



The Study of Polymorphism of ISSR Markers *Ulmus parvifolia* Jacq and *Ulmus laevis* pall

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ABSTRACT

Background: Representatives of the generic *Ulmus* spp. complex are widely used in protective plantings in the arid region, but their genetic profile has not been studied. The purpose of the research is to carry out a molecular genetic and physiological-biochemical analysis of *U. parvifolia* and *U. laevis* for further breeding work.

Methods: The work uses samples of *U. parvifolia* and *U. laevis* collected in cluster dendrological collections (cadastre No.34:34:000000:122 – Arboreal plant Nursery of the Federal Research Center for Agroecology of the Russian Academy of Sciences, Volgograd). DNA was extracted from leaves by CTAB method. ISSR primers were used to evaluate DNA polymorphism. Pigments and phenolic compounds were also determined in the extract from the leaves.

Result: 12 ISSR primers were selected, characterized by high efficiency of amplification of fragments of genomic DNA of two elm species. The maximum number of specific DNA fragments was observed using primers UBC 834 and UBC 840, the proportion of polymorphic loci for each species was 100%. It was found that the concentration of flavonoids in the leaves of *U. parvifolia* (53.95±12.88) significantly (by 49%) exceeded the content of flavonoids in the leaves of *U. laevis*. The assessment of the genetic profile of *U. parvifolia* and *U. laevis* and the relationship of genotyping with the content of metabolites in these plants will be the first stage in breeding work to create new forms resistant to arid conditions.

Key words: ISSR markers, Polyphenols, *Ulmus laevis* pall, *Ulmus parvifolia* jacq.

INTRODUCTION

Woody plants of the generic complex *Ulmus* spp., characterized by exceptional adaptive ability to various growing conditions, are widely represented in protective plantations in the dry-steppe region of Southern Russia (Kryuchkov *et al.*, 2014). Two species, *U. parvifolia* Jacq and *U. laevis* Pall, are most often used in protective forest plantations, urban landscaping due to their drought and frost resistance (Ozolin *et al.*, 1978; Chudnovskaya *et al.*, 2023).

One of the ways to increase the efficiency and durability of plantations in arid conditions is to create your own seed base in treeless areas and improve the quality of planting material. For successful breeding work, it is necessary to use an assessment of the genetic diversity of the selected objects (Chesnokov *et al.* 2016; Hoban *et al.*, 2021).

Phenotypic, biochemical, protein (enzyme) markers have long been used in the study of various aspects related to plant life, including the level of polymorphism (Singh, 2021). In addition to these methods, molecular genetic analysis using molecular markers has recently been used in breeding work, which makes it possible to identify genotypes, investigate taxonomy, phylogenetics and perform barcoding (Peng *et al.*, 2023). Intermicrosatellite (ISSR) and microsatellite (SSR) primers are currently the most widely used to assess intraspecific polymorphism (Munsaka, 2024). The ISSR (inter-simple sequence repeat) method, based on microsatellite DNA analysis, is used to assess genetic diversity within a species, as well as to compare different species (Gemmill *et al.*, 2021; Venkatesan *et al.*, 2021).

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The functional state of plants is most often assessed by the parameters of the photosynthetic apparatus, in particular the composition, content and ratio of pigments. Changes in the amount and ratio of pigments may be subject to seasonal dynamics, due to arid conditions and other influences. The assessment of the accumulation of polyphenolic compounds and flavonoids involved in the neutralization of reactive oxygen species indirectly indicates the work of the antioxidant system of plants (Lahrizi *et al.*, 2024).

Determining the content of photosynthetic pigments, the total amount of phenolic compounds and flavonoids in different elm species can be useful for evaluating the response of these plants to abiotic and biotic stresses

(Tarakhovsky *et al.*, 2013). A number of studies have noted the species specificity of these processes (Tognetti *et al.*, 2007).

Thus, carrying out molecular genetic analysis using ISSR markers can be an important step in developing a strategy for the protection of two elm species in their natural habitat, contribute to the identification of interspecific hybrids and also become the basis for the selection of the most resistant species for protective afforestation in the arid conditions of the Volgograd region. The analysis of the content of metabolites and pigments will allow us to assess the possible mechanisms of protection of these plants from various types of stress.

MATERIALS AND METHODS

The work described in the article was carried out in the laboratory of molecular breeding of the Federal State Budget Scientific Institution "Federal Scientific Centre of Agroecology, Complex Melioration and Protective Afforestation of the Russian Academy of Sciences". The collection of samples was carried out in the summer of 2024, molecular genetic and physiological biochemical analysis was carried out in September 2023-March 2024. The object of the study is the samples of *U. parvifolia* Jacq and *U. laevis* Pall collected on the basis of cluster dendrological collections (No 34:34:000000:122 - nursery of woody plants of the Federal Research Center for Agroecology of the Russian Academy of Sciences). The subject of the study is the determination of intraspecific genetic diversity and the content of a number of pigments and metabolites in samples. Genomic DNA was extracted from leaf samples weighing 50 mg using a modified CTAB method, the extractant cetyltrimethylammonium bromide (CTAB) with 2% β -mercaptoethanol (Popova *et al.*, 2022). The SpectrostarNano spectrophotometer (BMG Labtech, Germany) was used to measure the concentration of isolated DNA and to evaluate the spectral ratios A260/A280 and A260/A230 characterizing the quality of isolated DNA. A ready-made mixture of reagents with qPCRMix-HS (Eurogene) dyes was used for PCR. PCR was performed on an Applied Biosystems QuantStudio 5 thermal cycler (Thermo Fisher Scientific, USA), a reaction mixture of 5 μ l, deionized water - 16 μ l, a primer - 2 μ l, a DNA sample (concentration 50 ng/ μ l) - 2 μ l was added to one tube. Amplification mode: pre-denaturation at 95°C (10 min), followed by 40 amplification cycles: denaturation at 95°C (30 s), annealing (30 s) at 50°C (UBC 807, UBC 815, UBC 873, UBC 890); 51.4°C (UBC 834, UBC 836, UBC 840, UBC 843); 54.5°C (UBC 811, UBC 826, UBC 835, UBC 841); elongation at 72°C (60 s) and final elongation at 72°C (10 min).

Electrophoresis of the obtained amplicons was performed in agarose gel (1.8%) in a 1x TAE buffer, gel staining with ethidium bromide. DNA markers Step 50 plus and 100+bp DNA Ladder were used to determine the length of DNA fragments. Visualization of the reaction results after

electrophoresis was performed using the iBright CL1500 gel documentation system.

18 ISSR primers were tested, the frequency of polymorphisms and the number of bands formed by each primer were calculated individually using Image Lab 6.0.1 software. PCR loci based on ISSR were evaluated as present (1) or absent (0), each of which was considered independent.

Physiological and biochemical studies included the determination of pigments, the total amount of phenolic compounds and flavonoids.

The pigment content was determined in acetone extract by spectrophotometry. The concentration of chlorophylls (*a* and *b*) and carotenoids in the samples was calculated using the formulas (Lichtenthaler, 1987), then mg/g of crude weight was recalculated.

The spectrophotometric determination of the total amount of phenolic substances is based on the oxidation of phenolic groups of the alcohol extract of the test sample with the Folin-Chocalteu reagent in a saturated sodium carbonate medium. (Nikolaeva *et al.*, 2021).

The quantitative determination of flavonoids in raw materials is based on the method of differential spectrophotometry, based on the ability of flavonoids to form colored chelate complexes with 95% alcohol solution of aluminum chloride. The quantitative content of the sum of flavonoids was determined in terms of rutin (Sorokina *et al.*, 2013).

The statistical analysis of the obtained data of physiological and biochemical studies was carried out using the Statistica 12 program, the Kraskell-Wallis criterion. To calculate the data on genetic diversity, the POPGENE version 1.31 program was used.

RESULTS AND DISCUSSION

To identify the intraspecific genetic diversity of *U. parvifolia* Jacq and *U. laevis* Pall, we tested 18 ISSR primers for the first time and selected 12 with high efficiency of amplification of a larger number of fragments of genomic DNA. It was found that all the analyzed primers had 100% polymorphic loci for the samples *U. parvifolia* Jacq (Table 1) and *U. laevis* Pall (Table 2), which indicates a high level of heterogeneity of these plant populations.

The number of amplified fragments for *U. parvifolia* Jacq ranged from 3 to 18, for *U. laevis* from 4 to 11, depending on the primer. The size of the fragments ranged from 213 to 1230 bp for *U. parvifolia* and from 213 to 1378 bp for *U. laevis*. The maximum number of specific fragments was noted when using primers UBC 834 and UBC 840. For *U. parvifolia* Jacq samples, the number of amplified DNA fragments with UBC 834 primer was 18, the band size was 213-1230 bp, for *U. laevis* Pall samples the number of amplified DNA fragments with UBC 834 primer was 10, the band size was 213-515 pp (Table 1, 2).

Each analyzed ISSR marker locus formed a specific spectrum of amplification products in each individual of *U. parvifolia* and *U. laevis*.

Next, the frequency of occurrence of amplicons was estimated for each locus of *U. parvifolia* Jacq and *U. laevis* Pall, binary matrices were compiled based on the obtained amplicon spectra for further mathematical processing in POPGENE. Calculated molecular genetic parameters of *U. parvifolia* Jacq and *U. laevis*. The results are shown in the Table 3.

The next stage of the work was the assessment of the content of pigments and phenolic compounds in the leaves of smooth elm and small-leaved elm (Table 4).

The Kraskell-Wallis coefficient was used in the analysis of data on the content of phenols, flavonoids and photosynthetic pigments in the leaf blade of two elm species.

The content and ratio of different forms of photosynthetic pigments in chloroplasts can serve as one of the indicators of their photochemical activity and change under the influence of various types of stress.

The content of chlorophylls *a* and *b*, carotenoids and the ratio of the amount of chlorophylls to the content of carotenoids did not significantly differ in the two elm species, being within the limits typical for woody species. The total content of phenolic compounds in both species was similar, but the concentration of flavonoids in the leaves of small-leaved elm (53.95 ± 12.88 mg/g) significantly (by 49%) exceeded the content of flavonoids in the leaves of smooth elm (27.56 ± 7.98). As is known, flavonoids play an

Table 1: Analysis of ISSR spectra of *U. parvifolia* Jacq.

The primer	The boundary of the lengths of the spectrum loci, n.n.	The number of loci in the spectrum	The number of polymorphic loci	% polymorphism
807	303-1035	5	5	100
811	342-535	4	4	100
815	494-962	3	3	100
826	401-1155	7	7	100
834	213-1230	18	18	100
835	216-720	11	11	100
836	245-932	8	8	100
840	218-700	11	11	100
841	429-954	5	5	100
843	644-760	3	3	100
873	606-1039	4	4	100
890	228-581	7	7	100

Table 2: Analysis of the ISSR spectra of *U. laevis* Pall.

The primer	The boundary of the lengths of the spectrum loci, n.n.	The number of loci in the spectrum	The number of polymorphic loci	% polymorphism
807	303-1000	11	11	100
811	395-1168	4	4	100
815	619-1378	7	7	100
826	663-1155	10	10	100
834	213-515	10	10	100
835	228-1365	9	9	100
836	269-1011	10	10	100
840	230-1022	11	11	100
841	254-792	9	9	100
843	375-990	6	6	100
873	594-1029	9	9	100
890	249-715	10	10	100

Table 3: Indicators of intraspecific genetic diversity of *U. parvifolia* Jacq and *U. laevis*. Pall.

Samples	n_a	n_e	H_E	I	% полиморфизма
<i>U. parvifolia</i> Jacq	2	1.24 ± 0.12	0.18 ± 0.06	0.33 ± 0.08	100
<i>U. laevis</i> Pall.	2	1.11 ± 0.04	0.1 ± 0.03	0.2 ± 0.04	100

H_E : Expected heterozygosity; n_a : Absolute number of alleles per locus; n_e : Effective number of alleles per locus; I Shannon index. standard deviations are given in parentheses for all the above parameters.

Table 4: The content of pigments and phenolic compounds in the leaves of smooth elm and small-leaved elm, mg/g of raw weight.

Samples	Chlorophyll a	Chlorophyll b	Carotenoids	Chlorophylls a+b/Carotenoids	Phenolic compounds	Flavonoids ***
<i>U. laevis</i> Pall	0.56±0.13*	0.21±0.05	0.054±0.015	14.46±1.59	76.32±14.86	27.56±7.98
<i>U. parvifolia</i> Jacq	0.45±0.05	0.15±0.02	0.042±0.004	14.39±1.24	80.75±23.96	53.95±12.88

*The table shows the average values and standard deviations of the average values; **The concentration is expressed in mg-eq. gallic acid/g of crude mass; ***The concentration is expressed in mg-eq. routine/g of raw mass.

important role in protecting plants from bacterial, viral and fungal infections, from parasite penetration and insect damage. A number of authors note the greater resistance of *Ulmus parvifolia* Jacq to fungal pathogens and the development of graphiosis of ilmovs in comparison with *Ulmus laevis* Pall. (Firsov *et al.*, 2017; Kryukova *et al.*, 2018). It is possible that the increased content of flavonoids in the leaves of small-leaved elm causes greater stability of this species noted in literary sources.

CONCLUSION

As a result of this work, 18 ISSR primers were tested and 12 were selected, characterized by high efficiency of amplification of a larger number of fragments of genomic DNA. ISSR markers showed a high level of polymorphism and are suitable for determining the genotypic differences between the genotypes of *U. parvifolia* Jacq and *U. laevis* Pall growing in natural and artificial populations. The number of amplified fragments ranged from 3 to 18 and the size of the identified fragments ranged from 213 to 1,365 bp, depending on the primer. The maximum number of specific DNA fragments was noted when using primers UBC 834 and UBC 840. A high degree of polymorphism has been established for the samples *U. parvifolia* Jacq and *U. laevis* Pall. A comparative analysis of the content of pigments and phenolic compounds in two elm species was carried out. The concentration of flavonoids in the leaves of *U. parvifolia* Jacq significantly (by 49%) exceeded the content of flavonoids in the leaves of *U. laevis* Pall, which may be a consequence of genetic differences between these species and, possibly, causes a higher resistance of small-leaved elm to a number of phytopathogens. The results obtained can be used to identify individuals with economically valuable traits in breeding work, as well as in developing strategies for preserving biodiversity in natural populations of elms.

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conflict of interest

The authors declare that there is no conflict of interest. This article does not contain any research involving humans and animals as research objects.

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