

Effects of the interaction between shade and drought on physiological characteristics in *Calamus viminalis* seedlings

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Abstract

Recently, the endangerment of wild rattan population draws attention on the conservation and sustainable utilization of rattan resources. Rattan growing usually faces the light and water stress. Therefore, we aim to explore the combined effects of shade and drought on seedling growth, thus providing a theoretical ground for the conservation and artificial cultivation of the rattan. The combined effects of shade and drought on physiological and biochemical traits were studied in two-years-old *Calamus viminalis* seedlings. Photosynthetic indices including Pn, Gs, Tr, and Ci and physiological indices including MDA, SOD, POD, CAT, and Pro were measured under four levels of water treatments and four levels of shade. Shade, drought and their interaction have a significant effect on *C. viminalis* seedlings growth. Generally, moderate shade could alleviate the impact induced by drought. However, mild drought usually enhances the effect caused by shading. The result showed that the shade decreased Pn, Gs, and Tr but increased Ci, MDA content and Pro content. Either with the shading or drought increasing, the activity of SOD, POD, and CAT firstly increase and then declined. Drought reduced Pn, Gs, Tr, and Ci but increased the content of MDA and Pro. Overall, the result suggests that 25-50% shading and 65% RSWC water treatment are most beneficial for the growth of *C. viminalis* seedlings.

Keywords: adversity resistance; antioxidant enzyme; low irradiance; photosynthesis; rattan seedlings; water deficiency

Abbreviations: CAT: catalase; Ci: intercellular CO₂ concentration; Gs: stomatal conductance; MDA: malondialdehyde; Pn: Net photosynthetic rate; POD: peroxidase; Pro: proline; SOD: superoxide dismutase; Tr: transpiration rate

Introduction

Rattans are a group of climbing plants assigned to Family Palmae, Calameae, widely distributed in Indo-China and Chinese tropical and sub-tropical forests (Jiang *et al.*, 2007; Wang, 2015b). Rattans, so-called “green

gold”, are well known as an important non-timber product in southeastern Asia, most species of which are good material for handicraft and furniture. Moreover, some species’ shoots and fruits can be made into food products. Notably, the rattan plantation and trade are of enormous economic value and developmental potential (Li *et al.*, 2002; Jiang *et al.*, 2007; Jiang, 2013; Wang, 2015b). However, the over-exploitation of rattan resources leads to its habitat loss and a dramatic drop of the natural rattan populations, thus largely impeding the development of rattan industry and trade (Jiang *et al.*, 2007; Dong *et al.*, 2015; Chen *et al.*, 2017; Peng, 2017). The research to investigate physiological characteristics and the affecting factors of rattan seedling growth was called for better conservation of rattan resources and sustainable utilization.

Light and water supply are the most significant factors for the rattan seedling growing (Ingram and Bartels, 1996; Jiang *et al.*, 2007; Liu *et al.*, 2011). Many studies demonstrated that the seedlings of most rattan species are supposed to grow well under some extent of shade and different species are grown under a various degree of the irradiance (Mori, 1980; Yin *et al.*, 1988; Dransfield, 1992; Sun *et al.*, 2013). One-year-old *Calamus manan* seedlings grow well under 10%-15% shading (Mori, 1980). The most beneficial light condition for the growth of *C. simplicifolius* seedlings is 20%-50% relative light intensity while that of *C. teradactylus* is 50%-60% (Yin *et al.*, 1988). Contrary to the former species, *Daemonorops margaritae* and *C. rhabdocladus* seedlings require very high relative light intensity about 80% (Yin *et al.*, 1988, 1993).

The water deficiency or drought was the main factor resulting in a remarkable reduction of rattan population (Yin *et al.*, 1993; Li *et al.*, 2002). The growth of most rattan species needs sufficient water supply, such as *C. caesius* (Manocaran, 1981a,b, 1982a,b). Only a few species like *Daemonorops jenkinsiana* and *C. rhabdocladus* have relatively high drought tolerance and can survive in less humidity condition (Yin *et al.*, 1993; Li, 2003). The combine effects of shade and drought on woody seedling have often been described and there is growing evidence that the shade and drought have a significant interactive effect on plant growth (Sack and Grubb, 2002; Aranda *et al.*, 2005; Climent *et al.*, 2006). However, the opinions on whether the drought stress has a stronger impact on seedling growth in deep shade or at higher irradiance are divergent (Holmgren, 2000; Sack and Grubb, 2002). One of the popular opinions is that moderate shade can improve plant performance under drought (Rousset and Lepart, 2000). Tree seedling survival under dry conditions was higher in shade than in high light thus providing support for this opinion (Amissah *et al.*, 2015). In addition, the study on *Arthraxon hispidus* suggests that the interaction of some degree of shading and drought can facilitate the photosynthetic capacity, metabolic rate, and resistance potential (Sun *et al.*, 2018). As for rattans, previous studies mostly concentrated on the effect of irradiance or water supply solely on the growth of rattan seedlings (Yin *et al.*, 1988; Li *et al.*, 2002, 2003; Sun *et al.*, 2013; Dong *et al.*, 2015), however, the interactive effect of the two factors on the growth of rattan seedlings remains unknown.

C. viminalis, widely distributed in southern Asia, is one of the excellent rattan species for the rattan plantation and industry. However, the research about its physiological characteristics and responses to shade and drought stress are still lacking. In the present study, *C. viminalis* seedlings were subjected to 2×4 factorial (shade, drought) experiment to test the interactive effects of shade and drought on seedling growth and their physiological resistance. We aim to provide a theoretical ground and support for the conservation and artificial cultivation of *C. viminalis*.

Materials and Methods

Plant growth conditions and experimental site

Two-years-old *C. viminalis* seedlings were collected from Hainan Zhongteng Co., Ltd., Hainan province, China. Each seedling was separately transplanted to a pot (diameter: 21 cm; height: 19 cm) with the same amount of red clay soil obtained from Ganzhaling, Hainan province. The physiological conditions of the

soil were measured as follows. PH: 5.6, water content: 17.26%, organic content: 11.4 g·kg⁻¹, available N content: 127.75 mg·kg⁻¹, available phosphorus content: 7.13 mg·kg⁻¹, available potassium contents: 80.05 mg·kg⁻¹. The seedlings were grown at the greenhouse of Yongmao ecological breeding base, which located at Jiyang, Sanya in Hainan province (109°35'41"E, 18°19'21"N) from 5 April to 21 June 2018.

Experimental design

After one-month establishment phase of the seedlings, the seedlings were subjected to 2×4 factorial (shade, drought) experiment for a total of 16 treatment combinations and each treatment had 15 pots (8 May 2018). The experiment consists of four water regimes: 85% relative soil water content (CK, W0), 65% RSWC (mild drought, W1), 45% RSWC (moderate drought, W2) and 25% RSWC (severer drought, W3) and four shade treatments: full sunlight (CK), 25% shading, 50% shading, and 75% shading. For the water treatment, relative soil water content was measured by the soil moisture tester (TDR300, USA) at 15:00-18:00 every day and watered as needed to maintain soil moisture during the experiment. For the shade treatment, the seedlings are covered by the shade-cloth. Physiological indices were measured after 45 days of treatment.

Physiological traits measurement

Net photosynthetic rate (P_n), transpiration rate (T_r), intercellular CO₂ concentration (C_i), and stomatal conductance (G_s) were measured by a portable photosynthesis system *Li-6400* (Lincoln, USA) at 9:00-11:00 on sunny days. Five random replicates and from each of replicates three functional leaves were selected for measurement.

The content of MDA and Pro were measured by thiobarbital acid colorimetry and acid ninhydrin method respectively. The activity of SOD, POD, and CAT were measured using NBT method, guaiacol method and ultraviolet absorption method respectively. Three replicates were imposed for each index measurement.

Statistical analyses

All data were subjected to an analysis of variance and Duncan comparisons at a threshold $p=0.05$ to test the differences between the treatments with an *SPSS 20.0* (IBM, USA). All the graphs were created and processed by using *Origin 8.5*.

Results

Effects of the shade and drought on photosynthetic indices of Calamus viminalis seedlings

With the shading degree increasing, the photosynthetic indices including the net photosynthetic rate (P_n), stomatal conductance (G_s), transpiration rate (T_r) and intercellular carbon dioxide concentration (C_i) all experienced an early raise and a subsequent decline (Table 1). For 25% shading, T_r and C_i significantly increased whereas P_n and G_s showed no statistically significant difference ($p>0.05$). For 50% shading, P_n decreased significantly by 30%, however, T_r and C_i showed a significant increase by 12% and 8% respectively ($p<0.05$). A significant decrease was found for P_n , G_s , and T_r at 75% shading ($p<0.05$), where the decrease was 70%, 45%, and 11% respectively. P_n and G_s reached a peak at 25% shading while T_r and C_i experienced the greatest increase at 50% shading. Conclusively, all the photosynthetic indices were found significantly declined at 75% shading.

Drought decreased T_r and C_i . And P_n and G_s increased under W1 but decreased under W2 (Table 1). Under mild drought (W1), P_n and G_s significantly increased by 27% and 47% respectively compared to control group ($p<0.05$) but T_r and C_i markedly decreased by 9% and 12% respectively. Under moderate drought (W2), T_r and C_i showed a significant decline by 34% and 15% respectively but P_n showed no significant

decrease from the control group. When the seedlings submitted to the severe drought (W3), water deficiency induced a prominent decrease for Pn, Gs, Tr, and Ci by 67%, 64%, 65%, and 20% respectively.

Table 1. Effects of different levels of shade and drought on photosynthetic parameters of seedlings

| Index | Drought stress | Shade degree/% | | | |
|---|----------------|-----------------|----------------|----------------|-----------------|
| | | 0 | 25 | 50 | 75 |
| Photosynthetic rate/ $(\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1})$ (P_n) | W0 | 2.44±0.17ABa | 2.91±0.14Ba | 1.67±0.42ABb | 0.74±0.07Bc |
| | W1 | 3.13±0.15Ab | 4.55±0.52Aa | 2.01±1.12Ac | 1.12±0.15Ac |
| | W2 | 1.75±0.08Bb | 3.01±0.14Ba | 1.01±0.02Bc | 0.19±0.03Cd |
| | W3 | 0.81±0.05Cb | 1.89±0.06Ca | 0.72±0.11Cb | 0.17±0.09Cc |
| Stomatal conductance / ($\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) (G_s) | W0 | 0.11±0.04ABab | 0.13±0.07ABa | 0.08±0.01Abc | 0.06±0.01ABc |
| | W1 | 0.14±0.05Aa | 0.16±0.06Aa | 0.10±0.03Aab | 0.09±0.05Ab |
| | W2 | 0.09±0.01Bab | 0.12±0.03Ba | 0.09±0.02Aab | 0.07±0.03ABb |
| | W3 | 0.04±0.01Cb | 0.09±0.01Ba | 0.04±0.01Bb | 0.05±0.02Bb |
| Intercellular CO ₂ concentration / ($\mu\text{mol}\cdot\text{mol}^{-1}$) (C_i) | W0 | 317.86±17.22ABc | 322.53±5.69Ab | 344.31±9.45ABa | 312.66±11.29ABc |
| | W1 | 289.74±13.15Bc | 340.58±14.25Ab | 382.86±39.46Aa | 325.46±8.24ABc |
| | W2 | 271.04±10.41Bd | 332.36±7.44Ab | 370.87±6.39Aa | 316.28±21.67ABc |
| | W3 | 254.62±31.16Cc | 333.19±13.03Aa | 314.38±30.12Ba | 279.71±25.51Bb |
| Transpiration rate/ $(\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1})$ (T_r) | W0 | 2.04±0.13Ab | 2.11±0.42Aab | 2.28±0.17Aa | 1.81±0.05Ac |
| | W1 | 1.79±0.08Bb | 2.09±0.65ABa | 2.12±0.16ABa | 1.65±0.26ABb |
| | W2 | 1.35±0.09Cc | 1.82±0.08Bb | 2.09±0.23ABa | 1.24±0.17Bc |
| | W3 | 0.72±0.04Dc | 1.42±0.35Cb | 2.01±0.39Ba | 0.87±0.11Cc |

Note: Different capital letters in the same row indicate significant difference among the drought treatments at $p < 0.05$ level, and different lower case letters in the same row indicate significant difference among the luminousness at $p < 0.05$ level ($n=12$); W0: enough moisture; W1: mild drought; W2: moderate drought; W3: severe drought.

The interactive effects of shade and drought were significant for the growth of *C. viminalis* seedlings. The moderate shade can alleviate the drought influences on seedlings. Twenty-five shading can reduce the decrease of Pn, Gs, Tr, and Ci induced by drought, however, 50% shading enhanced the reduction of all the photosynthetic indices. Meanwhile, mild drought (W1) significantly increased Pn at 25% shading ($p < 0.05$), whereas significantly decreased Pn at 75% shading ($p < 0.05$). Moderate drought (W2) significantly reduced Pn, Tr at 75% shading ($p < 0.05$). A significant reduction of Pn, Tr, Ci, and Gs was observed by 93%, 51%, 55%, and 83% respectively under the severe water stress (W3) and lowest irradiance (75% shading) ($p < 0.05$). In concluding, *C. viminalis* seedlings exhibited the greatest Pn ($4.55 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) under 25% shading and mild drought (W1).

The double factor variance analysis suggests the shade and drought both have highly significant effects on photosynthetic indices ($p < 0.001$) (Table. 2). The examining value of F shows that the influence of shade has the ranking as follows $C_i > P_n > T_r > G_s$. The influence of drought has the same ranking as shade. A distinct interaction of shade \times drought is also shown in Table 2 ($p < 0.001$) with a ranking $T_r > C_i > P_n > G_s$.

Effects of shade and drought on the content of MDA in C. viminalis leaves

Shade led to a reduction of the content of MDA in *C. viminalis* seedlings under 25% and 50% shade degree by 10% and 8% respectively while a significant increase under 75% shade degree by 17% ($P < 0.05$) (Fig. 1). Drought stress increasingly raised the content of MDA in *C. viminalis* seedlings. Mild drought didn't induce a significant increase in the content of MDA ($p > 0.05$). Moderate drought and severer drought led to a significant increase in the content of MDA in seedlings by 31% and 42% respectively in response to water deficiency ($p < 0.05$).

Table 2. Double factor variance analysis on photosynthetic indices. The photosynthetic rate, stomatal conductance, intercellular CO₂ concentration and transpiration rate were measured in (Pn)/(μmol·m⁻²·s⁻¹), (Gs)/(mmol·m⁻²·s⁻¹), (Ci)/(μmol·mol⁻¹), and (Tr)/(mmol·m⁻²·s⁻¹) respectively

| Item | | Photosynthetic rate | Stomatal conductance | Intercellular CO ₂ concentration | Transpiration rate |
|-----------------|---|---------------------|----------------------|---|--------------------|
| Shade | F | 89.355 | 8.411 | 259.412 | 11.651 |
| | p | <0.001 | <0.001 | <0.001 | <0.001 |
| Drought | F | 94.656 | 16.343 | 342.176 | 78.243 |
| | p | <0.001 | <0.001 | <0.001 | <0.001 |
| Shade × Drought | F | 9.466 | 4.093 | 17.624 | 39.175 |
| | P | <0.001 | <0.001 | <0.001 | <0.001 |

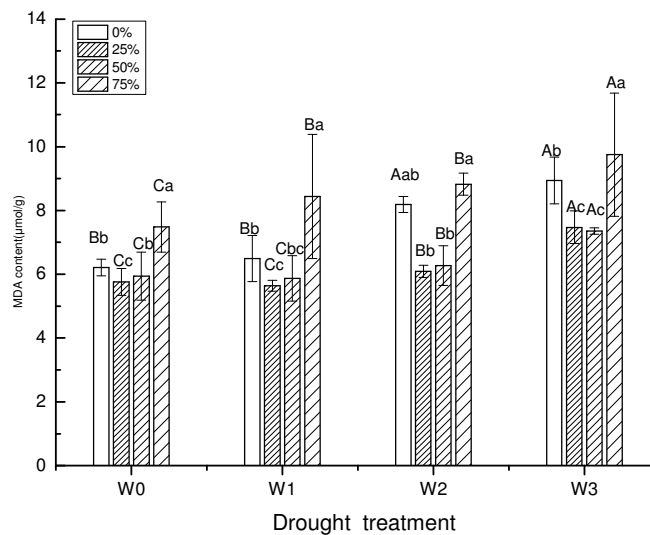


Figure 1. Effects of different shade and drought on MDA content of *C. viminalis*

Moreover, a significant interactive effect of shade and drought was found on the content of MDA ($p < 0.05$). Twenty-five shading can reduce the growing of MDA induced by drought. By contrast, more than 50% shading would assist in the promotion of MDA under drought stress in seedlings. On the other hand, mild drought stress promoted the increase of MDA significantly at 75% shading. The content of MDA increased significantly by 60% compared with the control group under the combined effect of severe drought and lowest irradiance.

The variance analysis indicates that shade and drought have a highly significant influence on the content of MDA in *C. viminalis* seedlings. The magnitude of influence ranks as follows Shade > Drought > Shade × Drought (Table 3).

Table 3. Double factor variance analysis on MDA

| Item | Shade | | Drought | | Shade × Drought | |
|--------------------------------|--------|-------|---------|--------|-----------------|--------|
| | F | p | F | p | F | p |
| Malondialdehyde (MDA)/(μmol/g) | 12.809 | 0.001 | 19.613 | <0.001 | 4.313 | <0.001 |

Effects of shade and drought on the activity of SOD, POD, and CAT of C. viminalis leaves

With shading increasing, the activity of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) increased first and then declined (Fig. 2). The activity of SOD increased significantly by 12% compared with the control group at 25% shading ($p < 0.05$). For 50% shading, the activity of SOD and POD have observed a prominent increase by 39% and 25% respectively ($p < 0.05$). However, the activity of SOD, POD, and CAT show no significant difference ($p > 0.05$) at 75% shading. Mild drought (W1) and moderate drought (W2) increased the activity of SOD significantly by 26% and 23% respectively ($p < 0.05$). On the contrary, the activity of SOD, POD, and CAT declined but not significantly under the severe drought (W3) ($p > 0.05$).

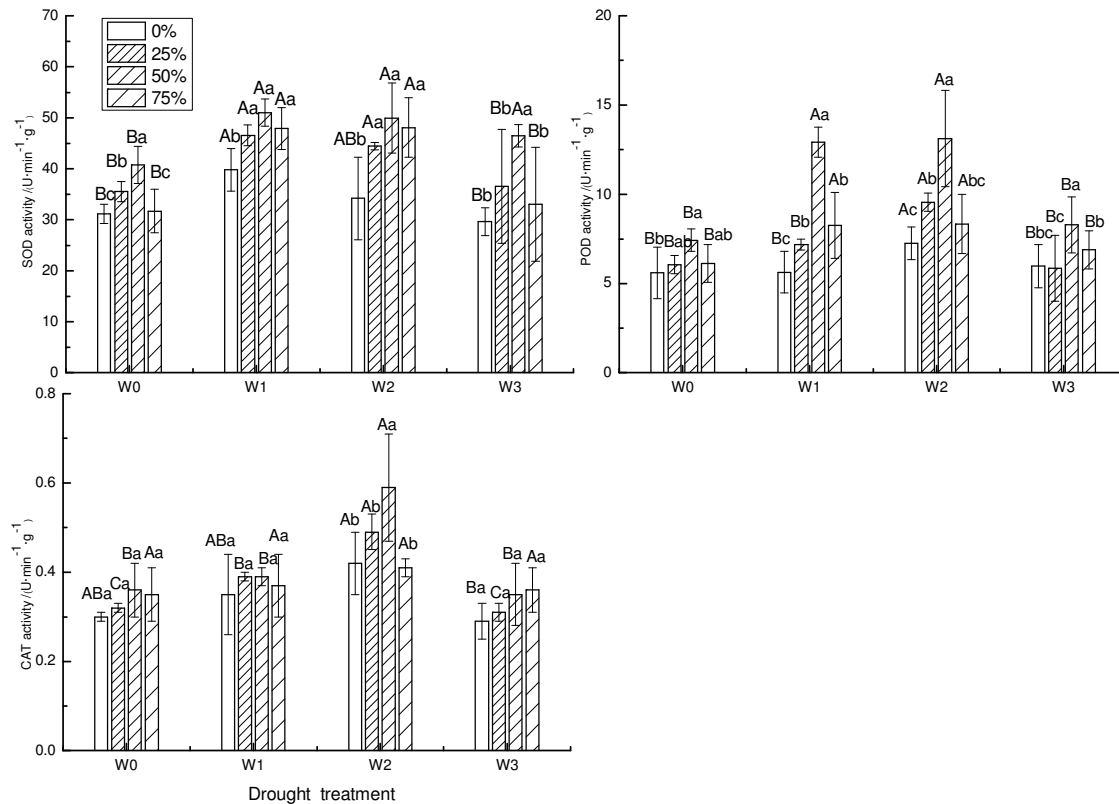


Figure 2. Effects of different shade and drought on the antioxidase activity of *C. viminalis*

The interactive effect of shade and drought was significant for the activities of SOD, POD, and CAT ($p < 0.05$). The greatest activity of SOD was observed when seedlings submitted to 50% shading and moderate drought, with an increase up to 66% of the control group. The activity of POD and CAT reached a peak at 50% shading and moderate drought, increasing by 134% and 97% respectively. The combined effect of deep shading (75% shading) and severer drought (W3) significantly declined the activity of SOD, POD, and CAT.

The variance analysis suggests that shade and drought have highly significant effects on the activity of SOD, POD, and CAT ($p < 0.01$) (Table 4). The effects of shading on antioxidase rank as follows SOD > CAT > POD and the effects of drought rank SOD > POD > CAT. The interaction of shade and drought was prominent for the activity of SOD and POD, however not significant for that of CAT, where the ranking of effects is SOD > POD > CAT.

Table 4. Two ways variance analysis of antioxidase activity. The superoxide dismutase, peroxidase activity and gatalase activity were measured in (SOD)/(U·min⁻¹·g⁻¹), (POD)/(U·min⁻¹·g⁻¹) and (CAT)/(U·min⁻¹·g⁻¹) respectively

| Item | | Superoxide dismutase | Peroxidase activity | Gatalase activity |
|-----------------|---|----------------------|---------------------|-------------------|
| Shade | F | 40.88 | 7.936 | 8.867 |
| | p | <0.001 | 0.001 | 0.002 |
| Drought | F | 103.451 | 9.832 | 6.126 |
| | p | <0.001 | <0.001 | <0.001 |
| Shade × drought | F | 4.632 | 3.109 | 1.438 |
| | p | 0.041 | 0.026 | 0.091 |

Effects of shade and drought on the content of proline of C. viminalis leaves

The content of proline was observed no significant difference when submitted to 50% shading (p>0.05). However, 75% shading increased proline significantly by 46% (p<0.05). The drought stress significantly increased the content of proline, which increases up to 46%, 77%, and 108% respectively under mild drought (W1), moderate drought (W2) and severe drought (W3) stress.

Shade and drought have a significant interactive effect on the content of proline (p<0.05). The increase of proline was reduced at 25% shading, however, increased at 50% and 75% shading. Drought stress greatly further increased the growing of the proline induced by shading. Mild drought (W1) significantly increased the content of proline at 50% shading. The severe drought (W3) and the lowest irradiances induced a significant increase of proline by 161% compared with the control group (Fig. 3).

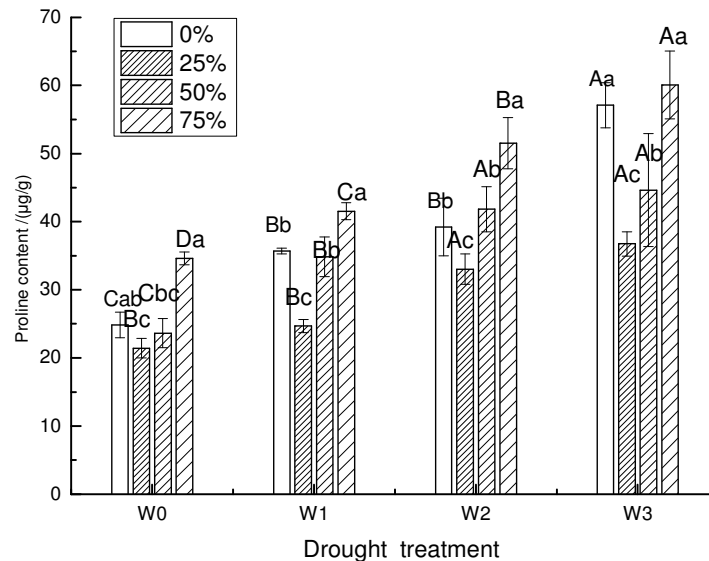


Figure 3. Effects of different shading and drought on proline content of *C. viminalis*

The variance analysis showed that shade and drought both have a highly significant effect on proline (p<0.01) (Table 5), ranking as Drought > Shade > Shade × Drought.

Table 5. Double factor variance analysis on proline content

| Item | Shade | | Drought | | Shade × Drought | |
|------------------------------|--------|--------|---------|--------|-----------------|--------|
| | F | p | F | p | F | p |
| Proline content (Pro)/(µg/g) | 12.809 | <0.001 | 19.613 | <0.001 | 4.313 | <0.001 |

Discussion

Effects of shade and drought on photosynthesis

Shading impacts the photosynthesis by reducing the light intensity and duration and regulating the transpiration rate and stomatal closure (Tang *et al.*, 2013). The present study shows that shading has a significant influence on the photosynthesis of *C. viminalis* seedlings. With the shading degree increasing, Pn, Gs, Tr, and Ci increased first and then declined. Pn and Gs reached a peak at 25% shading while Tr and Ci reached a peak at 50% shading (Table 1). Therefore, the growth of *C. viminalis* seedlings favors some extent of shading, especially among the range of 25%-50%. This result agrees with the general opinion that moderate shading could facilitate the growth of rattan seedlings but overshading is greatly harmful to its growth (Bogh, 1996; Yin *et al.*, 1998). However, different species have various shading tolerance. *C. manan*, *C. simplicifolius*, and *C. teradactylus* have similar shading tolerance with *C. viminalis*, approximately ranging from 20%-65% (Mori, 1980; Yin *et al.*, 1988, 1993; Sun *et al.*, 2013). *C. gracilis* seedlings can grow well under up to 75% shading (Dong *et al.*, 2015). By contrast, *C. rhabdocladus* seedling growth requires very high light intensity up to 80% full light (Yin *et al.*, 1993).

Water stress leads to the reduction of photosynthetic material transportation, thus directly decreasing the efficiency of photosynthesis (Lawlor, 1995; Han *et al.*, 2014; Yuan *et al.*, 2016). In the present study, with an increasing degree of water stress, Pn and Gs increased first but then declined. The result is not confirmed to the opinion that mild drought decreases photosynthetic capacity (Yang *et al.*, 2007; Dai *et al.*, 2009). Contrarily the mild drought (W1) could promote Pn and Gs to the greatest value in the present study (Table 1) (Deng *et al.*, 2016). And water stress started to decrease Pn and Gs from moderate drought (W2) but not significantly. A significant decline of Pn, Gs, Tr, and Ci was observed only when seedlings submitted to severe drought (W3) (Table 1). Therefore, the result suggests that *C. viminalis* seedlings have relatively high drought tolerance.

A significant interactive effect of shading and drought on the growth of *C. viminalis* seedlings was observed in the present study. Mild drought (W1) could generally improve the photosynthetic capacity. On the other hand, 25% shading could alleviate the decrease of photosynthetic capacity induced by drought. But the interaction of overshading and severer drought could lead to marked reduction of the photosynthetic capacity. The same result was also observed in *Arthraxon hispidus* (Sun *et al.*, 2018.)

Effects of shade and drought on the content of MDA

The content of MDA is an indicator of membrane lipid peroxidation, reflecting the degree of cell membrane damage in response to the light or water stress (Chen, 1989; Yang *et al.*, 2017). The MDA content would dramatically increase in the plant when the plant is at an adverse condition (Wei *et al.*, 2014; Liu *et al.*, 2018). In the paper, the MDA content at 25% shading was even lower than that of the control group (Fig. 1). The MDA content started to increase at 50% shading but still lower than that of the control group (Fig. 1). However, when the seedlings submitted to 75% shading, the MDA content remarkably increased to its maximum value (Fig. 1). Therefore, 25% shading could promote the performance of the seedling, but 50% shading is a threshold that would do harm to the growth of seedlings.

On the other hand, the drought stress induces a significant increase in MDA content (Wang *et al.*, 1990; 2006; 2013). Moderate drought and severe drought lead to a prominent increase of MDA content, suggesting an enhancement of lipid peroxidation in cells (Sang *et al.*, 2011; Qian *et al.*, 2015; Jia *et al.*, 2015, 2018). The similar result was found in the study of *Rhodiola kirilowii* seedlings (Yang *et al.*, 2017). The interactive effect of shading and drought is significant on the growth of *C. viminalis* seedlings. Low irradiance like 25% shading could lower the MDA content, thus alleviating the damage caused by drought on the cell membrane. However, more than 50% shading would augment the MDA content induced by drought.

Effects of shade and drought on the activity of SOD, POD, and CAT

The antioxidant enzyme system, including SOD (superoxide dismutase), POD (Peroxidase) and CAT (catalase), is an important and complicated protective mechanism of plants against the adverse condition (Wu *et al.*, 2016). When plants are facing water or light stress, the activity of SOD, POD, and CAT are promoted to decrease the damage induced by the environment stress (Wu *et al.*, 2016; Sun *et al.*, 2018). The activity of SOD, POD, and CAT generally show an increasing trend at the early stage under stress. The activity of SOD, POD, and CAT started to increase significantly at 25% shading and decline at 75% shading but not significantly (Table 4). Different plant species differ in the responding order and magnitude of the antioxidant enzyme system under stress. In *Arthraxon hispidus*, the activity of POD significantly increased at 10% shading whereas that of *C. viminalis* significantly increased at 30% shading (Sun *et al.*, 2018). Moreover, the activities of SOD, POD, and CAT in *Arthraxon hispidus* prominently decreased at 70% shading, however, those of *C. viminalis* declined at 75% shading but not significantly (Sun *et al.*, 2018.). Probably, the *C. viminalis* seedlings have a higher resistance than *Arthraxon hispidus* under low irradiance.

For the drought stress, the activity of SOD was promoted significantly when facing mild drought (W1) and the activity of POD increased significantly when seedlings submitted to moderate drought (W2) (Table 4). Under severe drought, the activity of SOD, POD, and CAT all experienced a decline but the decreases are not significant (Table 4). Consequently, *C. viminalis* seedlings may have powerful antioxidant enzyme regulating ability to protect plants from damage induced by adverse conditions. However, when the interactive effect of deep shading and severer drought obviously exceeds the threshold of the regulation of antioxidant enzyme system, the activity of SOD, POD and CAT significantly decline (Jing *et al.*, 2013; Li *et al.*, 2014; Ren *et al.*, 2017). This strong combined effect is also observed in *Arthraxon hispidus* (Sun *et al.*, 2018).

Effects of shade and drought on the content of proline

The content of free proline, an important osmolyte, plays a vital role in regulating the osmotic pressure of the plant cell and alleviating the damage induced by adverse condition (Ren *et al.*, 2000; Mahajan and Tuteja, 2005; Haffani *et al.*, 2014). In this study, Pro had lower content than the control group at 25% and 50% shading but increased significantly by 46% at 75% shading. That suggests the damage induced by full light and deep shading triggers the great accumulation of Pro in the plant (Arora and Saradhi, 2002; Xue *et al.*, 2011; Xie *et al.*, 2013).

The drought has a greater impact on the content of Pro than shading. The water stress significantly increased the content of Pro at mild drought, moderate drought, and severe drought by 46%, 77%, and 108% respectively (compared with the control group). The accumulation of Pro can protect plant cell from peroxidative processes induced by water stress through osmotic regulation (Shao *et al.*, 2006; Liu *et al.*, 2007; Sang *et al.*, 2011; Hou *et al.*, 2014). This result is supported by many studies in terms of maize, sorghum, *Arthraxon hispidus* and *Eucommia ulmoides* (Shao *et al.*, 2006; Liu *et al.*, 2007; Sun *et al.*, 2018). The interaction of shading and drought exert great influences on the content of Pro. Twenty-five percent shading could alleviate the impact induced by drought, but more than 50% shading enhances the accumulation of Pro caused by drought. This result is in full accordance with the recent study on *Arthraxon hispidus* (Sun *et al.*, 2018).

Conclusions

Shade, drought and their interaction have a significant effect on *C. viminalis* seedlings growth. Generally, moderate shade could alleviate the impact induced by drought. However, mild drought usually enhances the effect caused by shading. The interactive effect of deep shading and severe drought leads to a significant decrease of the photosynthetic capacity, the decrease of the activity of the antioxidant enzyme and the accumulation of MDA and Pro. When facing the light and water stress, the seedling adjusts itself by regulating photosynthetic indices, enhancing the activity of antioxidant enzyme system, and increasing Pro content to reduce the damage and gradually establish a protective mechanism to adapt the adverse conditions. The result shows that *C. viminalis* seedlings have relatively high shading and drought resistance and 25%-50% shading and 65% relative soil water content is most beneficial for *C. viminalis* seedlings growth. As a result, *C. viminalis* seedlings are more favorably planted in well-drained soil and moderate shade condition than poorly-drained soil and full light condition. When rattans are grown in the natural high-density forests, proper weeding and thinning the crown of the trees would facilitate their growth.

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Conflict of Interest

The authors declare that there are no conflicts of interest related to this article.

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