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Full Length Article

Effects of Drought Stress on Physiological Characteristics of *Cinnamomum camphora* Seedlings under Different Planting Densities

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Abstract

Drought can affect physiological characteristics of trees, whereas stand density affects soil water by tree transpiration, and high density stands have reduced water capture ability, which often cause drought stress. Understanding the mechanisms of drought stress is necessary for managing forest productivity. However, a little information is available for effect of drought and density interaction on tree physiology. The objective of the present study was to determine the effects of planting density on *Cinnamomum camphora* seedlings under drought stress. One-year-old *C. camphora* seedlings with different planting densities (10, 20, 40, 80 seedlings m^{-2}) were subjected to drought stress for 4, 8, 12, 16 and 20 days. Under drought stress, levels of soluble sugar, soluble protein, proline, and malondialdehyde increased and relative water content, chlorophyll content, and superoxide dismutase decreased according to seedling density. These results indicate that the drought resistance of this species decreased with increasing seedling density.

Keywords: *C. camphora* seedling; Density effect; Drought resistance mechanism; Physiological responses

Introduction

Drought is a major abiotic stress that influences growth and development of plants (Reddy *et al*., 2004) and productivity (Farooq *et al.,* 2009). Increasing greenhouse gas emissions are expected to increase the global atmospheric temperature by 2–7°C by the end of this century (Xiao *et al.,* 2003), which will likely lead to increasingly intense droughts owing to evaporative demand (Rebetez and Dobbertin, 2004). It is expected that the duration and frequency of future droughts will increase in many areas around the world (Bedel *et al.,* 2013). Plant competition from drought has been observed worldwide. Therefore, understanding plant responses to drought will be increasingly important (Reddy *et al.,* 2004). During tree growth, when a soil water deficit occurs, trees undergo a series of physiological and biochemical changes to reduce or avoid damage to cells from water insufficiency. These changes are reflected in trees' physical structures, photosynthetic mechanisms, osmoregulation and protective enzymatic activity. In recent years, many studies have been carried out on physiological characteristics of trees related to water loss, including water potential and transpiration (Sellin, 2001), photosynthetic mechanisms (Ouzounidou *et al.,* 2014), the chlorophyll fluorescence effect (Ohnishi *et al.,* 2005; Bae *et al.,* 2008), osmoregulation (Farooq *et al.,* 2009; Feng *et al.,* 2011) and protective enzyme activity (Wang *et al.,* 2009; Ouzounidou *et al.,* 2014; Sun *et al.,* 2016).

Population density is one of the most important selective pressures in nature (Japet *et al*., 2009) and influences interactions among plant individuals. Intraspecific competition due to high planting density affects plant nutrition, individual vigor, leaf photosynthetic rate, and photosynthetic carbon assimilation of the group (Li, 2000). Most studies have focused on plant growth and the distribution of biomass under different density conditions (e.g., Pfeifer–Meister *et al.,* 2008; Xue and Hagihara, 2008; Aikio *et al.,* 2009; Prasad *et al.,* 2011; Xue *et al.,* 2011, 2012). However, little information is available about the physiological and biochemical characteristics of plants under different densities. Stands with higher density need more soil water because of increased transpiration, which often cause drought stress to plants during dry seasons or droughts. Thus, determining appropriate planting density is important for successful stand management.

Cinnamomum camphora is a large evergreen broadleaved tree that grows up to 20–30 m tall. This species commonly occurs in stands in tropical and subtropical areas characterized by its rapid growth, lush foliage, strong wind and water logging resistance and weak drought tolerance. It is ideal for defusing noise, landscaping and conserving water and is also cultivated for camphor and timber production. However, little information is known about the effects of *C. camphora* on forest soil properties, mixed forests, and soil carbon density (He *et al.,* 2003; Jiang *et al.,* 2005; Wang *et al.,* 2009; Tian *et al.,* 2010). Although rainfall is abundant in regions of *C. camphora* distribution

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area, this species frequently faces unfavorable growth conditions during drought seasons, because *C. camphora* seedlings are often planted in mountain areas. Nowadays, there is a lack of knowledge about drought effect on *C. camphora* trees. In this study we investigate the physiological changes of *C. camphora* seedlings with different planting densities suffering from drought stress for understanding drought resistance mechanism of this species.

Materials and Methods

Experimental Site

The study was carried out at Yuejinbei Nursery (113°21′E, 23°09′N), South China Agricultural University, Guangzhou City, Guangdong Province, China. The region has a humid monsoon climate with short winters and long, hot summers. The average annual temperature is approximately 21.9°C and the monthly mean temperature ranges from 13.3°C in January to 28.1°C in July. Annual rainfall averages 1899.8 mm, and occurs mainly between April and October, accounting for 77% of annual precipitation. Average annual relative humidity is 77%. The experimental field had sufficient natural light exposure to grow seedlings. Physiological measurements were carried out at the Laboratory of Forest Cultivation, South China Agricultural University.

Experimental Materials

One-year-old seedlings of *C. camphora* came from Guosen Forestry Co. Ltd were planted according to 1, 2, 4, 8 seedlings per bag with a size of 35 cm in diameter and 30 cm in height, respectively, namely 10, 20, 40 and 80 seedlings m^{-2} (hereafter, densities 'I', 'II', 'III' and 'IV') during March 2014. The four density treatments were randomly arranged in four complete blocks, each treatment in each block represented by a tray of 100 bags, and the seedling growth substrate formed by Holland soil and yellow soil with the ratio of 1 to 1. The trial was conducted in a greenhouse. At the beginning of the experiment, the mean ground diameter, height and crown width of seedlings were 0.49±0.15, 45.2±2.57 and 20.25±2.24 cm, respectively.

Methods

The seedlings were exposed to natural precipitation as well as regular watering between March and December 2013. In December 2013, the seedlings were divided into drought and control groups. Physiological parameters were measured 0, 4, 8, 12, 16 and 20 days after ceasing watering. Five seedlings per group (control and drought) with fully expanded leaves were selected from each density group. At approximately 8:30 am on the day of harvest, the third to the eighth leaves from the top were collected to determine the physiological parameters. All samples were ground using grinding bowl and all physiological measurements were performed in triplicate. There were no significant differences in physiological parameters among the density groups at 0 days of drought stress.

Relative leaf water content was determined by weight. Chlorophyll, soluble sugar, soluble protein, proline (Pro), and malondialdehyde (MDA) contents were determined by spectrophotometry (Chen and Wang, 2002), anthrone colorimetry (Li, 2000), staining with Coomassie brilliant blue G-250 (Gao, 2006) and acidic ninhydrin and thiobarbituric acid reactions (Chen and Wang, 2002), respectively. Superoxide dismutase (SOD) activity was measured using the nitrotetrazolium blue chloride photoreduction method.

All statistical analyses were conducted with Microsoft Excel 2003 and SAS version 9.3. Tukey multiple comparisons were used to determine significant differences in physiological parameters among different density treatments. Differences were deemed significant when $P<0.05$.

Results

Relative Water Content of Leaves

With increasing drought duration, the relative water content of the seedlings clearly decreased according to seedling density (Fig. 1). The relative water content of leaves in group IV was significantly lower ($P<0.05$) than that of the other groups at all experimental stages. The relative water content of group III was considerably lower than those of groups I and II ($P<0.05$) starting at 8 days of drought stress. After 12, 16 and 20 days of drought stress, relative water content levels followed the planting density (i.e., the water levels were highest to lowest in density I> density II> density III> density IV).

Chlorophyll Content of Leaves

There was no significant difference in chlorophyll content among density groups at 4 days of drought stress. Chlorophyll content increased after 8 and 12 days, decreased after 16 days, and continued to decrease at 20 days of drought stress ($P<0.05$) (Fig. 2). Chlorophyll content decreased in the order density IV > density III > density I > density II at 8 and 12 days, in the order density II > density I > density III > density IV at 16 days, and in the order density I > density II and density III > density IV at 20 days of drought stress.

Soluble Sugar Content of Leaves

Leaf soluble sugar content increased to a significantly greater degree in group IV ($P<0.05$) than in the other densities at each every drought stage (Fig. 3). After 20 days of drought stress, the soluble sugar content in group IV increased by 1.17%, 1.12% and 0.87% more than those of groups I, II and III, respectively.

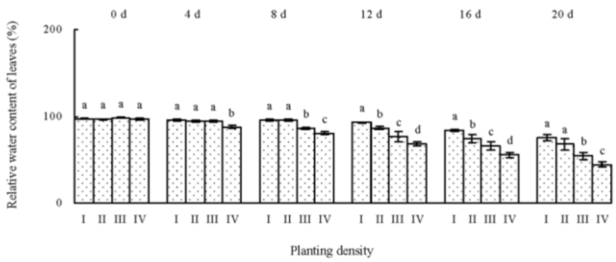

Fig. 1: Relative water content of *C. camphora* seedlings in various density groups under drought stress

Groups I, II, III, IV were planted at 10, 20, 40 and 80 seedlings·m^{-2}, respectively

Significant differences between treatments are indicated by different letters above bars, P<0.05. This also applies to the figures below

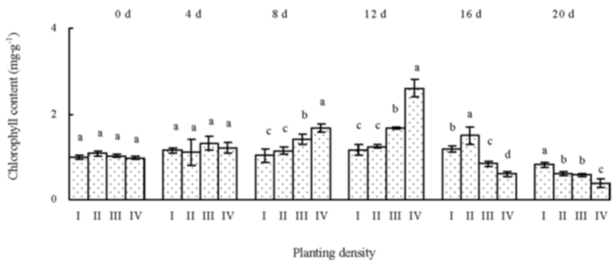

Fig. 2: Chlorophyll content of *C. camphora* seedlings in various density groups under drought stress

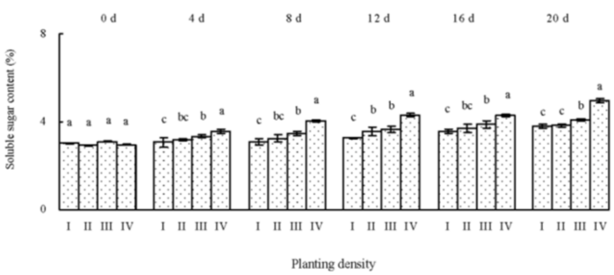

Fig. 3: Soluble sugar content of *C. camphora* seedlings in various density groups under drought stress

Soluble Protein Content of Leaves

The soluble protein content of seedlings increased during each stage of drought stress in all groups, except after 4 and 8 days of stress. Soluble protein in group IV increased to significantly greater extent (P<0.05) than that of the other densities after 10, 16 and 20 days (Fig. 4). The soluble protein content in density IV increased by 3.84, 2.38 and 1.50 mg·g^{-1} over density I, II and III in 20 days, respectively.

Proline Content of Leaves

Leaf proline content increased in all groups after 12, 16 and 20 days of drought stress (Fig. 5). During stress treatment, the leaf proline content of group IV was significantly higher than that of the other densities (P<0.05) starting at 4 days of

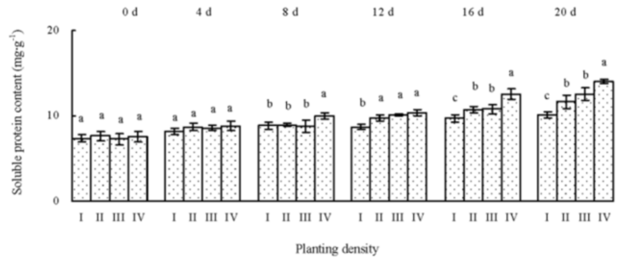

Fig. 4: Soluble protein content of *C. camphora* seedlings in various density groups under drought stress

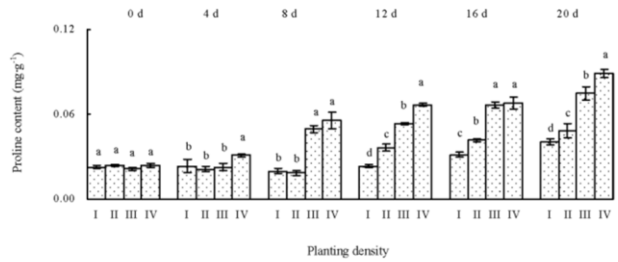

Fig. 5: Proline content of *C. camphora* seedlings in various density groups under drought stress

drought stress; however the proline content of groups III and IV were significantly higher than density I and II after 8, 12, 16 and 20 days (P < 0.05).

SOD Activity of Leaves

SOD activity did not change significantly after 4 days of drought stress in groups I, II and III. SOD activity increased followed by a decrease with increasing density at day 8 and decreased further with increasing density after 12, 16 and 20 days of drought stress (Fig. 6). In the latter two treatments this decrease was statistically significant (P<0.05).

MDA Content in Leaves

MDA content tended to increase with increasing density and the MDA content of group IV was significantly higher than that of the other groups (P<0.05) at each drought stage (Fig. 7).

Discussion

In this study, the relative water content of *C. camphora* leaves decreased with increasing drought duration and planting density, which is consistent with other studies (Ren *et al.*, 2005; Duan *et al.*, 2009). This indicates that water competition among seedlings was more acute in high-density seedlings than low-density seedlings, because the same quantity of soil water is available to more plants which means that the share of each plant decreases as the number of plants increases.

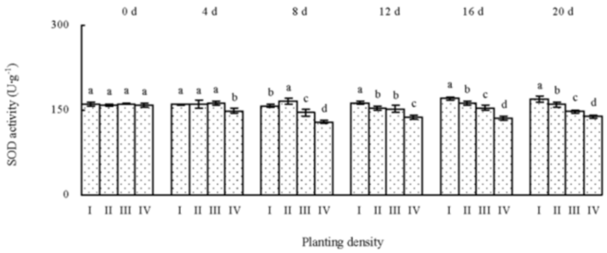

Fig. 6: Superoxide dismutase activity of *C. camphora* seedlings in various density groups under drought stress

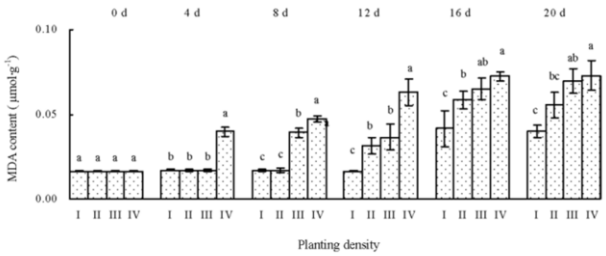

Fig. 7: Malondialdehyde content of *C. camphora* seedlings in various density groups under drought stress

Differences in leaf chlorophyll content among density groups were not significant 4 days after drought stress; however, chlorophyll content increased with density after 8 and 12 days. Beeflink *et al.* (1985) reported an increase in chlorophyll in onion leaves under drought stress. Mensah *et al.* (2006) found that the chlorophyll content of sesame leaves (*Sesamum indicum* L.) suffering from drought stress increased and then remained unchanged. The reason may be that chlorophyll of leaves is highly concentrated in high-density seedlings due to their active transpiration with greater total leaf area, or their relation to the compensation of environmental factors and the effect of super compensation (Yu *et al.,* 2007). In this study, with increasing drought duration, chlorophyll content of seedlings was reduced in each density group and this decrease was great in high-density seedlings, indicating that competition is acute among high-density seedlings, suggesting that intense competition for water occurred among the higher density seedlings, which may have damaged the chloroplasts of these seedlings. The balance between synthesis and decomposition of chlorophyll may have been affected by drought stress. Further, it is likely that the catabolic enzymes of chlorophyll were mainly synthesized (Zhao *et al.,* 2006), and the quantity of chlorophyll cells in low-density seedlings was greater than in higher density seedlings.

The soluble sugar content of seedlings continuously increased with drought duration in all density groups. This probably occurred because the decrease in osmotic potential of protoplast by increasing the soluble sugar can help cells absorb water and protect cells from damage. Soluble sugar content also increased significantly with increasing density, indicating that the high-density seedlings suffered more acute drought stress, leading to an earlier response in these seedlings (i.e., adjusting their soluble sugar content to a greater degree).

Soluble protein content increased continuously with increasing density for the duration of the drought stress experiments, except at 4 and 8 days of drought stress. This increase in soluble protein content likely maintained the lower osmotic potential of cells, enabling them to resist damage. Additionally, the high-density seedlings suffering more acute drought stress may have increased their soluble protein to a greater extent, because doing so provides a structural matrix to bind intracellular water and, thus, improves the water-holding capacity of tissues (Gong *et al.,* 2012).

Proline content in all density groups increased continuously during drought stress, indicating that a significant amount of proline was accumulated, probably to regulate cell water content; this mechanism helps plants resist injury from drought stress (Gao *et al.,* 2004). Increases in proline content under drought stress have been reported in pea cultivars (Alexieva *et al.,* 2001) and drought-tolerant petunia (*Petunia hybrida*) varieties (Yamada *et al.,* 2005). In this study, the proline content of seedlings clearly increased with increasing density, which suggests that higher density led to greater water competition during the later stages of drought stress.

SOD content continuously decreased with increasing density starting at 12 days of drought stress, indicating that low SOD content in high-density seedlings may be unfavorable to resisting drought stress. This is likely because competition for water is acute among high-density seedlings. Decreasing SOD activity with increasing density may increase lipid peroxidation and cause instability in membrane permeability. The results of this study are in accordance with observations in wheat by Zhang and Kirkham (1995) and in maize by Jiang and Zhang (2002), whose studies attributed the depletion of antioxidants under drought stress to decreased SOD activity (Bartoli *et al.,* 1999).

MDA content increased with increasing drought duration and seedling density, indicating that cells underwent lipid peroxidation in response to severe stress.

Conclusion

Drought affects the physiological characteristics of seedlings; however, seedlings appeared to counteract the adverse effects of drought through a combination of mechanisms such as adjusting levels of substances that regulate osmosis and protective enzyme activity. We found that parameters of drought resistance in *C. camphora* seedlings decreased with increasing density, which suggests that water competition among seedlings was more intense for high-density seedlings. Therefore, planting density is an important consideration for management of *C. camphora* seedlings in arid environments.

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