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RESEARCH ARTICLE

Effect of laser pretreatment on germination and membrane lipid peroxidation of Chinese pine seeds under drought stress

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Abstract The germination of laser-irradiated Chinese pine seeds was carried out under drought stress. The activities of superoxide dismutase (SOD) and peroxidase (POD), and the content of malondialdehyde (MDA) were determined. Results showed notably increased germination percentage, root length, vitality index and fresh weight. The SOD and POD protective enzyme system activity of the Chinese pine seedlings obviously rose. It can be concluded that the germination and juvenile resistance of Chinese pine seeds under drought stress are enhanced after laser processing.

Keywords laser irradiation; drought stress; Chinese pine seeds; membrane lipid peroxidation

1 Introduction

Drought is the main adverse factor that restricts agricultural production. Statistics have indicated that farming and forestry losses brought on by drought are the leading consequences of natural disasters in the world. Therefore, research on plant adaptability in arid conditions is always a focus of many investigators. Recently, many research findings have indicated that laser can be used to pre-treat plant seeds in order to enhance the rate at which seeds turn into seedlings, the field rate of seedling emergence, the neatness of seedling emergence, the promotion of seedling growth, the germination rate, vigor index, etc., even under poor surroundings (Chen et al., 2002; Chen et al., 2003; Cui et al., 2002; Liu et al., 1995; Wu et al., 1998; Wu et al., 1999; Wu et al., 2002; Wu et al., 2003; Xu et al., 2002).

Chinese pine is the tree of choice for greening and reforestation of barren hills in most of the arid and half-arid areas in Northern China, but its germination rate is low and its seedling growth is slow. It is therefore important for economic and ecologic reasons to enhance the germination rate, to promote seedling growth, and especially to enhance the drought-resistance characteristics of Chinese pine seedlings. In this research, a He–Ne laser was used to irradiate Chinese pine, and polyethyethylene glycol (PEG6000, molecular weight 6000) were used to simulate water stress. Our main objective was to study the effect of laser pretreatment on the activities of the defense enzymes and on membrane lipid peroxidation of the Chinese pine seeds while these were being germinated under water stress. Our rationale was that this study will have practical significance in the investigation of the biological effects of drought on germination and on seedling drought-resistance.

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2 Materials and methods

2.1 Materials

Chinese pine seeds (Shaanxi) were selected for uniformity of size, fullness of pellets, and luminosity. Seeds were disinfected with low concentration K_2MnO_4 for 3–5 min, and washed thoroughly under tap water for 10–15 min. Unfruitful grain and sundries were removed, and the seeds were soaked at 42°C for 24 h. Finally, after spontaneous insulation, the seeds were mixed evenly and stochastically divided into five groups before treatment was given.

2.2 Methods

Three of the five groups were stochastically selected and labeled as treatment groups T_1, T_2 and T_3, corresponding to treatment times of 40, 60 and 100 s respectively. These were then irradiated using a He–Ne laser with a wavelength of 632.8 nm, an output power of 10.8 mw, and a light beam round spot diameter of 6.5 cm. Three replicates were carried out for each treatment group. The other two groups acted as

the control (untreated with He–Ne laser) and were labeled as CK_1 (untreated with He–Ne laser and cultivated normally), and CK_2 (untreated with He–Ne laser but cultivated under water stress conditions). Water stress was simulated during seed germination at 24°C by using PEG6000 solution (osmotic pressure 1.0 MPa). Three replicates were also performed for each treatment. Nutrient fluid was added in sufficient amounts every day to the CK_1 group for normal cultivation, while PEG6000 was added once daily to the CK_2 group and to the treatment groups T_1, T_2 and T_3 in order to maintain certain nutrient fluids and keep the coercion osmotic pressure invariable.

2.3 Study sites and methods

The germination rate, vigor index and fresh weights of the seedlings were determined by conventional methods. The number of germinating seeds was recorded daily, while the germination rate (G), the root length (S), and other relevant data were determined on the 7th day; the vigor index was determined by using the formula [germination rate (G) × average root length (S)].

Superoxide dismutase (SOD) activity was assayed by monitoring inhibition of the photochemical reduction of nitro blue tetrazolium (NBT), according to the method of Wang et al. (1983). One unit of SOD activity (u/g) was defined as the amount of enzyme required to cause 50% inhibition of NBT reduction as monitored at 560 nm. Peroxidase (POD) activity was determined as according to the method of Liu et al. (1993). Malondialdehyde content (μmol/g) was measured according to the method of Wang et al. (1989). The degree of membrane lipid peroxidation was expressed as the number of micromoles of MDA per gram of fresh leaves.

3 Results and analysis

3.1 Effect of laser irradiation on germination of Chinese pine seeds under drought stress

Table 1 shows the germination of Chinese pine seeds under normal conditions, under water stress, and under water stress after laser pretreatment.

Table 1 shows that germination of Chinese pine seeds was obviously inhibited, with the germination rate, root length, vigor index and fresh weight of the seedlings tending to decline under drought stress. In the water stress control group CK_2, the germination rate, root length, vigor index and fresh weight of seedlings were decreased by 34.85%, 57.52%, 73.48%, and 46.52%, respectively, compared with the normally cultivated control group CK_1. On the other hand, the germination rate, root length, vigor index and fresh weight of seedlings increased significantly in the laser pretreatment groups T_1, T_2, T_3, with the vigor indexes up by 57.74%, 81.19%, and 62.26%, respectively, as compared to the water stress control group CK_2. Laser processing of Chinese pine seeds can therefore enhance seedling drought resistance.

3.2 Effect of laser on the activities of the defense enzymes (SOD, POD) and MDA content

Under water stress simulated by PEG6000, there were significant changes in the activities of the defensive enzymatic system during the germination of the Chinese pine seeds. Figure 1 shows that in the water stress group (CK_2), activities of SOD and POD decreased significantly compared with the normal control CK_1, dropping by 35% and 43% respectively. However, the activities of SOD and POD in the laser

Table 1 Effect of laser irradiation on germination of Chinese pine seeds under drought stress

Group	Germination rate/%	The elevated scope comparing CK_2/%	Root length/cm	The elevated scope comparing CK_2/%	Vigor index	The elevated scope comparing CK_2/%	Seedlings fresh weight/g	The elevated scope comparing CK_2/%
CK_1	66	–	2.26	–	149.16	–	2.73	–
CK_2	43	–	0.92	–	39.56	–	1.46	–
T_1	52	20.93	1.20	30.43	62.40	57.74	1.96	34.25
T_2	56	30.23	1.28	39.13	71.68	81.19	2.14	46.58
T_3	55	27.91	1.17	27.17	64.35	62.66	2.02	38.26

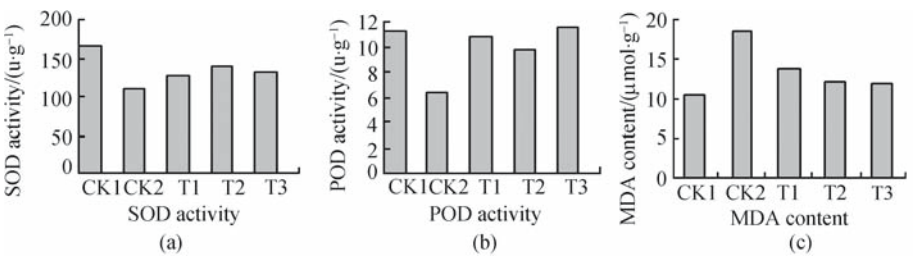

Fig. 1 Effect of laser irradiation on vitality during germination of Chinese pine seeds under drought stress

pretreatment groups were significantly higher than in the water stress group CK_2 during the Chinese pine seed germination.

Malondialdehyde is the product of membrane lipid peroxidation, and its amount in the cell is an important indicator of the degree of membrane injury (Zeng et al., 1997). The higher the MDA content, the worse the injury to the membrane system is. From Fig. 1(c), we can see that during germination, MDA content in the drought stress groups were higher than that in the CK_1 group. However, the MDA content in the laser pretreatment groups T_1, T_2, and T_3 were significantly lower than that in the untreated control group CK_2, under the same water stress conditions during the germination. The results show that laser treatment of Chinese pine seeds could enhance the activities of SOD and POD, and lead to a decline in the MDA content, thus mitigating the damage caused by water stress on the membrane system.

4 Discussion

Laser as a new technology has been widely used in farming and forestry, horticulture, genetics, bioengineering, etc. Previous results of laser irradiation of several tens kinds of plants have indicated that laser irradiation could deactivate seed dormancy, improve the germination rate and germination vigor, boost young seedling growth, make plants precocious and give high yield, and strengthen plant adaptation to the environment. This, in turn, has been the focus of much research in recent years. The results of this work show that germination rate, vigor index and fresh seedling weights were significantly enhanced in Chinese pine seeds under water stress after He–Ne laser pretreatment. Furthermore, our experimental results indicated that, during Chinese pine seed germination under water stress conditions after He–Ne laser pretreatment, the activities of the defense enzymes SOD and POD were significantly improved in every treatment group compared with that of the control group. This shows that there is a relationship between the increase in Chinese pine seed vigor and the enhancement of enzymatic activities during the process of germination. This is consistent with the findings of Wang et al. (1997) and Cai et al. (1994). Laser treatment increased the metabolism of Chinese pine seeds by enhancing enzymatic activities during germination. This in turn created beneficial physiological and biochemical effects causing the enhancement of seed vitality under water stress. Enhancement of seed vigor was demonstrated by germination rapidness, root system expansion, and improvement of fresh seedling weight. This thus establishes a material basis for the potential ability of seed germination and seedling growth to show strong vitality amid drought resistance.

Since Mcocord and Fridovich proposed the free radical injury theory, research on the products of membrane lipid peroxidation and the defensive enzymatic system in relation to drought resistance has received universal attention. The free radical injury theory proposed that free radical processes and active oxygen generation and scavenging occur in plant cells under normal conditions. When cells are placed in adverse conditions, or in the process of senescence, the metabolic system regulating generation of free radicals is destroyed, resulting in the surplus of free radicals. A product of this surplus leads to the initiation or aggravation of ongoing membrane lipid peroxidation, causing damage to the membrane system and increasing the content of MDA. Malondialdehyde, the end product of membrane lipid peroxidation, has been utilized very often as a suitable biomarker for lipid peroxidation (Dat et al., 1998; Sergi et al., 2003; Han et al., 2002; Li et al., 1993; Wang et al., 2004; Wang et al., 2004). Superoxide dismutase and POD are important components of the defensive enzymatic system that act as free radical scavenging agents. Superoxide dismutase causes an O^{2-} dismutation reaction, producing O_2 and H_2O_2, while POD subsequently decomposes H_2O_2. The activities of SOD and POD declined when seeds were germinated under water stress, thus resulting in the accumulation of free radicals, and causing damage to the membrane system and resulting in decreased drought resistance of the seedlings. The results of this study indicate that the activities of SOD and POD declined and the MDA content increased under water stress. However, the activities of SOD and POD increased, while the content of MDA decreased, in the laser treatment groups compared to the control group CK_2. These are consistent with the findings of Cao et al. (2003) and Zhu et al. (2001). This might be one of the main reasons for the increase in the observed biological effects and drought resistance of the seedlings. All of these indicate that laser pretreatment of seeds in order to increase the biological effects on seed germination under water stress, and increasing drought resistance of seedlings have important application prospects.

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