835	AGAGAGAGAGAGAGAGYC	54.0
UBC836	AGAGAGAGAGAGAGAGYA	51.5
UBC841	GAGAGAGAGAGAGAGAYC	54.0

Results and Discussion

Analysis of intraspecific genetic diversity in *G. triacanthos* seedlings by five ISSR markers showed homogeneity of the seed population, which confirms their common biotype. Based on the obtained data of amplicon spectra, binary matrices were compiled for further mathematical processing in POPGENE to estimate the intraspecific genetic diversity in *G. triacanthos* seedlings (Fig 1).

Genetic parameters of seedlings of *G. triacanthos* are given in Table 2. The effective number of alleles for the primers used ranged from 1.23 to 1.76, indicating the close relatedness of the oospores under study. It was revealed that seedlings of *G. triacanthos* seedlings had the highest effective number of alleles at the UBC 841 locus, which shows greater individuality of the genetic structure compared to other primers. One of the key parameters for assessing the closely relatedness of individuals of *G. triacanthos* was applied by Ney genetic distance estimation, which showed low values indicating genetic homogeneity. The Shannon index also showed low values ranging from 0.27 to 0.62, indicating low intraspecific diversity. As a result, loci UBC 811 and UBC 841 were identified whose parameter values were 1.5-2 times higher than those of UBC 808, UBC 835 and UBC 836.

The results of the analysis show homogeneity of the genetic structure, which will increase the representativeness of the data obtained for other morphophysiological parameters under experimental conditions.

Study of morphological parameters of seedlings *G. triacanthos*

Under unfavorable conditions, seeds of *G. triacanthos* seeds developed passively due to low light conditions. The stage of germinal root growth and seedling formation was slow. However, all subphases of organogenesis of seeds of *G. triacanthos* passed 98% of the individuals, thus forming seedlings under standard criteria. The height of plants was several times higher than in favorable conditions, this is explained by the fact that seedlings tried to find an additional light source (Fig 2A).

The greatest number and length of leaves were recorded in the seedlings that grew under favorable conditions ($p<0.05$). This variant did not need anything, thus developed its biomass (maximum leaf length) and underwent growth and development much more intensively ($p<0.05$) (Fig 2B). This is in agreement with the results of growing seedlings *Gleditsia caspica* L. (Nourmohammadi *et al.,* 2019).

Study of the content of pigments of the photosynthetic system of seedlings *G. triacanthos*

The content of chlorophyll sums in *G. triacanthos* leaves differed between seedlings in favorable and unfavorable conditions 7 days after sprouting and after 15 and 30 days no significant differences were found (Fig 3A). After 7 days under favorable conditions, the content of chlorophyll sum in *G. triacanthos* seedlings was 3 times higher compared to

Table 2: Indicators of genetic diversity of seedlings *G. triacanthos.*

Locus	n_e	h	I
	Me [Q1 ÷ Q3]		
UBC 808	1.23 [1.22 ÷ 1.86]	0.18 [0.17 ÷ 0.46]	0.33 [0.32 ÷ 0.67]
UBC 811	1.53 [1.26 ÷ 1.70]	0.35 [0.17 ÷ 0.41]	0.53 [0.26 ÷ 0.60]
UBC 835	1.32 [1.05 ÷ 1.87]	0.22 [0.05 ÷ 0.46]	0.37 [0.10 ÷ 0.66]
UBC 836	1.27 [1.00 ÷ 1.62]	0.17 [0.11 ÷ 0.38]	0.27 [0.12 ÷ 0.57]
UBC 841	1.76 [1.37 ÷ 1.98]	0.43 [0.27 ÷ 0.49]	0.62 [0.44 ÷ 0.69]
General polymorphism	1.45 [1.11 ÷ 1.81]	0.31 [0.09 ÷ 0.45]	0.49 [0.20 ÷ 0.64]

Note: n_e – Effective number of alleles per locus; h – Ney genetic distance; I – Shannon index; standard deviations are given in parentheses for all the above parameters.

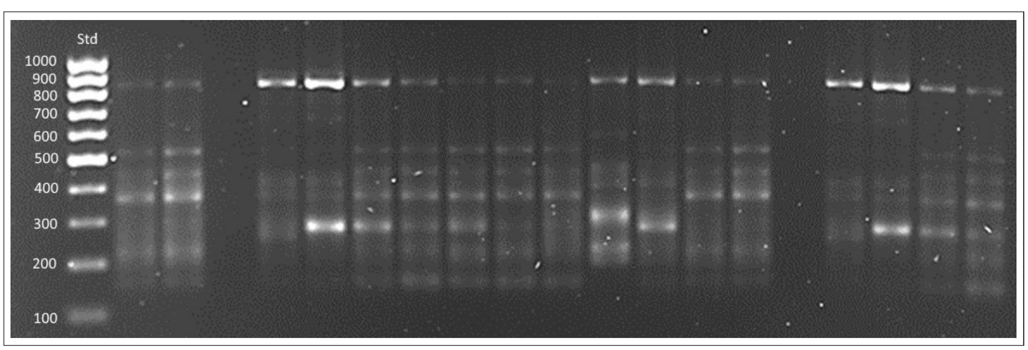
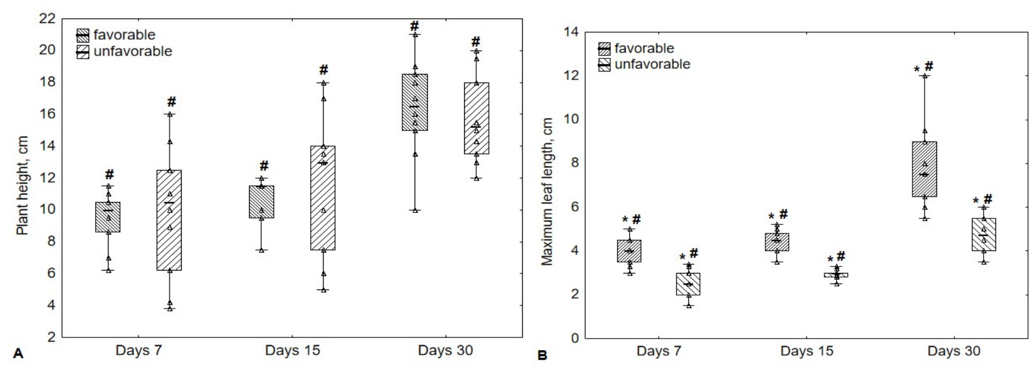
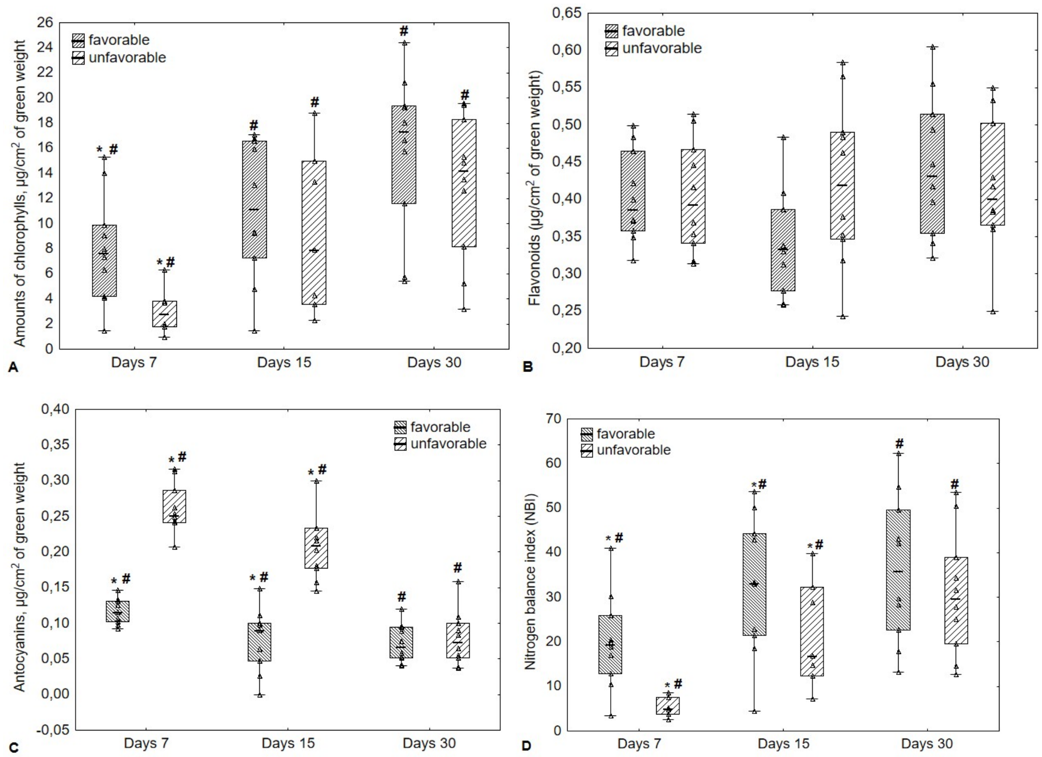


Fig 1: Example of electrophoregram of amplification products of seedlings *G. triacanthos* with ISSR primer UBC 841, Std molecular weight marker (100-1000 bp).



A - Plant height, B - Maximum leaf length, statistically significant differences between * - "favorable" and "unfavorable" conditions, # - between time intervals. Mann-Whitney, Kruskal–Walliss test at p<0.05.

Fig 2: Morphological parameters of seedlings *G. triacanthos*.



A- Amount of chlorophylls, B- Flavonoids, C- Anthocyanins and D – NBI. Significant differences between * - "favorable" and "unfavorable" conditions, # - between time intervals. Mann-Whitney, Kruskal-Walliss test at p<0.05.

Fig 3: The content in the plant leaves *G. triacanthos*.

individuals under unfavorable conditions (p<0.05). At the same time, there was a tendency to increase the sum of chlorophylls in *G. triacanthos* leaves in each experimental group by 30 days (p<0.05).

There were no statistically significant differences in flavonoid content in *G. triacanthos* individuals under favorable and unfavorable conditions throughout the experiment (Fig 3B). This may be primarily due to the fact that flavonoids are responsible for the functioning of the plant immune system (Middleton, 2009), also under laboratory conditions, no disease or leaf lamina lesions were observed.

Under unfavorable conditions, *G. triacanthos* individuals had 2 and 4 times higher anthocyanins content after 7 and 15 days than seedlings growing under favorable conditions (p<0.05)(Fig 3C). It was found that there was no difference in anthocyanins content after 30 days between the experimental groups ($p>0.05$). This indicates an important role of anthocyanins in the early stages of ontogenesis in the formation of adaptive potential (Cappellini *et al.,* 2021).

In *G. triacanthos* individuals growing under favorable conditions, the value of nitrogen balance index on 7 days was 4 times higher ($p<0.05$) than in unfavorable conditions and on 15 days the difference was reduced by 2 times ($p<0.05$)(Fig 3D). After 30 days, no statistically significant differences were observed. During the whole experiment, there was an increase in the index value of nitrogen balance index ($p<0.05$).

CONCLUSION

Data on the growth and development of *G. triacanthos* seedlings at the early stages of ontogenesis grown under conditions of light deficiency were obtained. Genetically homogeneous seedlings of *G. triacanthos* formed a specific adaptive response to the conditions of light deficiency. According to the results of pigment system analysis, a pronounced reaction of *G. triacanthos* seedlings to an important factor of growth and development - illumination - was revealed by changes in the content of the sum of chlorophylls and anthocyanins. The study of morphological parameters of *G. triacanthos* seedlings in different conditions showed that the lack of light is a limiting parameter for accelerated growth and development of seedlings in laboratory conditions. Individuals of *G. triacanthos* in unfavorable conditions undergo all stages of ontogenesis with lower intensity than individuals in favorable conditions.

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Conflict of interest

All authors declare that they have no conflicts of interest.

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