Genome Sequencing and Drought Resistance Expression Analysis of Mulberry Hybrids

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Abstract: Drought is one of the most harmful abiotic stresses to plant growth, which directly causes a substantial reduction in the yield of crops worldwide. The purpose of this paper is to study and analyze the genome sequencing and drought resistance expression of mulberry hybrids. Using the mulberry seeds of the F1 generation of the hybrid combination of Gui Sangyou and Cesha series that have been collected at this stage with good phenotype and resistance, and the characters can be stably inherited, the seeds were treated with LNaCl solution, and the germination rate after treatment, The data of germination potential, germination index, salt damage index and other traits were used to comprehensively evaluate the salt tolerance of various mulberry resources; the seedlings were treated with the culture medium containing 45% PEG6000, and the survival rate of the seedlings of each mulberry hybrid combination after treatment was compared to evaluate its drought tolerance. The two kinds of resistance data were comprehensively analyzed, and the comprehensive resistance of different mulberry germplasm resources was classified. Using transcriptome sequencing (RNA-seq) technology, mulberry trees were sequenced under drought conditions. Simple measurements of the sequenced data are performed, along with identification assays and associated bioinformatics analysis.

Keywords: Mulberry Hybrid; Hybrid Combination; Genome Sequencing; Drought Resistance Expression

1. Introduction

The drought resistance of plants is a relatively general concept in ecology and biology, which generally refers to the drought resistance of plants under the condition of water deficit [1]. It is of great significance for ecological sustainable development and food security to explore the molecular mechanism of drought resistance and drought tolerance of drought-resistant plants, and cultivate drought-resistant plants and crops that can efficiently utilize water [2]. Therefore, exploring how plants regulate the expression of drought resistance genes, identify key genetic determinants and improve crop drought resistance are the key tasks and main goals to meet the scientific field of plant stress resistance research [3].

The cost of whole-genome sequencing has declined rapidly and is increasingly being used in large clinical research projects and introduced into routine clinical care [4]. Morris H R outlines steps for whole-genome sequencing of patient tissues in neurology clinics and emphasizes that close links between clinicians and laboratories are essential [5]. The unprecedented level of bacterial strain identification provided by whole-genome sequencing (WGS) presents new challenges for the utility and interpretation of the data. Waldram a analyzed the whole genome sequences of 1445 Salmonella isolates isolated between April and August 2014, belonging to the most common serotypes in England and Wales. Single-linked SNP thresholds at 10, 5, and 0 levels were explored and statistically significant epidemiological associations were identified in 17 clusters [6]. Drought has a great impact on mulberry, manifested in transpiration, the activity of many enzymes and the rise and fall of malondialdehyde, plant hormones, etc. Drought can seriously affect the growth and development of mulberry trees, and long-term drought can even lead to the death of mulberry trees. Therefore, the selection of drought-resistant mulberry varieties is of great significance for drought resistance and desertification resistance in the northwest region [7].

Here, RNA-seq analysis was performed on two mulberry specimens under drought conditions and under normal growth conditions. We selected a number of different cultivars to identify and analyze multiple expression in mulberry under drought-tolerant and normal growth conditions. The results showed that the expression of 7 selectable genes increased to a certain extent under drought conditions compared with the normal condition, and only one WRKY6 was different from the normal condition. This not only provides a good database for the study of functional mulberry genes, but also provides a basis for the training of mulberry genes and functional genes, and provides a comprehensive database for collecting all mulberry genes.

2. Genome Sequencing and Drought Resistance Expression of Mulberry Hybrid Combinations

2.1 Whole-genome Sequencing Data Processing Methods

All genome sequencing technologies can rapidly measure the genetic information of an organism, which is very important for the study of biological sciences such as humans, animals and plants. The location information generated by the tracking technology is very reliable and highly accurate. Over the past decade, the following techniques have made great strides from initially time-consuming, high-cost to short-term, low-cost, high-performance [8].

(1) High-throughput sequencing technology

This technique will guide the development of new technologies that can compare data on hundreds of thousands of DNA molecules at a time. Technology is now increasing speed and reducing cost. Therefore, it is more and more widely used in related fields. Tracking technology promotes the development of scientific research, and the introduction of platform technology solves problems related to places [9].

High-tech technology is applied to the generated cDNA to obtain the content of mRNA fragments of different genes in a specific model by modifying the transcription of mRNA, which is the mRNA or mRNA-Seq process. High-resolution detection of various scripts can be performed by Deep sequence technology, collectively referred to as RNA-Seq [10].

(2) Third-generation sequencing technology

The technology refers to a novel monocyte and single-molecule genome sequencing technology based on high-performance sequencing technology. It mainly includes Helico Bioscience Single Molecule Sequencing Technology (TSMS), Pacific Bioscience Single Molecule Real-Time Molecule Sequencing Technology (SMRT) and Oxford Nanopore Single Molecule Sequencing Technology.

(3) Sequencing data processing process

Raw sequencing fastq files are required to align and compare species reference sequences in fasta format. This is usually done using the bwa tool. Next, convert it into genotype data that can be parsed by other tools. The specific steps of the operation are: index the reference genome, find and compare read sequences, perform tasks in each read file, convert sam to bam, and for the final sam or bam, export the alignment difference positions and can use GATK to directly compare each read. Chromosomes perform np calls.

2.2 Plant Drought Resistance Mechanism

When water dries up or there is a prolonged drought, the shape of the leaves changes in response to climate change, water conservation, and reduced transpiration from water. Excessive shade, blemishes, and so-called fences on the back wall of the epidermis, can reduce air loss, reduce transpiration, and reduce damage. Drying is the movement of plant leaves to prevent overwatering during droughts. Some plants can block the rotation of the sun, rotating the angle and orientation of the plant, dramatically changing the orientation of the leaves to align with the direction of the sun. In the event of dehydration, plants can also effectively reduce instantaneous water loss by slowing leaf growth, rotating leaves, changing direction, and losing old leaves.

When plants are affected by drought, many types of oxygen (ROS) are delivered under ROS molecules, disrupting many skin proteins or essential properties of yeast, resulting in increased plaque and ions or severe damage and plant death. After long-term transformation, plants have developed a stable and complete immune system that can retaliate against oxygen that acts and protects from

oxidative damage, thereby promoting the normal function of the nucleus accumbens. There are two kinds of antioxidants in plants, one is the enzyme system, such as SOD, POD, etc.; the other is the non-immune system containing glutathione, ascorbic acid and carotenoids.

3. Investigation and Research on Genome Sequencing and Drought Resistance Expression of Mulberry Hybrids

3.1 Materials and Reagents

In this paper, the F1 of the hybrid combinations are marked as Cesha-za 1, Cesha-za 2, Cesha-za 4, Cesha-za 5, Cesha-za 6, Cesha-za 7, and Cesha-za 8, Cesha-Miscellaneous 10, Cesha-Miscellaneous 11, Cesha-Miscellaneous 12 and Cesha-Miscellaneous 13.

The mulberry seeds of Guisangyou No. 12 and Guisangyou No. 62 are provided by the Sericulture Development Station of M Autonomous Region. The test site is shown in Figure 1. The female parent of Guisangyou 12 is Sha 2, the male parent is F1 which is a cross between 7722 and Lun 109, and the female parent of Guisangyou 62 is 7862 and the male parent is 7722.

Figure 1: Salt tolerance identification test points

Weigh 5gHgCl2 powder, dissolve it in 500mL sterile ddH2O, and store at room temperature for later use.

Weigh 12g and 11.7g of NaCl respectively, dissolve in 1LddH2O, sterilize in a pressure cooker at 121℃ for 20min, and store at room temperature for later use.

Weigh the corresponding quality of PEG6000, dissolve it in 1L of prepared Hoagland's nutrient solution, prepare the culture solution of 200g/L, 300g/L, 400g/L of PEG6000, and store it in a 4C refrigerator for later use.

3.2 Seedling Drought Stress Test

The optimal treatment concentration of PEG6000 was determined, and the seedlings of the tested materials with basically the same growth status were treated, and the growth status of the seedlings was investigated and the survival rate was calculated. The seedlings that did not become soft and wilted after the treatment were regarded as highly drought-tolerant seedlings, placed in a Petri dish (containing filter paper) containing Hoagland's nutrient solution for one week, and then transplanted into the soil (normal water holding capacity), at normal culture in an artificial climate chamber. The first softened and wilted seedlings were regarded as seedlings with low drought tolerance, and were transferred to a Petri dish containing Hoagland's nutrient solution (with filter paper) for 1 week, and then transplanted into soil (with normal water holding capacity), and then The natural drought treatment was carried out until the seedlings showed obvious symptoms of drought stress (about 10

days, the relative soil water loss rate was about 70%), and the drought tolerance of the high and low drought-tolerant seedlings was investigated.

3.3 Measurement Method and Data Analysis

The effect of different concentrations of PEG6000 on the growth of F1 seedlings of the tested mulberry hybrid combination was analyzed by the statistics of the survival rate of seedlings, and the drought tolerance of the tested mulberry seedlings was analyzed by the number of days when the survival rate was lower than 50%.

A five-level scoring method was used to comprehensively evaluate the salt tolerance of the F1 generation of different mulberry hybrid combinations tested. Its calculation formula is:

$$D = \frac{H\max - H\min}{5} \quad (1)$$

$$S = \frac{H - H\min}{D} + 1 \quad (2)$$

In the formula, Hmax is the maximum value of each index, Hmin is the minimum value of each index, H is an arbitrary value of each index, D is the score range, s is the F1 index score of each tested mulberry hybrid combination in different measurements, with The total score of different indexes of F1 of each tested mulberry hybrid combination was used as the evaluation basis.

3.4 Construction of Sequencing Library

8 μg of total RNA was purified using the RNeasy Microkit according to their protocol. The resulting product was used for sequencing library construction. According to the "Illumina TruSeq RNA Sample Preparation Kit", the purified total RNA was subjected to mRNA isolation, fragmentation, first-strand cDNA synthesis, second-strand cDNA synthesis, end repair, and addition of A at the 3' end to complete the construction of the sequencing sample library.

4. Genome Sequencing of Mulberry Hybrids and Analysis and Study of Drought Resistance Expression

4.1 Effects of Drought Stress on the Growth of Seedlings of Different Mulberry Hybrid Combinations

Based on the five-level scoring method, the total score of the salt tolerance of the tested mulberry seeds at the germination stage was obtained by calculation. According to the total score, the salt tolerance of the 13 mulberry seed groups at the germination stage can be divided into 4 types: the total score > 30, which is a very salt-tolerant type; the total score is between 20 and 29, which is a high salt-tolerant type; the total score is between 20 and 29. Between 10 and 19, it is a moderate salt-tolerant type; if the total score is less than 10, it is a salt-sensitive type.

When the tested mulberry seedlings were subjected to drought stress, the number of days when the survival rate was lower than 50% was negatively correlated with the drought tolerance ranking. The stronger the drought tolerance, the later the stress symptoms appeared as shown in Figure 2. To sum up, with the increase of the degree of drought stress, the survival rate of each tested mulberry seedling decreased gradually; the tolerance of F1 seedlings of different tested combinations to drought stress was significantly different.

Among the F1 of the 13 mulberry hybrid combinations tested, Guisangyou 62 was extremely salt-tolerant but sensitive to drought, while Cesha-za 4, Cesha-za 5 and Guisangyou 12 belonged to the types with high salt tolerance and high drought tolerance. Sha-za 6 is extremely salt-tolerant and extremely drought-tolerant, while Cesha-za 12 is extremely drought-tolerant but sensitive to salt, as shown in Table 1.
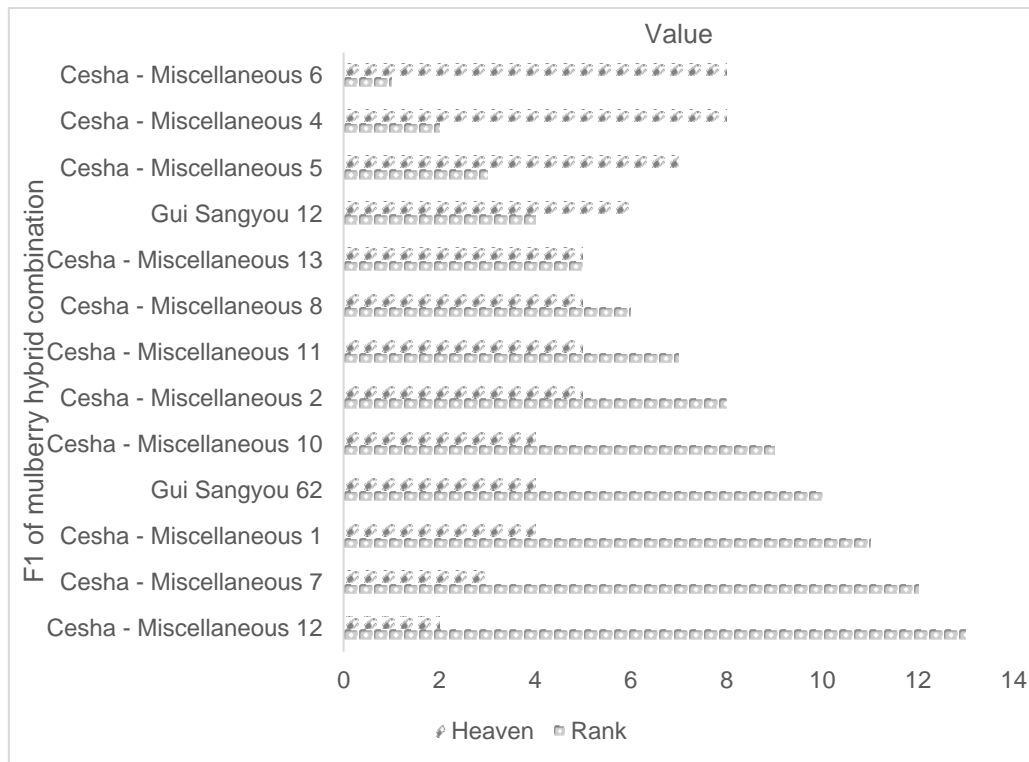
Table 1: Comparison of the number of days when the survival rate of F1 seedlings of 13 mulberry hybrid combinations was less than 50% and the ranking of drought tolerance

F1 of mulberry hybrid combination	Heaven	Rank
Cesha - Miscellaneous 6	8	1
Cesha - Miscellaneous 4	8	2
Cesha - Miscellaneous 5	7	3
Gui Sangyou 12	6	4
Cesha - Miscellaneous 13	5	5
Cesha - Miscellaneous 8	5	6
Cesha - Miscellaneous 11	5	7
Cesha - Miscellaneous 2	5	8
Cesha - Miscellaneous 10	4	9
Gui Sangyou 62	4	10
Cesha - Miscellaneous 1	4	11
Cesha - Miscellaneous 7	3	12
Cesha - Miscellaneous 12	2	13

Figure 2: Comparison of the number of days when the survival rate of F1 seedlings of 13 mulberry hybrid combinations was less than 50% and the ranking of drought tolerance

The obtained high and low drought tolerance seedlings of each tested mulberry hybrid combination were recovered and transplanted to the soil for further cultivation for 2 months, and then watered to saturation and subjected to natural drought. After 10 days, the leaves of the low drought-tolerant seedlings were obviously yellower than the leaves (A) of the high drought-tolerant seedlings, which also proved that PEG6000 was used to simulate drought stress at the seedling stage. feasible.

4.2 Transcriptome Sequencing of Mulberry Drought Stress

In order to identify the differentially expressed genes and their expression in normal and drought-stressed samples screened by transcriptome RNA-seq technology in this study, we randomly selected seven differentially expressed genes and used quantitative PCR to analyze these differentially expressed genes Expression under drought stress and normal conditions. We used the relative quantification (ddCt) method to analyze the quantitative PCR results, and the analysis results are shown in Figure 3.
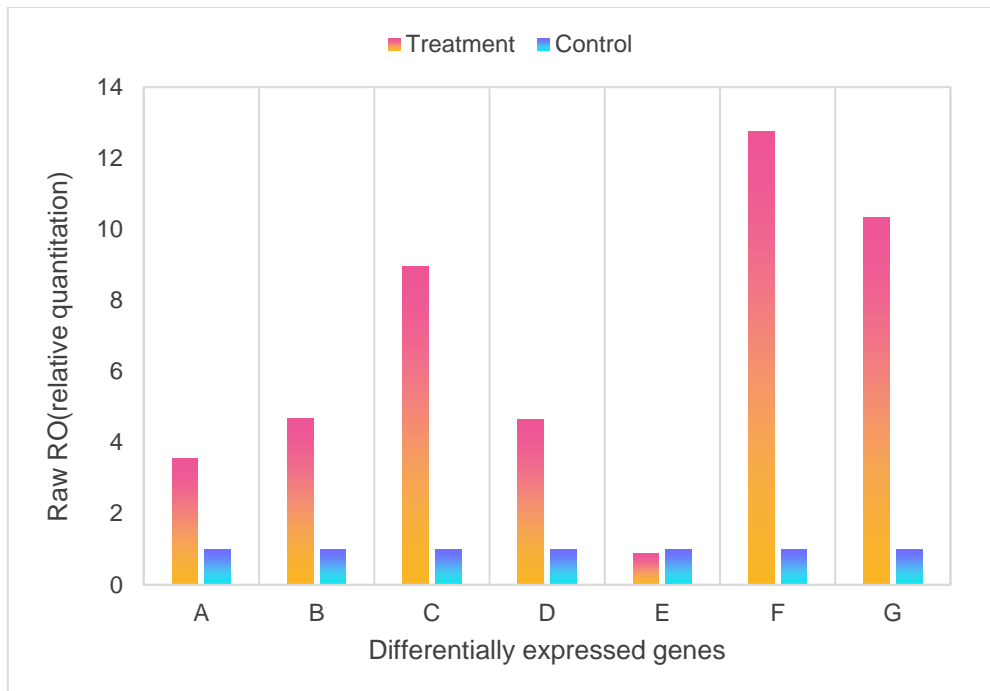
Figure 3: Relative quantitative analysis results of several differentially expressed genes

RQ Control=1, the value of RQ sample means that the expression level of this gene under drought stress conditions is a multiple of the expression level under normal conditions. For example, 3.54 in A means that the expression level of A under drought conditions is 3.54 times that under normal conditions, and so on. A: Dehydrin, B: EH2, C: AP2, D: NAC4, E: WRKY6, F: GBL, G: HSF30.

The expression of the selected seven genes all increased to a certain extent under drought stress, and only one WRKY6 gene had little difference with normal conditions. Among them, the GBL gene had the greatest increase in expression, which was more than 12 times that of normal conditions. This gene may be a galactose-binding lectin in mulberry (black mulberry).

5. Conclusion

By studying tree structure and physiological drought resistance, mastering the drought resistance mechanism of trees, and taking various measures to cope with drought, we can effectively resist drought, improve the survival rate of trees, and help cultivate drought resistance. In this paper, the hybrid combination of Cesha series and Guisangyou 12 and Guisangyou 62 were selected as test materials, which can improve the screening efficiency of salt- and drought-tolerant mulberry hybrid combinations. As far as the F1 of Cesha series mulberry hybrid combination and the hybrid F1 of Guisangyou series are concerned, the former has stronger drought tolerance and the latter has stronger salt tolerance. The results of this experiment have practical significance for promoting the development of ecological mulberry industry. In addition, this experiment evaluated the F1 salt and drought tolerance of each mulberry hybrid combination based on the comprehensive indicators of mulberry seed germination traits and the survival rate of seedlings. The reliability of the method was verified through plant experiments, and different types of salt tolerance and drought tolerance were obtained. Materials, changes in physiological and biochemical parameters such as photosynthesis, respiration, protective enzyme activity, and osmotic regulation of these materials under salinity and drought conditions require further study.

Acknowledgements

Authors'Contributions

Jianguo Shi conceived and designed the study. Shanshan Song and Jingjing Wang performed the experiments. Haofeng Gao and Ben Liu contributed materials/analysis tools. Shanshan Song and Jingjing Wang revised the paper. All authors approved and helped shape the final manuscript.

Availability of data and materials

The data-sets used and/or analysed during the current study available from the corresponding author on reasonable request.

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