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EVALUATION OF STRUCTURAL HOMOLOGY OF ABScisic ALDEHYDE OXIDASE IN WOODY PLANTS
USED IN AGROFORESTRY ON THE SOUTH OF RUSSIA

Research article

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Abstract

Territorial degradation and desertification leads to a reduction in the diversity of woody plants. Therefore, it is important to increase species diversity in arid areas by introducing new species. To increase the efficiency of introducing new species, it is necessary to study the genetic resources of woody plants that are resistant to adverse environmental conditions. The goal of the work was to study the abscisic-aldehyde oxidase enzyme as a potential genetic marker in woody plants used in agroforestry and introduction in arid areas. The UGENE program was used to obtain results from multiple alignments using the ClustalW algorithm, while a phylogenetic tree was constructed by the maximum likelihood method using MEGA 11 software. The amino acid sequence of the abscisic-aldehyde oxidase enzyme differed in *Populus trichocarpa*, which is the most drought-resistant species among the studied trees. Phylogenetic analysis of this enzyme in *Populus trichocarpa* highlights evolutionary changes and a distinct amino acid sequence compared with other species. The results showed that the abscisic-aldehyde oxidase enzyme can be a potential genetic marker for determining drought resistance in plants. Abscisic-aldehyde oxidase can be used as one of the criteria for selecting woody plant species for introduction into dry climatic conditions.

Keywords: abscisic-aldehyde oxidase, woody plants, multiple alignment, phylogenetics, introduction.

ОЦЕНКА СТРУКТУРНОЙ ГОМОЛОГИИ АБСЦИЗОВОЙ АЛЬДЕГИД-ОКСИДАЗЫ У ДРЕВЕСНЫХ РАСТЕНИЙ, ИСПОЛЬЗУЕМЫХ В АГРОЛЕСОМЕЛИОРАЦИИ НА ЮГЕ РОССИИ

Научная статья

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Аннотация

Деградация территорий и опустынивание приводят к сокращению разнообразия древесных растений. Поэтому важно увеличить видовое разнообразие в аридных районах путем внедрения новых видов. Для повышения эффективности ввода новых видов необходимо изучить генетические ресурсы древесных растений, устойчивых к неблагоприятным условиям среды. Целью работы было изучение фермента абсцизо-альдегид-оксидазы как потенциального генетического маркера у древесных растений, используемых в агролесомелиорации и при внедрении в аридные районы. Для получения результатов множественного выравнивания с помощью алгоритма ClustalW была использована программа UGENE, а филогенетическое дерево построено методом максимального правдоподобия с помощью программы MEGA 11. Аминокислотная последовательность фермента абсцизо-альдегид-оксидазы отличалась у *Populus trichocarpa*, который является наиболее засухоустойчивым видом среди исследованных деревьев. Филогенетический анализ этого фермента у *Populus trichocarpa* выявил эволюционные изменения и отличия в аминокислотной последовательности по сравнению с другими видами. Полученные результаты показали, что фермент абсцизо-альдегид-оксидаза может быть потенциальным генетическим маркером для определения засухоустойчивости растений. Фермент абсцизо-альдегид-оксидаза может быть использован в качестве одного из критериев отбора видов древесных растений для интродукции в засушливые климатические условия.

Ключевые слова: абсцизо-альдегид-оксидаза, древесные растения, множественное выравнивание, филогенетика, внедрение.

Introduction

Climate change leads to desertification and degradation of territories, which manifests itself in a decrease in the biodiversity of woody plants [1]. In this regard, increasing the species diversity of arid territories through introduction is relevant [2]. Increasing the efficiency of introduction involves searching for original and promising materials based on the study of the genetic resources of woody plants that are resistant to a complex of adverse environmental factors [3].

There are various criteria for the selection of promising woody plants, one of which is the physiological and biochemical parameters of the species [4]. Currently, such a criterion as the molecular genetic criterion is actively used, which allows screening of resistant plant individuals at an early stage [5] and studying the formation of adaptive reaction mechanisms at a fundamental level [6].

Abscisic-aldehyde oxidase (AAO) is an enzyme that plays an important role in plant life processes under various adverse environmental conditions. This enzyme is involved in the formation and metabolism of abscisic acid (ABA). ABA is a plant hormone that affects processes such as seed and fruit maturation, seed germination, stomatal closure, and adaptation to stress conditions [7], [8].

The role of AAO is to catalyze the oxidation of abscisic aldehyde to ABA. AAO is activated in response to stress, leading to an increase in ABA levels in plants. A high ABA level acts as a signal of external stress and activates a cascade of reactions aimed at protecting the plant from adverse environmental conditions. During periods of water stress, ABA levels increase, causing stomata to close to reduce water loss. Features of ABA synthesis and metabolism are also determined by the environmental conditions. For example, the activity of enzymes involved in ABA formation and conversion increases during drought or other stress. Therefore, AAO plays an important role in plant protection and adaptation to stress conditions [9], [10], [11].

The study of the role of AAO and ABA in plant metabolism is of great importance for understanding the mechanisms that ensure the survival and thriving of plants in arid conditions. Based on this, it is possible to select species for introduction into arid areas prone to desertification.

Thus, the aim of this study was to identify differences in the structural homology of AAO in native and introduced woody plants used in agroforestry in the southern territories of Russia.

Research methods and principles

The AAO enzyme was chosen as a potential gene marker whose expression may reflect changes in the state of drought-stressed plants. Woody species were selected from the forest seed bank organization program for protective afforestation in the southern regions of Russia [12], [13]. Known drought-tolerant plants were taken into account. As a result, five plants with different numbers of AAO enzyme isoforms were found for further study: 2 isoforms of *Malus sylvestris* (wild apple), 3 isoforms of *Populus trichocarpa* (black cottonwood), 2 isoforms of *Prunus mume* (Japanese apricot), 1 isoform of *Pyrus x bretschneideri* (Chinese pear), and 2 isoforms of *Quercus robur* (common oak). Within the *Populus* genus, three species with the enzyme were found: *Populus alba* (white poplar), *Populus euphratica* (Euphrates poplar), and *Populus trichocarpa* (black cottonwood). To align the sequences, the amino acid sequences of the first isoforms of the selected plants were used:

1. *Malus sylvestris*: XP_050141529.1;
2. *Populus alba* A0A4V6A3J8;
3. *Populus euphratica* XP_011005929.1_1;
4. *Populus trichocarpa*: XP_024455533.2;
5. *Prunus mume*: XP_008230901.1;
6. *Pyrus x bretschneideri*: XP_048429325.1;
7. *Quercus robur*: XP_050284917.1.

Data on the structure of nucleotide and amino acid sequences of the studied genes of woody plants were obtained from the open-access databases UniProtKB [14], NCBI Protein [15], and NCBI Gene [16]. Amino acid sequences were obtained based on the coding region of the gene. The search was conducted using synonymous constructs (Abscisic aldehyde oxidase[All Fields] AND ((“Amorpha”[Organism] OR Amorpha[All Fields])) OR (“Quercus”[Organism] OR Quercus[All Fields]) OR... (“Fraxinus”[Organism] OR Fraxinus[All Fields])) AND alive[prop]. Bioinformatic analysis was performed to identify differences between genes. It included multiple alignment of the amino acid sequences of the studied genes using the Ugene program (UNIPRO, Russia) and the ClustalW algorithm (BLOSUM62 matrix). The results of the alignment were evaluated by the percentage of identity, points (score), and the presence of common blocks (structural regions). Some sequences with the suffix “like” in the gene name were selected for alignment, as only such forms were present in the plant. This was because there were a limited number of responses to the query for the studied gene across the genera. Pseudogenes were not considered. A phylogenetic tree was constructed using the maximum likelihood method based on amino acid sequences, the JTT+G model, and bootstrap support in the MEGA11 program (MEGA, Japan).

Main results

As a result of analyzing the similarity of the enzyme in woody plants, we found potential differences that may affect the plant's ability to adapt to drought (see Table 1, Fig. 1, 2, 3).

Table 1 - Similarity of amino acid sequences of abscisic-aldehyde oxidase in woody plants according to the results of multiple alignment

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	Species	<i>Malus sylvestris</i>	<i>Populus trichocarpa</i>	<i>Prunus mume</i>	<i>Pyrus x bretschneideri</i>	<i>Quercus robur</i>
Species	Drought tolerance*	+	+++	++	++	++
<i>Malus sylvestris</i>	%	100	69	83	97	75
	score	1409	972	1173	1363	1059

	Species	<i>Malus sylvestris</i>	<i>Populus trichocarpa</i>	<i>Prunus mume</i>	<i>Pyrus x bretschneideri</i>	<i>Quercus robur</i>
<i>Populus trichocarpa</i>	%	69	100	70	70	73
	score	972	1409	988	983	1034
<i>Prunus mume</i>	%	83	70	100	83	77
	score	1173	988	1409	1173	1078
<i>Pyrus x bretschneideri</i>	%	97	70	83	100	76
	score	1363	983	1173	1409	1068
<i>Quercus robur</i>	%	75	73	77	76	100
	score	1059	1034	1078	1068	1409

Note: «+++» - high, «++» - moderate, «+» - low drought tolerance

Populus trichocarpa is highly drought-resistant, while *Malus sylvestris* is poorly adapted to drought. The lowest percentage of similarity between the amino acid sequences of these trees is 69%. The similarity percentage between *Populus trichocarpa* and *Prunus mume*, *Pyrus x bretschneideri*, *Quercus robur* is approximately at the same level – 71%. The sequence of *Malus sylvestris* is most similar to *Pyrus x bretschneideri*. *Prunus mume* shows a high sequence similarity with both *Malus sylvestris* and *Pyrus x bretschneideri*. Since these trees are fruiting plants, their genetic sequences will be more similar to those of non-fruiting plants.



Figure 1 - Alignment of amino acid sequences of *Malus sylvestris*, *Populus trichocarpa*, *Prunus mume*, *Pyrus x bretschneideri*, and *Quercus robur*. Areas with a conservative level of more than 85% are colored dark gray

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The amino acid sequence of *Populus trichocarpa* significantly differs in positions 4–19, 580–590, and 1352–1368. The presence of distinctive regions as the result of multiple alignment may indicate that these differences can play a role in functional variations between proteins. In this case, it is assumed that the difference in the sequence of the enzyme AAO may affect the process of ABA synthesis during drought. However, the enzyme has numerous conserved regions in its sequence among the considered tree species.

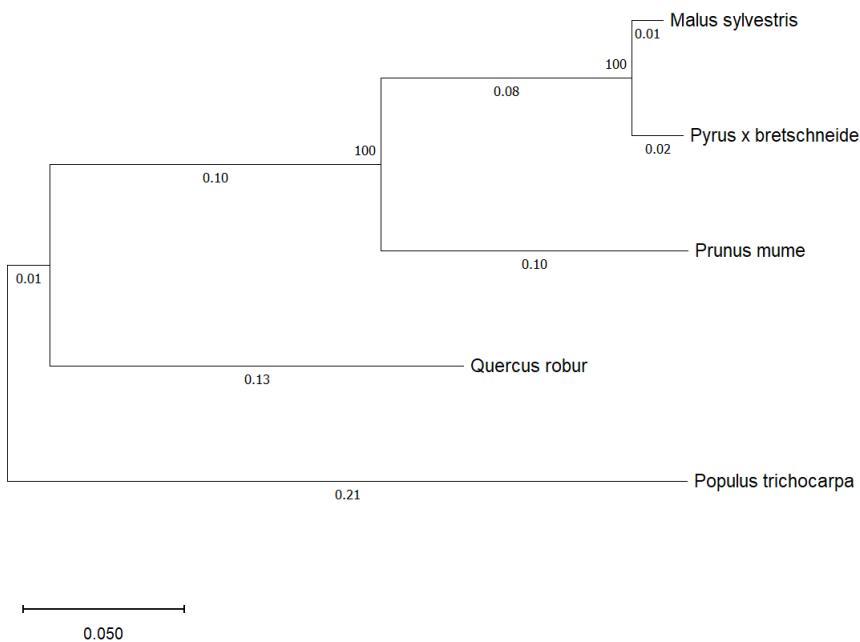


Figure 2 - Phylogram of the enzyme abscisic-aldehyde oxidase in woody plants
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Phylogenetic analysis showed that *Populus trichocarpa* does not form clades with other tree species, highlighting its genetic difference from the other woody plant species. The clade with *Malus sylvestris* and *Pinus x bretschneideri* has 100% bootstrap support. The clade with *Malus sylvestris*, *Pinus x bretschneideri*, and *Prunus mume* has the same bootstrap support. These high values indicate a significant genetic similarity among these species. The absence of bootstrap support values for other nodes indicates that there is not enough data to calculate values. Closer to the common ancestor is *Quercus robur*, which means its sequence is more conservative among the plants. *Pyrus x bretschneideri*, *Prunus mume*, and *Populus trichocarpa* are further away from the common ancestor.

The evaluation of the structural homology of AAO in *Malus sylvestris*, *Populus trichocarpa*, *Prunus mume*, *Pyrus x bretschneideri*, and *Quercus robur* showed differences in several regions of the enzyme's amino acid sequence. This, in turn, may potentially influence the activity of the enzyme and the amount of its product involved in the formation of adaptive responses to drought [17], [18]. Differences in the enzyme's structure were also found in *Populus trichocarpa*, which exhibits high drought resistance compared to other examined plants, potentially affecting its drought tolerance. Phylogenetic analysis demonstrated the distinctness of *Populus trichocarpa* from other tree species, which may indicate its genetic divergence from other plant species.

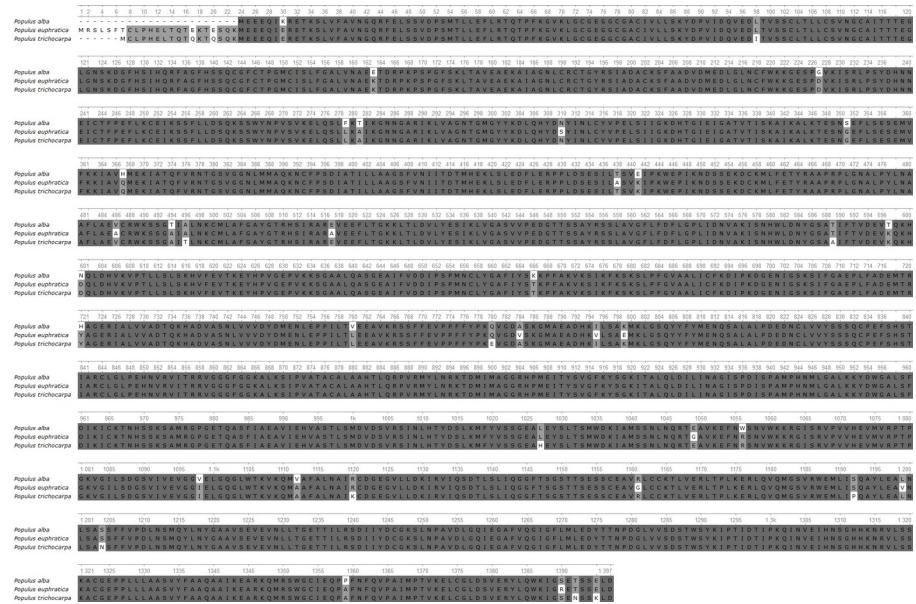


Figure 3 - Alignment of amino acid sequences of *Populus alba*, *Populus euphratica*, and *Populus trichocarpa*. Areas with a conservative level of more than 83% are colored dark gray

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To find the same distinctive positions in the genus *Populus*, multiple alignment of the enzyme was carried out. The analysis revealed that the sequences between poplar trees are more than 95% similar. The whole sequence is conservative; there are only single substitutions in positions. The domain structures also overlap [19]. However, a region at the beginning of the sequences stands out: 1-23. Nevertheless, the genetic variability within the species can be further studied, as these variations may be associated with differences in ecological conditions or genetic diversity.

Discussion

To find the same distinctive positions in the genus *Populus*, multiple alignment of the enzyme was carried out. The analysis revealed that the sequences between poplar trees are more than 95% similar. The whole sequence is conservative; there are only single substitutions in positions. The domain structures also overlap [19]. However, a region at the beginning of the sequences stands out: 1-23. Nevertheless, the genetic variability within the species can be further studied, as these variations may be associated with differences in ecological conditions or genetic diversity.

Conclusion

Populus trichocarpa is one of the most drought-tolerant plants among the studied species of woody plants. The amino acid sequence of the enzyme AAO in *Populus trichocarpa* differs partially from other studied woody plants. The presence of these unique differences may influence the functional properties of the enzyme. In addition, phylogenetic analysis based on this enzyme shows that *Populus trichocarpa* is relatively isolated from other studied plants. It can be hypothesized that *Populus trichocarpa* has evolved and developed a unique amino acid sequence of the AAO enzyme. The amino acid sequence similarity between *Populus alba*, *Populus euphratica*, and *Populus trichocarpa* is over 95%. It is unknown whether these differences have functional significance in the enzyme. Presumably, these changes may be related to environmental differences or are a result of genetic diversity among poplar species.

Финансирование

Создание биоресурсной коллекции видов растений для борьбы с деградацией аридных регионов России.

Конфликт интересов

Не указан.

Рецензия

Все статьи проходят рецензирование. Но рецензент или автор статьи предпочли не публиковать рецензию к этой статье в открытом доступе. Рецензия может быть предоставлена компетентным органам по запросу.

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Creation of a Bioresource Collection of Plant Species to Combat the Degradation of Arid Regions of Russia.

Conflict of Interest

None declared.

Review

All articles are peer-reviewed. But the reviewer or the author of the article chose not to publish a review of this article in the public domain. The review can be provided to the competent authorities upon request.

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