

PHYSIOLOGICAL CHARACTERISTICS OF *ELAEOCARPUS SYLVESTRIS* SEEDLINGS UNDER DIFFERENT DENSITIES OF PLANTS

JIE LI, WEILONG HUANG, TONGTONG ZHOU AND LI XUE*

College of Forestry and Landscape Architecture, South China Agricultural University, Guangzhou-510642, P. R. China

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Abstract

Physiological indexes of the different seedling densities of one-year-old *Elaeocarpus sylvestris* (Lour.) Poir were determined. The results showed that with the increasing density, changes in the content levels of chlorophyll, soluble sugar, and malondialdehyde (MDA) in the leaves of the seedlings were not significant. The soluble protein content and superoxide dismutase (SOD) activity increased and then decreased, whereas proline content decreased. Decreases in the contents of soluble protein, proline, and SOD indicate a weakening of the response of the high-density seedlings against drought stress, and that 40 seedlings m⁻² should be the recommended planting density.

Introduction

Population density is one of the important selection pressures for plants in nature (Japhet *et al.* 2009), and it is closely related to levels of environmental resources like light, water, and nutrients (Maherali and Delucia 2001) which affect the growth, morphology, and survival of plants. Density may maintain the reasonable utilization of resources for populations and may maintain the stability of the population by regulating the number and growth of individuals within a population (An and Shangguan 2008). Population density is directly related to afforestation quality and is the key factor affecting the structure and growth of stands, woodland utilization rate, and stand productivity (Qin *et al.* 1999, Yu *et al.* 1999). Studies on density control started in 1960s in different regions of the world (Wang and Peng 1985). However, studies on seedlings density started in China initially on crops and later on various tree species (Li *et al.* 2001, Zhang *et al.* 2001).

At the present time, most researches on plant competition focuses on plant growth under different densities. For example, Xue and Hagihara (2002) studied the competition density effects of Masson pine plantations by simulating the growth characteristics of tree species using density effect models. There are relatively a few studies on the effect of seedling density on growth, for example, Cicek *et al.* (2007) studied that the seedbed density effect on *Fraxinus angustifolia* seedlings, An and Shangguan (2008) reported the biomass accumulation of *Robinia pseudoacacia* seedlings with different densities, Woeste *et al.* (2011) analyzed the growth law of Woeste (*Juglans nigra*) seedlings, and Chen *et al.* (2005) reported the density effect on the survival and growth of seedlings of *Myricaria laxiflora*, Yan *et al.* (2012) described the density effect on the survival and growth of *Quercus liaotungensis* seedlings. Studies of potted plant density have focused on crops. Wu *et al.* (2010) studied the competition effect of weedy rice on the physiological characteristics of the root system and the yield of cultivated rice using a pot experiment method.

*Author for correspondence: <forxue@scau.edu.cn>.

Seedling density affects plantation productivity and influences the quality and yield of seedlings. It also affects the physiological and biochemical characteristics of plant leaves. Current studies on plant physiological and biochemical characteristics under different densities mainly focus on crops and medicinal herbs, such as *Zea mays* (Chen 2005), *Triticum aestivum* (Cha 2007), and the immature fruit of *Poncirus trifoliata* (Li 2008). The photosynthetic pigment content in plant leaves reflects the photosynthetic capacity of plants, and chlorophyll is an important pigment molecule in the photosynthetic pigment. Different seedling densities affect the chlorophyll content of leaves by changing light. Studies of the density effect on chlorophyll in plant leaves have focused on cash crops. There have been a few reports on the chlorophyll content of seedlings at different densities. Some studies have shown that chlorophyll content decreases with increased density (Duan *et al.* 2007). Under stress conditions, cells actively yield osmotic adjustments, like soluble sugar, soluble protein, and proline. This increases the concentration of soluble substances and reduces the water potential, so that cells continuously absorb water from extracellular sources, allowing plants to grow normally. Superoxide dismutase (SOD) activity is an antioxidant enzyme that plays an important role in the process of scavenging oxygen reactive species. Hu (2009) found that as density increases, the soluble protein content decreases, the malondialdehyde (MDA) content increases, and the SOD activity first increase and then decrease.

Elaeocarpus sylvestris is a species of fast-growing, broad-leafed, evergreen timber and landscape tree. Its characteristics include strong adaptability, ease of breeding, and efficient water conservation ability. Studies of *E. sylvestris* cover morphological characteristics, physiological characteristics (Xue *et al.* 2012), tissue culture (Shi *et al.* 2004), and genetics (Zeng *et al.* 2003), whereas the effect of seedling density on the physiological characteristics of this species is rarely reported. In this study, we examined the physiological characteristics of *E. sylvestris* seedlings under different densities, which can enrich the population knowledge of this species. It is hypothesized that the increase in seedling density would affect the plant's physiological characteristics. The objectives of the study are: to assess the physiological parameters of *E. sylvestris* seedlings under different seeding density conditions, and, to assess the plant growth behavior under different seedlings density conditions.

Elaeocarpus sylvestris is a fast-growing evergreen broad-leaved timber and landscape tree species with characteristics of strong adaptability, easy to breed, and good water conservation ability. The studies on *E. sylvestris* include morphological characteristics, physiological characteristics (Xue *et al.* 2012), tissue culture (Shi *et al.* 2004), genetic (Zeng *et al.* 2003), whereas the seedling density effects on physiological characteristics of this species has not been reported. In this study, physiological characteristics of *E. sylvestris* seedlings under different densities were studied, which can enrich population knowledge of this species.

Materials and Methods

The experimental field site was the Yuejin Nursery of the South China Agricultural University in Guangzhou City, South China (113°21'E, 23°09'N). This area has a subtropical monsoon climate. The annual average temperature is 21.9°C and the average relative humidity is 77%. The rainy season spans April to October and the average annual rainfall is 1899.8 mm.

The experimental materials were one-year-old *E. sylvestris* seedlings. They were planted 1, 2, 4 and 8 seedlings per bag. The bags were 35 cm in diameter and 30 cm in height, and represented 10, 20, 40 and 80 seedlings/m². The seedling soil consisted of humus soil and loess in a 1 : 1 ratio. The soil pH was 7.11 ± 0.01, soil organic matter, and the total contents of N, P and K were 60.43 ± 0.12, 0.82 ± 0.01, 0.25 ± 0.01, 12.58 ± 0.11 g/kg, respectively. The experiment was conducted from March to December, 2014. The mean ground diameter, seedling height, and crown of the seedlings were 0.5 ± 0.1, 45.01 ± 5.2 and 15.9 ± 2.4 cm, respectively.

In September, 2014 the third to eighth functional leaves of each seedling were selected and the physiological indexes were measured. Chlorophyll, soluble sugar, and soluble protein contents were determined by spectrophotometry, the anthrone colorimetric method, and the Coomassie brilliant blue method, respectively (Li 2000). Proline content, superoxide dismutase (SOD) activity and malondialdehyde (MDA) were determined by the acidic ninhydrin method, the nitroblue tetrazolium (NBT) photoreduction method, and the thiobarbituric acid method of determination, respectively (Chen and Wang 2002). All indexes were measured three times.

All statistical analyses were performed using Excel 2010 and the Statistical Analysis System (SAS 9.3).

Results and Discussion

The chlorophyll content of the seedling leaves in the 1 and 8 seedlings/bags was slightly higher than at the other two densities, but the difference was not significant (Fig. 1). The soluble sugar content in the 4 seedlings/bag was slightly lower than the other density seedlings, but the difference among the four density seedlings was not significant (Fig. 2).

The soluble protein content in the seedlings decreased in the order of: 4 seedlings/bag > 8 seedlings/bag > 2 seedlings/bag > 1 seedling/bag. Among these, the soluble protein in the 4 and 8 seedlings/bags was significantly higher than the other density seedlings ($p < 0.05$) (Fig. 3). The proline content in *E. sylvestris* seedlings decreased in the order of: 1 seedling/bag > 2 seedlings/bag > 4 seedlings/bag > 8 seedlings/bag. The proline content in the 1 seedling/bag was significantly higher than the 4 and 8 seedlings/bags ($p < 0.05$) (Fig. 4). SOD activity of the seedlings decreased in the order of: 4 seedlings/bag > 8 seedlings/bag > 2 seedlings/bag > 1 seedling/bag. The SOD activity in the 4 and 8 seedlings/bags was significantly greater than the other density seedlings, and the SOD activity in the 2 seedlings/bag was significantly higher than the 1 seedling/bag ($p < 0.05$) (Fig. 5). The content of MDA in all densities of seedlings was similar, and there was no significant difference among them (Fig. 6).

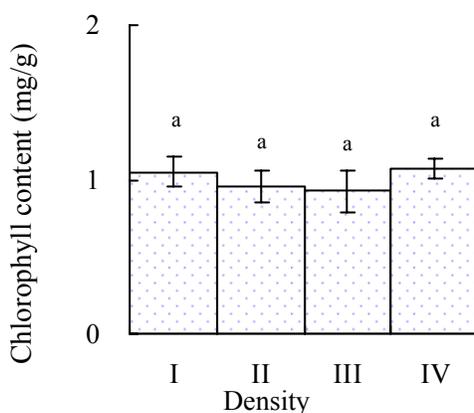


Fig. 1. Leaf chlorophyll content of the seedlings with different densities. I, 1 seedling/bag; II, 2 seedlings/bag; III, 4 seedlings/bag; IV, 8 seedlings/bag. The same letter indicates that the difference is not significant ($p < 0.05$).

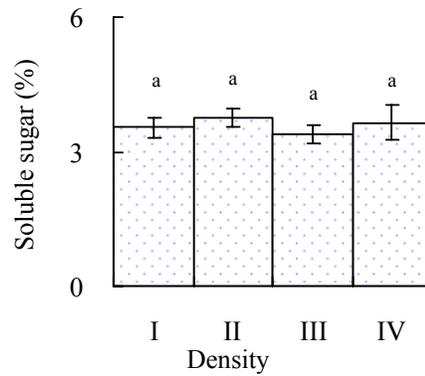


Fig. 2. Leaf soluble sugar content of the seedlings with different densities. I, 1 seedling/bag; II, 2 seedlings/bag; III, 4 seedlings/bag; IV, 8 seedlings/bag. The same letter indicates that the difference is not significant ($p < 0.05$).

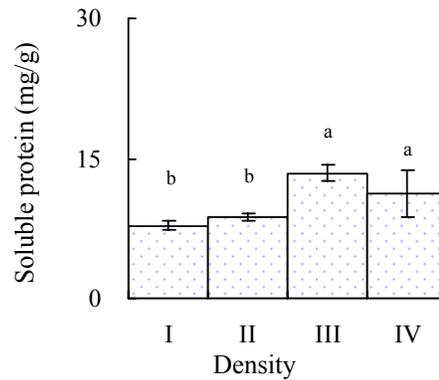


Fig. 3. Leaf soluble protein content of seedlings with different densities. I, 1 seedling/bag; II, 2 seedlings/bag; III, 4 seedlings/bag; IV, 8 seedlings/bag. The same letter indicates that the difference is not significant ($p < 0.05$).

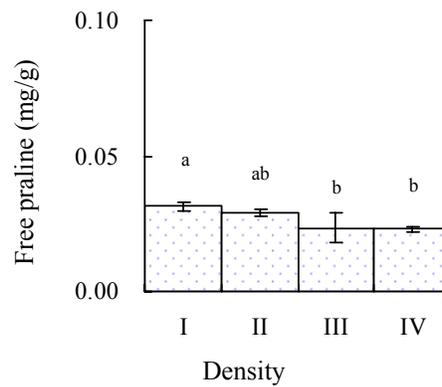


Fig. 4. Leaf proline content of the seedlings with different densities. I, 1 seedling/bag; II, 2 seedlings/bag; III, 4 seedlings/bag; IV, 8 seedlings/bag. The same letter indicates that the difference is not significant ($p < 0.05$).

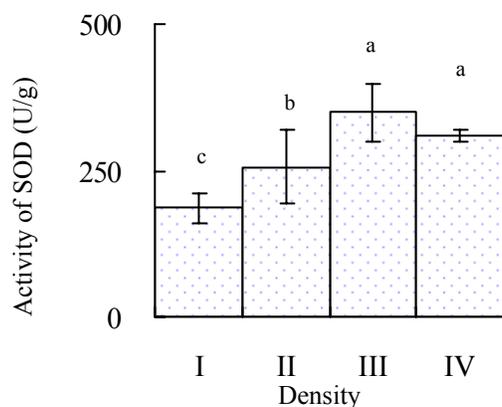


Fig. 5. Leaf SOD activity of the seedlings with different densities. I, 1 seedling/bag; II, 2 seedlings/bag; III, 4 seedlings/bag; IV, 8 seedlings/bag. The same letter indicates that the difference is not significant ($p < 0.05$).

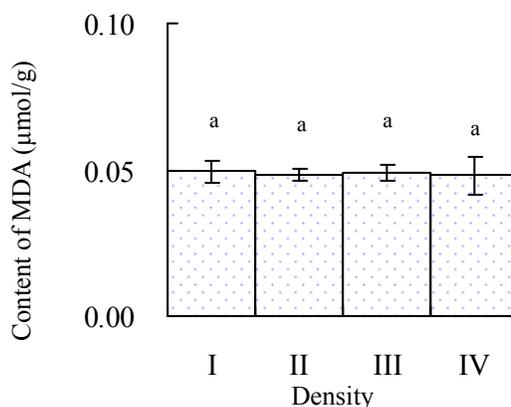


Fig. 6. Leaf MDA content of the seedlings with different densities. I, 1 seedling/bag; II, 2 seedlings/bag; III, 4 seedlings/bag; IV, 8 seedlings/bag. The same letter indicates that the difference is not significant ($p < 0.05$).

Qi *et al.* (2004) studied the density effect on the photosynthesis of maize and found that the contents of chlorophyll a, b, a + b, and carotenoid pigment decreased in maize as density increased. Duan *et al.* (2007) reported that chlorophyll content decreased with the increasing density. In this study, the effect of density on chlorophyll content is not obvious. The reason for this may be that the seedling size is small, and there is not aggressive water competition among the seedlings at the different densities, making the density have little effect on chlorophyll content.

Soluble sugar is an ideal osmotic adjustment substance in plant cells, which can alleviate the damage of the membrane system under stress. There were no obvious changes to the soluble sugar content in the leaves of the seedlings, because the seedling size is small, and the effect on the osmotic adjustment substances is not yet reflected.

Soluble protein is an important osmotic adjustment substance under environment stress. It also may increase the water holding capacity of the cells to maintain the low osmotic potential and resistance to damage. Hu (2009) found that the soluble protein content decreased in spring corn as the density increased. The decrease range was greater in less tolerant varieties. In present study, with increasing density, the soluble protein content increased first and then decreased in 1.5-year-old *E. sylvestris* seedlings, which indicated that within a certain density range, the soluble protein increased to resist light and nutrient stresses. However, higher density seedlings may result in the degradation of protein and directly influence protein synthesis with seedling growth (Duan *et al.* 2007).

The proline mainly exists in the cytoplasm, as it is an important osmotic adjustment substance in cells. Proline has two functions in the anti-inverse: maintaining the osmotic balance between the protoplast and the environment; maintaining the integrity of the membrane structure. Proline content decreased with increasing seedling density, indicating that high density may be a disadvantage for the osmotic adjustment of the seedlings.

SOD is a key enzyme in the protective enzyme system. It can effectively remove free radicals from plants and delay leaf senescence (Duan *et al.* 2007). For varieties with strong resistance, the decrease range was small when the SOD activity decreased, and the increase range was great when the SOD activity increased (Li 2008). With increasing density, the SOD activity of *E. sylvestris* seedlings first increased and then decreased, showing that a correct high density can improve SOD activity in order to resist the environmental stress caused by high density, whereas when the density is too high, the resistance of the seedlings to stress decreases, resulting in a decrease in SOD activity.

Cell membranes are the most sensitive part of a cell's environmental response. They play a role in regulating cell systems. The cell membrane permeability changes when plants are stressed by light intensity, temperature, water, and other factors. As density increases, the damage to the plant cell membrane becomes more and more serious, and the MDA content of the leaves increases (Li 2008). As a product of lipid peroxidation of plant membrane, MDA can bind protein and cause cross-linking for protein molecules to other protein molecules, which damages the plasma membrane, causing serious damage to the enzyme and the membrane, and ultimately destroying the membrane structure (Wang *et al.* 1986, Chen 1991). Environmental stress can increase the content of MDA. The increasing range of MDA content is small in tolerant plant types and large in non-tolerant plant types (Duan *et al.* 2007). There was not a significant difference in the MDA content among different density of *E. sylvestris* seedlings, which indicates that the content of MDA was not affected by the density effect of *E. sylvestris*.

In conclusion, in *E. sylvestris* seedlings, the effects of drought on the physiological characteristics in 10, 20 and 40 seedlings m⁻² were mild, whereas the effects in 80 seedlings m⁻² were severe. Present results showed that soluble protein, proline, and SOD may be involved in the competition effect for soil water for seedlings exposed to drought stress. Forty seedlings m⁻² may be a suitable density for 1.5-year-old *E. sylvestris* seedlings. The results of this research may help in improving functions related to the density effect on seedlings under drought stress, which will improve density management for these seedlings.

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