Response of Interspecific Hybrid Species of Manchurian Ash to MJ and NO Signals and Preliminary Study on the Formation Mechanism of Drought Resistance Advantage

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Research Article

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Abstract

Background: In this study, sodium nitrate (SNP, a donor of nitric oxide) and methyl jasmonate (MJ) were used as exogenous hormones. The experiment was conducted with the offspring (interspecific hybrid) D110 of ash and ash, and their respective parents (non-interspecific hybrid) D113 and 4-3 as experimental materials. The experiment set up three experimental groups of drought stress, exogenous hormone SNP and MJ, and a control group under normal growth (non-drought stress), to study the physiological indicators and gene expression of manchurian ash.

Result: The results showed that under drought stress and exogenous application of hormone SNP or MJ, there were significant differences between hybrids and parents in plant growth, photosynthesis, defense enzyme activity, hormone content and gene expression.

Conclusions: This experiment provides a new theoretical support for the existing hormone breeding methods of manchurian ash, which can improve the drought resistance of manchurian ash and increase its survival rate in the wild. Increasing the growth rate and breeding efficiency of manchurian ash brings new ideas.

Background

Fraxinus mandshurica is a deciduous tree belonging to Fraxinus Linn. It is distributed in Daxing'an Mountains, Xiaoxing'an Mountains and Changbai Mountains in China. [1] And it is an excellent and precious tree species with fast growth and high economic value among broad-leaved trees. It has a wide range of uses and good materials. It is a famous commercial wood. It was recognized as a class II key protected wild plant by the state in 1994. [2]

As one of the main factors restricting the development of agriculture and forestry, drought not only affects the growth and metabolism of plants, but also affects the yield and reproduction of cash crops. Therefore, the study on drought resistance of plants is of great significance [3].It is of great theoretical significance and application value to explore the mechanism of plant resistance to drought stress and find out the measures to alleviate the drought stress of plants for further cultivating new varieties of drought-resistant manchurian ash.

In the preliminary work, our research group has obtained interspecific hybrids of Fraxinus mand shurica Rupr and Fraxinus Americana Linn, selected combinations with strong drought resistance [4-5], and studied the advantages of their drought resistance physiological and circadian genes in regulating the drought resistance of hybrid varieties. Studies have shown that the improved hybrid D110 is more adaptive to adversity probably due to the change of ABA signaling pathway in drought conditions. In addition, under drought conditions, the change of circadian gene expression may be one of the molecular mechanisms of heterosis [6].

Jasmonic acid (JA) and nitric oxide (NO) are the main signaling molecules in response to plant stress, which play a significant role in both biological and abiotic stress and in plant growth and development. When there is external stimulation, jasmonic acid has physiological effects such as signaling and initiation of stress resistance gene expression. Jasmonates, as environmental signaling molecules, not only participate in the regulation of plant growth and development under normal conditions, but also participate in the response and defense of plants to stress when induced by environmental stress. The results showed that exogenous methyl jasmonate could improve photosynthetic rate, transpiration and stomatal conductance of maize seedlings, enhance antioxidant enzyme activity and reduce malondialdehyde content, so as to alleviate plant damage caused by drought stress[7]. Nitric oxide (NO) is involved in plant responses to a variety of environmental stresses. Studies have shown that transgenic arabidopsis thaliana lines transgenic with rat neural NO synthase (nNOS) exhibit high levels of antioxidant enzyme activity, and the photosynthetic capacity, stress tolerance and hormone content of plants are all affected by nNOS transgenic. SNP is a commonly used NO donor, as a signaling molecule, NO is involved in ABA increase plant superoxide dismutase (SOD), peroxidase (POD) and ascorbic acid peroxidase activity of anti-aging ways [8], and reduce the accumulation of reactive oxygen species under abiotic stress, improving the function of plant resistance to oxidation damage. Moreover, MJ can rapidly activate the antioxidant protection system of woody plants, remove reactive oxygen species, reduce the level of oxidative stress, and effectively alleviate the damage caused by drought stress [9]. Moreover, studies have shown that low concentration of MJ can significantly improve the activity of protective enzymes in leaves and enhance plant stress resistance[10]. However, the current reports on the improvement of plant drought resistance by exogenous JA and NO mainly focus on herbaceous plants, involving few woody plants.

The circadian clock is a kind of timing system, which can provide a mechanism for organisms to adapt to the 24-hour day and night cycle. In the daily life of organisms, Circadian clock can enable organisms to achieve the corresponding biological activity by activating the relevant genes at a specific time point and synchronously expressing them. Studies on model plant arabidopsis thaliana have shown that: LHY (late elongated hypocotyl),TOC1 (timing of CAB expression 1) and CCA1 (circadian clock associated) interactions play a vital role in the maintenance of circadian rhythms [11].

In order to further explore the drought resistance mechanism of this hybrid combination, the response of NO and jasmonic acid to signal molecules and the expression characteristics of related genes under drought stress were studied in this paper. To explore the difference in response characteristics of interspecific hybrids of manchurian ash with respect to parents when exogenous NO and jasmonic acid were applied, the reasons for the formation of hybrid drought-resistance advantage, and the correlation with gene expression of rhythm, and to analyze its role, so as to provide new ideas for revealing the drought-resistance advantage mechanism of interspecific hybrids of manchurian ash.

Methods

Material

This article selects the drought-resistant obvious advantages of interspecific hybrid material D110, female parent provenance in Heilongjiang province with ridge manchurian ash (Fraxinus mandshurica Rupr.) champion tree D113, male parent for the provenance of Xinjiang big leaf ash (Fraxinus Americana Linn.) code 4-3, in manchurian ash free from female parent and big leaf ash free pollination progeny as for parental controls, selects the potted seedlings, born five years and take its blade as experimental material.

Drought treatment

The plants were planted in plastic buckets with a diameter of 53 cm and a height of 55 cm in a pot in a big shed. The planting medium was the mixed soil with fine sand mixed with humus soil in the nursery of the forest farm at a rate of 3:1. The drought stress was realized by water deficit. The standard of water deficit was set as 25% by relative water content, in which relative water content = soil actual water content/soil field water holding capacity, and the field water holding capacity of experimental soil was about 30%. Three seedlings were selected for each combination as the treatment group and three seedlings as the control group. Each morning and evening, Hydra Probe II Soil Moisture and Salinity Sensor (sdi-12 / RS485) are used to control its water content at the set level (relative water content 25%), and it is continuously stressed for 12 days before rehydration. Observe and record the growth status and in dry processing day 0, 3 days, 6 days, 9 days, 12 days and 9 points out every 3 days after water, use liquid nitrogen frozen after - 80 °C refrigerator. Set aside. To study the effects of drought stress and rehydration on the expression of drought-resistant genes in plants.

Exogenous application of SNP and MJ

The plants with robust growth and uniform growth were selected, and the SNP (sodium nitrate as the donor of NO) solution and MJ (methyl jasmonate as the donor of JA) solution with the concentration of 1 mmol/l were added in the way of irrigation at 0d after the drought treatment, and then the materials were selected at a fixed point at 12d after the drought treatment.

Determination of photosynthesis parameters

The photosynthetic index was determined by selecting 3 middle leaves of the third pair from the top to the bottom of each plant, and the fixed light intensity was 1000 mol m $^{-2}$s.$^{-1}$, sample room CO_2 400 molCO $_2$Mol,$^{-1}$, the measured leaf area is 6cm^2 , gas flow rate of 500 mols $^{-1}$Then, the li cor 6400XT photosynthetic system (USA) was used to measure the photosynthetic activity of the tested materials before and after the drought treatment and after rehydration, and the net photosynthetic rate (mol CO) was mainly recorded$_2$ m $^{-2}$s.$^{-1}$), intercellular CO_2Concentration (mu molCO_2Mol,$^{-1}$), stomatal conductance (molH_2Om^{-2}s.$^{-1}$) and transpiration rate (mmolH_2Om^{-2}s.$^{-1}$) four indicators. The photosynthetic index of each plant was measured three times per leaf.

MDA content and activity of SOD and POD were determined

Superoxide dismutase (SOD) activity was determined by NBT photoreduction method, and POD activity was determined by guaiacol method. The content of malondialdehyde (MDA) was determined by thiobarbituric acid (TBA) colorimetry.

Real-time quantitative RT-PCR

RNA extraction, reverse transcription and PCR amplification of cDNA were performed using plant RNA out extraction kit and DNA scavenging kit of TAKARA company. Total RNA was extracted and purified from samples at different stages. RNA was assayed by concentration, electrophoresis and reverse transcription. The expression levels of LHY, TOC1 and TU (internal reference) genes were detected simultaneously by fluorescence quantitative PCR of the cDNA. Real-time fluorescence quantitative PCR was performed by fluorescence quantitative PCR instrument, and 2 $^{-\Delta\Delta CT}$To calculate the relative expression of the gene.

Primers sequence used in this experiment

Data processing and statistics

The experimental data were saved and calculated by Microsoft Excel 2007 software, analyzed by IBM SPSS statistics 19 software, and analyzed by Duncan's new complex range method. The significance difference test $P<0.05$.

Results

Effects of exogenous NO and MJ on relative growth of Fraxinus mandshurica under drought stress

By measuring plant height (Fig. 2.1.a) and ground diameter (Fig. 2.1.b) of hybrid combinations after drought stress, it was found that drought stress had a significant inhibitory effect on plant height growth of hybrid combinations. However, there was no obvious effect on the increment of ground diameter. The plant height growth of hybrid D110 was higher than that of non-hybrid D113 and 4-3, which indicated that the growth of hybrid D110 was better than that of parent under drought stress, and the drought resistance of hybrid D110 was stronger than that of non-hybrid D113 and 4-3.The results showed that the plant height growth of D110 hybrid was 1.3 times higher than that of non-hybrid D113 under drought stress or drought stress, while exogenous MJ, was added at the same time as drought stress. The plant height growth of the hybrid D110 was 3.0 times of that of the non-hybrid D113, which indicated that the treatment of MJ could make the plant have stronger drought resistance. After adding exogenous SNP and MJ, the inhibitory effect of drought stress on plant growth was obviously weakened.

Effects of exogenous NO and MJ on photosynthesis of Fraxinus mandshurica under drought stress

Photosynthesis was inhibited and photosynthetic rate decreased under drought condition. It is generally believed that varieties with strong drought resistance can maintain relatively high photosynthetic rate or net photosynthetic rate, so net photosynthetic rate is a reliable indicator for drought resistance identification.

From figure 2.2, the photosynthesis indexes of hybrid D110 and non-hybrid D113 and 4-3 were measured under drought stress and rehydration for every 3 days (12 days) after drought stress and rehydration for 3 days (15 days). It was found that drought stress inhibited the net photosynthetic rate of hybrid D110 ,non-hybrid D113 and 4-3 (Fig. 2.2.a, Fig. 2.2.b).The net photosynthetic rate of the hybrids and their parents decreased gradually with time, and reached the lowest at 12 days, but after rehydration, the inhibition was obviously weakened, the net photosynthetic rate was obviously increased, and the intercellular CO2 concentration was significantly increased. Stomatal conductance and transpiration rate were similar to net photosynthetic rate.

Under drought stress, the net photosynthetic rate of hybrid D110 decreased to some extent when exogenous SNP or MJ was added, but the decrease range of D110 was lower than that of control, and the net photosynthetic rate of hybrid D110 was higher than that of control. It shows the advantage of drought resistance.

Under drought stress, the net photosynthetic rate of progeny D110 added with exogenous SNP or MJ, was 6.4% or 24.1% higher than that of drought stress, indicating that hybrid progenies were more sensitive to exogenous MJ signals. However, when exogenous SNP and MJ were applied to parents, the variation of photosynthesis was not as obvious as that of hybrid progeny, which indicated that hybrid progenies were more sensitive to exogenous signals and had drought resistance advantages than their parents.

Effects of exogenous NO and MJ on the content of malondialdehyde (MDA) in water under drought stress

The damage (or senescence) of plants under stress is closely related to the membrane lipid peroxidation induced by the accumulation of reactive oxygen species. Malondialdehyde (MDA) is one of the most important products of membrane lipid peroxidation. Enzyme binding or crosslinking inactivates the biofilm, thus destroying the structure and function of the biofilm. Therefore, the degree of membrane lipid peroxidation can be understood by measuring the content of MDA, and the damage degree of membrane system and plant resistance to stress can be indirectly measured.

From Fig. 2.3,the content of MDA (Fig. 2.3) of D113 and 4-3 of hybrid progeny D110 and non-hybrid progeny was determined under drought stress and exogenous application of SNP and MJ. The results showed that the MDA content of D110 and D113 and 4-3 increased in different degree under drought stress, but with the rehydration, the MDA content decreased, and the D110 decreased 36.3%. Parent D113 decreased 30.7% and parental 4-3 decreased 27.1%.The results showed that drought stress caused damage to the membrane system of all hybrid combinations to varying degrees, but with the rehydration, the damage degree decreased to a certain extent, and the degree of decline of hybrid progenies was greater than that of parental control. The results showed that the damage degree of membrane system of hybrid progenies was lower and the drought resistance was higher. Under drought stress or exogenous application of SNP and MJ, the MDA content of the hybrid D110 was more stable than that of its parent D113 and 4-3, which indicated that the hybrid progenies had obvious drought-resistance advantage over their parents.

Effects of exogenous NO and MJ on antioxidant enzyme activity of Fraxinus mandshurica under drought stress

When plants are in bad environment or senescence, there will be a large amount of reactive oxygen species accumulation in plants, which will lead to damage to the membrane system, and then affect the normal growth of plants. Superoxide dismutase (SOD) combined with peroxidase (POD) can scavenge oxygen free radicals and reduce oxidative damage of active oxygen groups to plants. The determination of SOD, POD content can indirectly show the degree of oxidative damage in plants. Which shows the drought resistance of the plant.

From figure 2.4, under the conditions of drought stress and exogenous application of SNP and MJ, the activities of antioxidant enzymes SOD (Fig. 2.4.a) and POD (Fig. 2.4.cnd) were significantly increased compared with those of the control group without drought treatment. However, the content of MDA decreased significantly. The increase of the activity of SOD and POD and the decrease of MDA content in D110 were higher than those in D113 and 4-3 of their parents, which indicated that the ability of each hybrid to resist drought environment was stronger than that of its parents. After 3 days of rehydration, D110 could maintain a high level of SOD and POD activity, especially in the activity of POD. After drought, the POD activity of hybrid progenies treated with SNP and MJ increased respectively 41.5%, 120.4%, 173.5%, which may indicate that after rehydration, there are still a large number of active oxygen free radicals need to be removed, maintaining a higher SOD enzyme activity and POD enzyme activity can help plants recover from stress better.

After treated with SNP and MJ for 9 days, the activity of SOD enzyme of hybrid D110 was higher than that of drought stress and control, and increased by 12.2% and 25.1% than that of drought stress, respectively. The non-hybrid progeny D113 was treated with SNP and MJ for 9 days. Compared with drought stress, the activity of SOD enzyme increased by 4% and 8%, respectively. After 9 days of treatment with SNP and MJ, the activity of SOD enzyme in 4-3 hybrid was increased respectively by 7.7% and 10.1%. After treated with SNP and MJ for 12 days, the enzyme activity of POD was increased respectively by 10% and 33. 3% than that of drought stress. The enzyme activity of POD was increased by 15. 7% and 19. 9% than that of drought stress after SNP and MJ treatment for 12 days. After 12 days of treatment with SNP and MJ, the activity of POD enzyme in the 4-3 hybrid was increased respectively by 8.9% and 11.33%, compared with drought stress. The preliminary results showed that the hybrids could produce more antioxidant enzymes and enhance the survival rate of plants under stress, and the plants treated with MJ had more drought-resistant advantages than those treated with SNP.

Effects of exogenous NO and MJ on the expression of Rhythm Gene in Fraxinus mandshurica under drought stress

As a "big switch", rhythm gene can recognize the original regulation of gene upstream of stress signaling pathway and regulate the response of plants to abiotic stress. The determination of gene expression can reflect the drought resistance of plants at molecular level.

After drought stress and exogenous SNP and MJ treatment, the expression of rhythm gene LHY,TOC1,LOX,NIR was determined by drought stress on D110 hybrid and D113 parent.

LOX can synthesize the precursor of MJ and its metabolites are oxygen free radicals and reactive oxygen species which can destroy the membrane structure of cells and participate in the process of plant senescence. The results showed that LOX genes synthesized during germination could increase its expression level through abscisic acid pathway[12].In order to cope with the environmental pressure caused by global climate change and excessive nitrogen application, it is very important to improve the water and nitrogen use efficiency of crops. It is reported that higher nitrogen uptake can mitigate the destructive effects of drought stress. After drought stress, the nitrogen and proline contents of transgenic plants were higher than that of wild type control, and the activity of nitrite reductase (NIR) was also higher during nitrate assimilation. The results showed that higher nitrate transport and assimilation activity were helpful to improve drought resistance of transgenic plants [13]. Nitrogen (N) is the main plant nutrient and plays an important role in determining plant growth and productivity. Plants need nitrogen to synthesize important molecules, such as protein, nucleic acid and chlorophyll. Most plant species can absorb nitrate ($NO3^-$) and ammonium ($NH4$). Overexpression of some genes in N pathway can increase plant tolerance to abiotic stress. For example, salinity, drought and extreme temperature all affect the absorption and assimilation of nitrogen (N) in plants. Most of the genes encoding nitrate transporter proteins and enzymes responsible for N assimilation and reactivation (such as nitrite reductase NIR) are down-regulated under abiotic stress. Especially under long-term stress (24 hours), this may be one of the reasons for the decrease of plant growth and development under abiotic stress [14].

From Fig. 2.5, it can be seen that the gene expression of the hybrid D110 with the exception of LOX has a certain change with the passage of time, reaching the peak value at 12 days. The expression of LOX gene was very low under drought stress and increased rapidly after rehydration. Under drought stress, the expression of LHY in hybrid D110 was 400 times higher than that of parent D113 when exogenous SNP and MJ were applied to the hybrid. The NIR was 8.5 times higher, and the TOC1 was 2.3 times higher, which indicated that the drought resistance advantage of the hybrid was much greater. The LHY gene of the hybrid D110 continued to increase under SNP treatment and reached its peak at 12 days. The MJ treatment decreased first and then increased, reached a low point at 6 days and a peak at 12 days. When exogenous SNP was applied, the expression of LHY was 17 times higher than that of MJ. The expression of TOC1 gene decreased at first and increased at the beginning of SNP treatment, and had a low point at 9 days and reached its peak at 12 days after SNP treatment. The expression of TOC1 gene decreased at first, reached a low point at 6 days and reached a peak at 12 days after treatment with exogenous MJ. The difference between the expression of LHY and TOC1 was that the expression of TOC1 decreased to a low point when the expression of LHY gene reached its peak from 3 to 9 days, and they formed a form of mutual inhibition. The expression of LHY and TOC1 genes in parent D113 increased at first and reached its peak at 12 days. When SNP and MJ were applied , the expression of LHY and TOC1 genes was higher than that of drought, which indicated that exogenous hormones had a certain effect on the improvement of drought resistance of plants.

The gene expression of the hybrid D113 is higher than that of the parent, and the mechanism of the drought-resistant advantage of the hybrid is preliminarily explained, and the mechanism of the different physiological conditions of the hybrid and the parent gene is preliminarily explained, and the reason for the drought-resistant advantage of the hybrid is also disclosed.

Effects of exogenous NO and MJ on hormone content in Fraxinus mandshurica under drought stress

Plant hormones are organic compounds that naturally exist in plants. Even at low concentrations, plant hormones can coordinate a wide range of physiological processes, including growth and development, as well as responses to abiotic and biological stresses.

Abscisic acid (ABA) plays a key role in the response of plants to drought stress. ABA is known to mediate drought response by regulating the expression of drought response genes and stomatal closure. These hormones regulate signal transduction pathways and gene expression through rapid induction or inhibition of transcription [15]. Exogenous ABA, which increases the content of free polyunsaturated fatty acid (PUFA) under stress, can stimulate the activity of lipoxygenase and promote the production of JA. However, JA binds or methylates with amino acid isoleucine to produce methyl jasmonate MJ. Peroxidation of membrane lipids was activated to promote the formation of tolerance when rice leaves were damaged [16]. Therefore, the increase of ABA concentration under drought stress is beneficial to improve the survival rate of plants.

Auxin IAA is a common chemical signal in all vascular plants. IAA can regulate many aspects of plant growth and development, including cell division and elongation, and organ development at cell and whole plant level [17]. This physiological regulation is achieved by signal transduction that results in changes in the expression of many genes. Gibberellin GA can inhibit cell proliferation by increasing the action intensity of cell cycle inhibitor. GA can not only inhibit the growth, but also promote the survival of plants under pressure by limiting the accumulation of reactive oxygen (ROS). Thus delaying cell death [18].

It can be seen from chart. a, b and c that the variation trend of IAA, ABA and GA is similar, with the increase of the number of days of drought stress, and the concentration is obviously higher than that of the control group, and the content of the three hormones is almost the highest at 12d. And remained at a higher concentration at the time of the hydration 3d (15d). Under drought stress, after 12 days of treatment with exogenous SNP and MJ, the ABA, IAA, GA in the hybrid D110 was significantly higher than that in the control group, and the increase of ABA content was the most obvious, the average increase was 37.9%, 33.9%, 51.2% in comparison with the control group. The content of ABA in parent D113 was increased by 3.3%, 13.1%, 0.9% in comparison with the control group. The content of ABA in the hybrid D110 was much higher than that of the parent D113, which indicated that the hybrid progeny could respond to drought stress more quickly at the hormone level, and had the advantage of drought resistance, and maintained a high concentration after rehydration. It shows that the plant needs a certain time to recover after rehydration.

Cytokinin is known as a plant hormone that modifies root morphology and increases root biomass to enhance drought resistance by reducing root-crown-axis ratio (CTK level) [19]. From figure d, it was found that the concentration of CTK decreased under drought stress and was significantly lower than that of control group, and reached the lowest value after 12 days of drought treatment, and the content of CTK increased obviously after rehydration. And after 12 days of drought treatment, compared with the control group, the CTK content of the hybrid D110 decreased by 10.4%, and that of the parent D113 decreased by 8%. The content of CTK in the hybrid progenies decreased significantly at 9 days, while that of the parent D113 decreased slightly at 12 days, which proved that the hybrids could respond more quickly and strongly to drought stress. Increase plant survival in arid environments by adjusting hormone content. The difference of hormone between hybrids and their parents is one of the reasons why hybrids have the advantage of drought resistance.

Discussion

Drought is a kind of environmental stress which has a serious effect on seed germination, plant growth and productivity. Plants with strong drought tolerance are ideal targets for studying drought related genes, proteins and metabolites [20]. In this study, the interspecific hybrids (D110) and their parents (D113 and 4-3) were treated with drought stress, and the physiological indexes were determined after exogenous SNP or MJ, was applied to the interspecific hybrids (D110) and their parent free pollination progenies (D113 and 4-3).

Under the experimental conditions, drought stress had a significant effect on photosynthesis of hybrid and parent free pollination progeny. Under drought conditions, the stomatal conductance of hybrid D110 was increased by 72.9% and 98.4% compared with that of parent free pollination progeny D113 and 4-3, and the transpiration rate of D110 was increased by 33.2% and 18.6% compared with that of D113 and 4-3, respectively. The two factors impelled the net photosynthetic rate of hybrid D110 under drought stress was increased by 38.4% and 50.9% than that of D113 and 4-3, respectively. The results of photosynthesis test showed that D110 had better osmotic regulation ability and it is consistent with previous studies [21].At the same time, the increase of net photosynthetic rate can also be one of the reasons for drought resistance of hybrid. The net photosynthetic rate of hybrid progeny D110 was increased by 22. 8% and 10. 4%, respectively, compared with drought stress by exogenous SNP and MJ, indicating that the response speed and amplitude of the same plant to MJ were higher than that of SNP. This showed that exogenous SNP and MJ could alleviate the decrease of photosynthetic activity of leaf mesophyll cells of Fraxinus mandshurica under drought stress, and the effect of MJ was more significant. Under drought stress, Fraxinus mandshurica could maintain high photosynthesis ability.

The results showed that exogenous SNP and MJ could increase the activity of SOD and POD in Fraxinus mandshurica under drought stress, and the effect of MJ on enhancing the enzyme activity was better than that of SNP. The result was similar to that of oat treated with exogenous NO [22].With the prolongation of drought stress time, the decomposition rate of protein in plant was higher than that in synthesis, and the protein content decreased, and the content of free proline in plant increased due to the decomposition of protein. Proline is a compatible osmotic agent that controls osmotic regulation and reduces cell membrane pressure and it also acts as an enzyme function protectant and free radical scavenger. The ability of plants to overcome oxidative stress depends on the activity of antioxidant enzymes such as SOD,POD. It has been pointed out that the cultivar with high drought tolerance has lower MDA content under stress, while MDA is mainly caused by membrane lipid peroxidation [23].

The main function of SOD as oxygen free radical absorbent is to decompose active oxygen group and slow down the speed of membrane lipid peroxidation. POD could separate the H2O2 produced by SOD into H2O and oxygen molecules and maintain the reactive oxygen species at a lower level, so as to improve the survival rate of plants in arid environment.

Because the decomposition rate of protein and lipids is higher than the synthetic rate, plants can produce a large amount of free amino acids and polyunsaturated fatty acids (PUFA) in dry environment. However, a large number of metabolites produced by PUFA oxidation are collectively called lipid oxide (such as JA). Many of them are bioactive compounds, which may be directly involved in plant defense, communication with other organisms or plant development, defense response and stress adaptation signal transduction [24].

The plant hormone abscisic acid (ABA) plays an important role in many physiological processes, such as seed dormancy, seed germination, seed growth and response to biotic and abiotic stress. One of the effects of ABA on adverse environmental conditions is to regulate stomatal movement [25]. Under the condition of drought stress and exogenous application of SNP and MJ, the expression of three endogenous hormones of gibberellin IAA and abscisic acid ABA in hybrid D110 was significantly higher than that in parent D113. When exogenous MJ was applied to the same plant, the increase speed and amplitude of the three hormones were higher than that of exogenous SNP. The results showed that under drought stress, when exogenous MJ and SNP were applied, the CTK content of hybrid D110 was lower than that of parent D113, and the content of CTK in D110 increased significantly after 15 days of rehydration. The results showed that the hybrid D110 still had strong cell division ability and strong resilience after drought stress, which was helpful to the survival of Fraxinus mandshurica in arid environment.

Genes LHY and TOC1, which as the core oscillator of plant clock system, constitute a transcriptional inhibition loop. The responses of LHY and TOC1 to drought stress show an increasing trend of variation [26]. The expression of rhythmic genes in D110 and D113 progenies of SNP and MJ, hybrids under drought stress or not increased significantly, but the growth rate of D110 hybrid progenies was larger than that of D113 parents, and the response speed was also faster than that of D110 progenies. The results showed that the drought resistance of hybrids was related to the expression of rhythmic genes. Moreover, the expression of LHY in D110 increased during 3 to 9 days, while the expression of TOC1 decreased, which was consistent with the characteristics of inhibition between the two genes. Lipoxygenase (LOX) gene and TOC1 gene are important genes of plant response to drought stress. LOX activity is considered as a biological marker of plant physiological state. Normally, LOX is a cascade key enzyme. The regulation of LOX gene expression by cascade end products. The regulation of MJ can induce LOX expression and increase the oxidation rate of PUFA, which is an important mechanism to promote the metabolism of oxygen-lipozyme. There was a positive correlation between the concentration of ABA and the content of LOX transcription products in plants under drought stress [16]. When exogenous SNP and MJ are applied under drought stress, the expression of LOX gene in D110 and D113 progenies of exogenous SNP and MJ, hybrids were decreased in varying degrees, but the extent of decline was much greater in hybrid progenies than that of their parents. However, the expression of NIR gene increased in different degrees, and the increase of cross progeny was much larger than that of parents. Due to the expression of LOX gene could damage the cell membrane system, and the lower expression level in hybrid progenies showed that the hybrids could maintain the survival rate of plants better than their parents. NIR was mainly involved in the absorption and transformation of N in nutrient synthesis. The high expression of NIR in hybrid progenies indicated that the hybrids could produce more energy to maintain growth in arid environment.

Conclusion

To sum up, the physiological indexes and gene expression of interspecific hybrid D110 and parent D113 were significantly different under drought stress and exogenous application of SNP and MJ, which showed that the interspecific hybrid D110 could enhanced plant photosynthesis and the enzyme activity of POD, SOD, it was used to remove excess reactive oxygen species and regulate the expression of related hormones to maintain the integrity of cell membrane. The combination of various factors made the interspecific hybrids have more advantages in drought stress. Moreover, the expression of rhythm genes LHY, TOC1 and drought response related genes NIR, LOX of D110 and parent D113 were different, which could further explain the reason and mechanism of drought resistance heterosis of interspecific hybrid D110. Exogenous SNP and MJ increased the drought resistance of hybrids and their parents to varying degrees, and the response speed and amplitude of Fraxinus mandshurica to MJ was greater than that of SNP. At present, Drought is a natural disaster that occurs at random, so the study on the drought resistance of Fraxinus mandshurica can provide a theoretical basis for solving the drought stress problem of Fraxinus mandshurica at various growth stages. And it also provides a foundation for the cultivation of Fraxinus mandshurica and the increase of its yield.

Abbreviations

SNP: sodium nitrate; MJ: methyl jasmonate; JA: Jasmonic acid; NO: nitric oxide; nNOS: neural NO synthase; SOD: Superoxide dismutase; POD: Peroxidase; MDA Malondialdehyde; NIR: nitrite reductase; N: Nitrogen; PUFA: polyunsaturated fatty acid; ROS: reactive oxygen; ABA: Abscisic acid.

Declarations

Acknowledgement

Not applicable

Availability of data and materials

All data generated or analysed during this study are included in this published article. The experimental materials are the seeds obtained in our laboratory, and the complete plants were cultured in the greenhouse of Northeast Forestry University. We abide by relevant regulations.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

CY conceived the idea of the study and designed the experiments.CY performed most of the experiments.CY,ZXT,HLMcollected and analysed the data.CY,SF involved in writing the manuscript. All authors read and approved the final manuscript.

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Tables

Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.

Figures

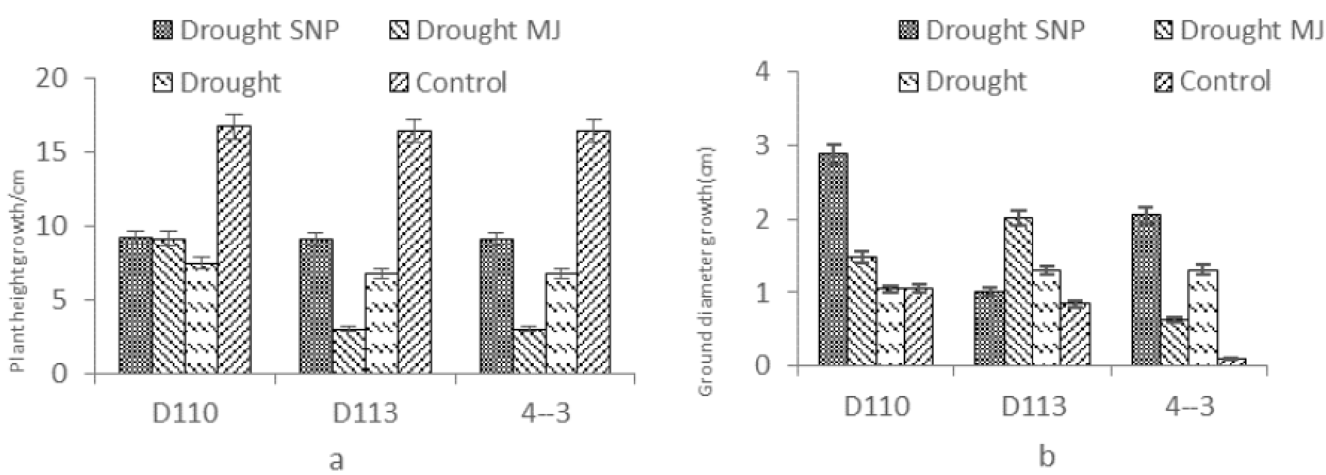

Figure 1

growth of Fraxinus mandshurica under drought stress by exogenous NO and MJ, Fig. a is the growth of plant height, and Fig. b is the growth of ground diameter

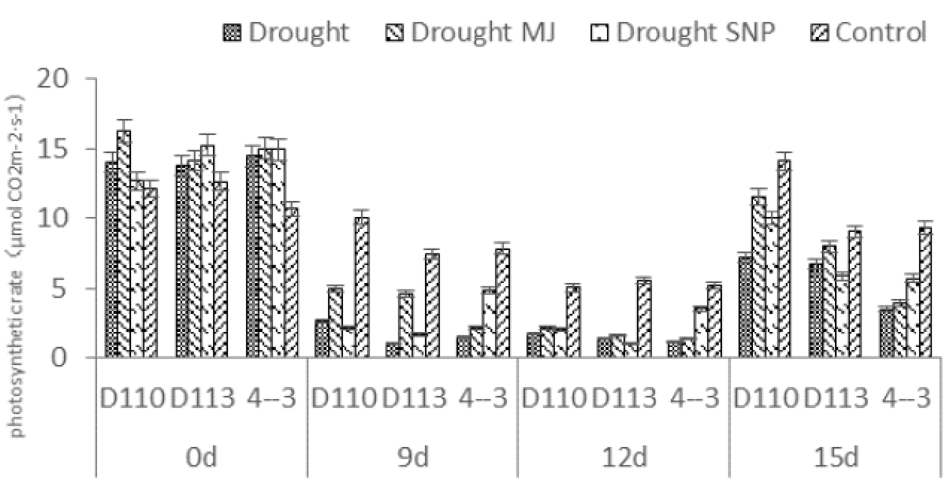

Figure 2

photosynthesis of Fraxinus mandshurica under exogenous drought stress by exogenous NO and MJ.

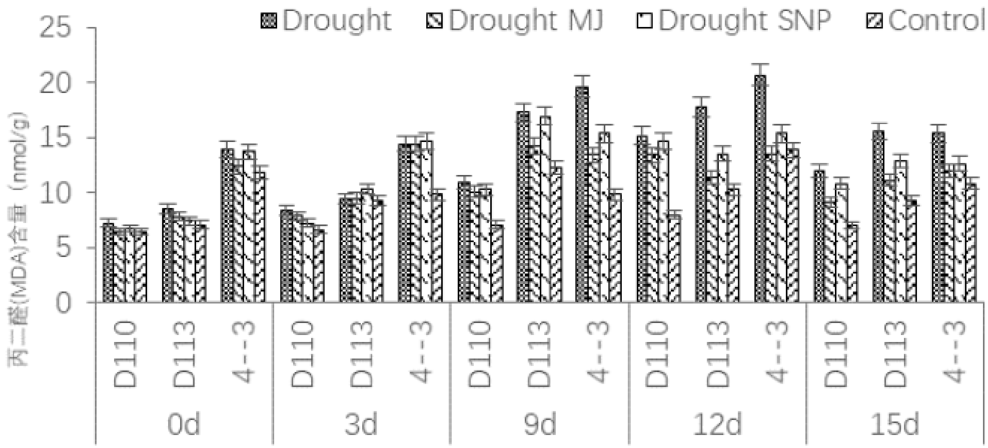

Figure 3

the content of malondialdehyde (MDA) in Fraxinus mandshurica under exogenous drought stress by exogenous NO and MJ.

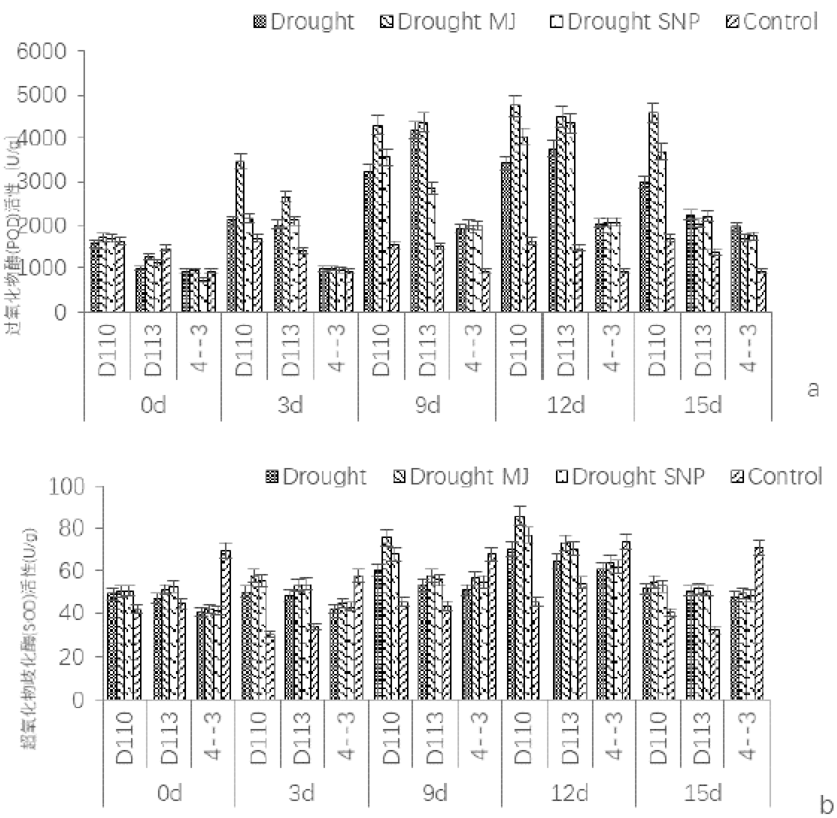

Figure 4

activity of antioxidant enzymes POD and SOD in Fraxinus mandshurica under exogenous drought stress by exogenous NO and MJ�Fig. A shows the change of antioxidant enzyme SOD from 0 to 15 days under drought condition. Fig. B shows the change of antioxidant enzyme POD in drought condition from 0 to 15 days.

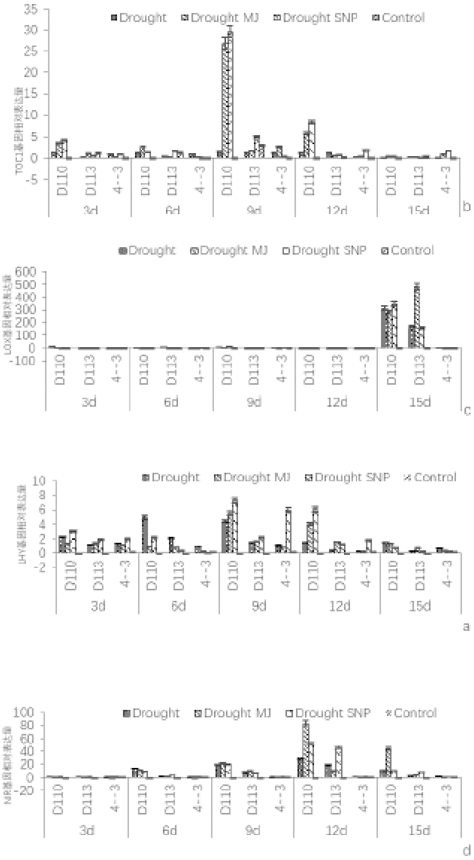

Figure 5

expression of circadian gene in Fraxinus mandshurica under exogenous drought stress by exogenous NO and MJ. Fig. A shows the expression of rhythmic gene LHY in 0-15 days. Fig. B shows the expression of rhythmic gene TOC1 in 0-15 days. Fig. C shows the expression of NO gene LOX from 0 to 15 days. Figure d shows the expression of MJ gene NIR from 0 to 15 days.

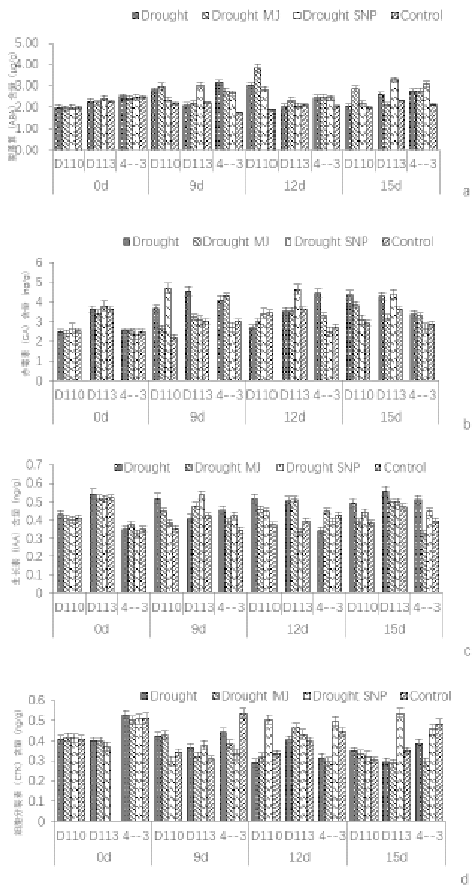

Figure 6

effects of exogenous NO and MJ on hormone content in Fraxinus mandshurica under drought stress. Fig. A shows the change of plant hormone abscisic acid ABA from 0 to 15 days under drought stress, and figure b shows the change of gibberellin GA during 0-15 days under drought stress. Fig. C shows the change of IAA from 0 to 15 days under drought stress, and the change of cytokinin CTK from 0 to 15 days under drought stress.

Supplementary Files

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