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Research Article

Response of *Cryptocoryne pallidinervia* Engler (Araceae) on light intensity and water depth

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Abstract
The study of the effect of different light intensities and water depths on the growth pattern, biomass allocation and photosynthetic rate of *Cryptocoryne pallidinervia* collected from Sungai Prasak, Keranji, Lundu, Sarawak, Malaysia, was conducted. Plants grown under tree canopy condition produced more leaves and lateral shoots than those grown under 50 % and 75 % shading. By increasing shade to 75 % level, the plants grew taller with larger leaves. The expansion of individual leaves at 75 % shade required 51 days to reach maximum size. The growth pattern such as leaf weight ratio (LWR), petiole weight ratio (PWR), rhizome weight ratio (RhWR) as well as root weight ratio (RWR) and biomass allocation such as specific leaf area (SLA), leaf area ratio (LAR), dry matter production (DMP), leaf area duration (LAD) and net assimilation rate (NAR) were significantly affected by shading. Plants grown in the depth of 15 cm were significantly taller and required a longer period to achieve maximum leaf expansion than those grown at 0 cm and 7 cm water depth. Growth pattern and biomass allocation were also significantly affected by water depths. Plants grown under 75 % shading and those grown at 0 cm water depth resulted in higher maximum quantum yield. However, plants at 75 % shade and those at 7 cm water depth had higher photosynthetic production at 300 μmol quanta $m^{-2}s^{-1}$ than those from other shade regimes and water depths.

Keywords: *Cryptocoryne pallidinervia*, light intensity, water depth, growth pattern, photosynthesis.

Introduction
Species of *Cryptocoryne*, also known as Keladi Air by locals in Sarawak, are mostly endemic aquatic plants. According to Jacobsen (1985), ten species of *Cryptocoryne* were recorded from Sarawak viz. *C. auriculata* Engl., *C. bullosa* Engl., *C. ciliata* (Roxb.)Kunth, *C. ferruginea* Engl., *C. grabowskii* Engl., *C. keei* N. Jacobsen, *C. lingua* Engl., *C. pallidinervia* Engl., *C. striolata* Engl. and *C. zonata* De Wit. Another four new species of *Cryptocoryne* recently found in

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Sarawak were *C. uenoi* Sasaki (Sasaki, 2002), *C. yujii* Bastmeijer (Bastmeijer, 2002), *C. zaidiana* Ipor & al. (Ipor et al., 2005a), *C. fusca* De Wit (Ipor & al. 2006), and a new interspecific hybrid *C. ×batangkayanensis* Ipor & al. (Ipor & al. 2015).

Jacobsen (1985) described three different habitats in which the *Cryptocoryne* grow, *vis.* the inner tidal zone as an amphibious life form, slow to fast running water as an aquatic life form and at the bank of smaller rivers as a rheophytic life form. Most of the *Cryptocoryne* are evergreen perennial herbs with procumbent to erect rhizomes and short to long subterranean runners.

Cryptocoryne pallidinervia is characterized by the limb of the spathe that has prominent, red protuberances and a broad, yellow collar zone with red spots, and on the spadix the male and female flowers are adjacent to each other (Arends et al., 1982; Bastmeijer, 2015). The leaves are cordate in shape and often somewhat bullate.

The physical appearance possessed by the species of *Cryptocoryne* have promoted the potential of these plants as aquarium plants. Species of *Cryptocoryne* are some of the commercially important aquatic species used in the aquarium plant trade, which have made them marketable products on the global market (Rataj & Horeman, 1977; Mansor, 1994). Besides that, the *Cryptocoryne* species are of great importance in aquatic ecology. In their natural habitats, they provide nutrients to other aquatic organisms. During the photosynthetic process, oxygen is supplied to the water thus lowering the BOD (biological oxygen demand) of the water. To some extent there is also an uptake of excessive nutrients from the water if not too contaminated and thus helps to clean it. The occurrence of *Cryptocoryne* in a stream is to some extent also an indication of a certain level of cleanness of the water. According to Jacobsen (1985), many *Cryptocoryne* can grow well under a thick canopy. The condition of low light intensity and high humidity level under the canopy promotes growth of these particular species. *Cryptocoryne* can even grow at high intensity of shading (approximately 90 % shading).

Cryptocoryne pallidinervia is mainly found in peat swamp areas (Figure 1). However, swamp areas have been substantially destroyed and transformed for agriculture and extensive forest harvesting. These activities have significantly destroyed its habitats and obviously caused drastic decline on populations. Ipor et al. (2007) had briefly attempted using polymerase chain reaction M13 universal primer of DNA fingerprinting of *C. pallidinervia* in Sarawak. The

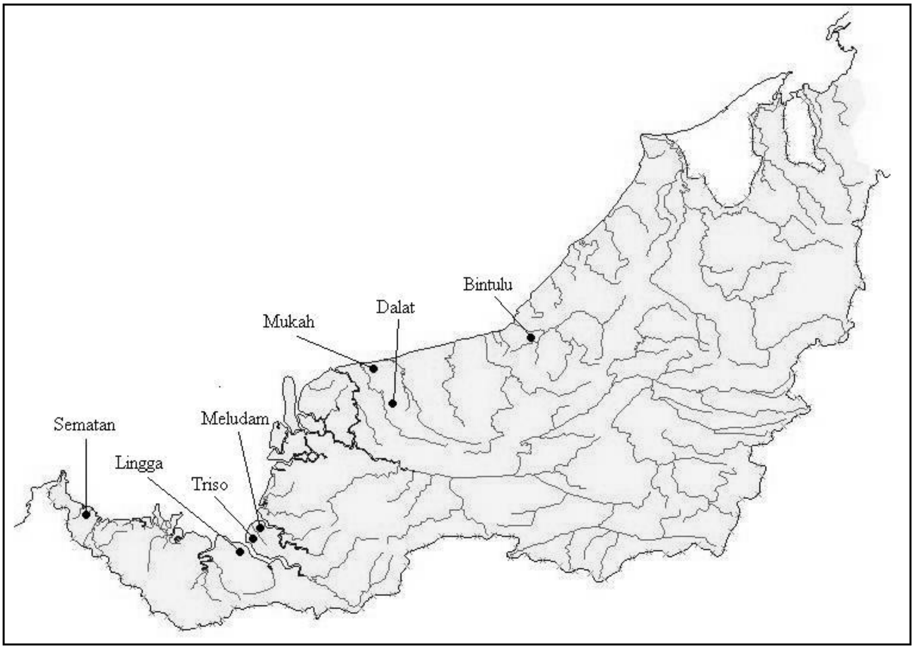

Figure 1. Seven areas of *C. pallidinervia* natural habitats found in Sarawak, Malaysia.

objective of this study is to determine the effects of different light intensities and water depth levels on the growth pattern of *C. pallidinervia*. The study also included the assessment of growth pattern, biomass allocation, photosynthetic rate, growth measurement and individual leaf expansion of *C. pallidinervia*.

Materials and Methods

Sampling and Study Area

Sampling of *C. pallidinervia* plants and soil was made at Sungai Prasak, near Kampong Keranji, Lundu, Sarawak, Malaysia. The plant and soil samples were then brought back to Universiti Malaysia Sarawak (UNIMAS) for plant material preparation for greenhouse experiments.

Propagation of planting materials

The lateral shoots were raised from mother plants throughout the entire study. The potting was a mixture of sandy loam soil and peat swamp soil from the sampling site. The selected lateral shoots were transplanted in pots (14 cm diameter and 9 cm height) and placed inside trays (47 cm x 24 cm). Two weeks after transplanting, placement of selected uniform plants to respective shade levels and water depths was carried out.

Light intensity

Three different light regimes which were under tree canopy shading condition, 50 % and 75 % shading conditions. Different intensity of lathe netting was used to obtain the 50 % and 75 % shading conditions. The pots were placed at 10 cm water depth (water depth measured from water surface to the media surface in the pot) for all light regimes. The plants were then subjected to growth assessment and biomass allocation analysis.

Growth measurement

Twenty uniform plants from every different shade level were labeled prior to growth assessment. The parameters that included plant height and total leaf number were taken every two weeks.

Development of individual leaves

In another assessment, the development of individual leaves was also studied. Each individual leaf was measured every 3 days using LI-3000A Portable Area Meter without severing the leaves. The measurement was continuously carried out until the consistent area obtained from three consecutive readings.

Biomass allocation

Five plants from each light regime were selected randomly and harvested after 30 days of transplanting. The leaf area of the individual plants was determined before drying the samples using the AT Delta-T scan equipment. The leaf, root, petiole and rhizome were separated prior to oven drying at 60°C for seven days to determine their dry weight. Similar harvest or assessment was done after 60 days of transplanting. The biomass allocation assessment was determined using the method described by Patterson & Flint (1983).

Water depth

Three different water depths used in this experiment were 0 cm, 7 cm and 15 cm. The depths were measured from the media surface to the water surface. For each treatment, 20 plants were placed in the designated water depth. All water containers containing plants at the respective water depth were placed under 50 % shade condition. The designated water levels were maintained and monitored every day. Similar assessment as in the light response experiment such as growth assessment and biomass allocation was conducted for their response to different water depths.

Photosynthesis measurement

The photosynthesis measurements were done in both light intensity and water depth experiments. In measuring the photosynthetic rates, "WALZ Diving-PAM Flourometer" equipment was used. Three uniform leaves were selected from each plant to determine their maximal fluorescence yield. Each light regime and water depth comprised five plants. Each leaf was dark-adapted for 10 minutes prior to photosynthetic measurements. The light curve (electron transport rate vs. photosynthetic active radiation) was also determined. The light curve experiment was done in a darkened room. Leaves were dark-adapted using dark-leaf clip for 10 minutes prior to measurement.

Data analysis

The experiments were conducted twice in completely randomized blocks with five replicates. The data were pooled and statistically subjected to least significant difference or Tukey's test. Significance in the text refers to the 95 % confidence level.

Results

Response to shading

It was observed that tree canopy shading and 75 % shading has approximately similar light intensity within the 13–hour-measurements, only that at 1400 hours the light intensity for tree canopy shading increased to a higher level than that under the 50 % and 75 % shading due to direct penetration of sunlight on the samples. The light regime of 0% shading was not applied in this study as the direct sunlight condition was not favourable for *C. pallidinervia* cultivation. It was observed that *C. pallidinervia* was a heat sensitive plant as it was not tolerant to direct sunlight.

Plants grown under tree canopy and 75 % shade regime were significantly taller than those at 50 % shade regime. From the 4th week, plants under tree canopy condition were significantly taller than those under 50 % and 75 % shading. Between the second and fourth weeks, plants under 75 % shade regime experienced a great change of plant height, however, it also grew well after week 4 (Figure 2).

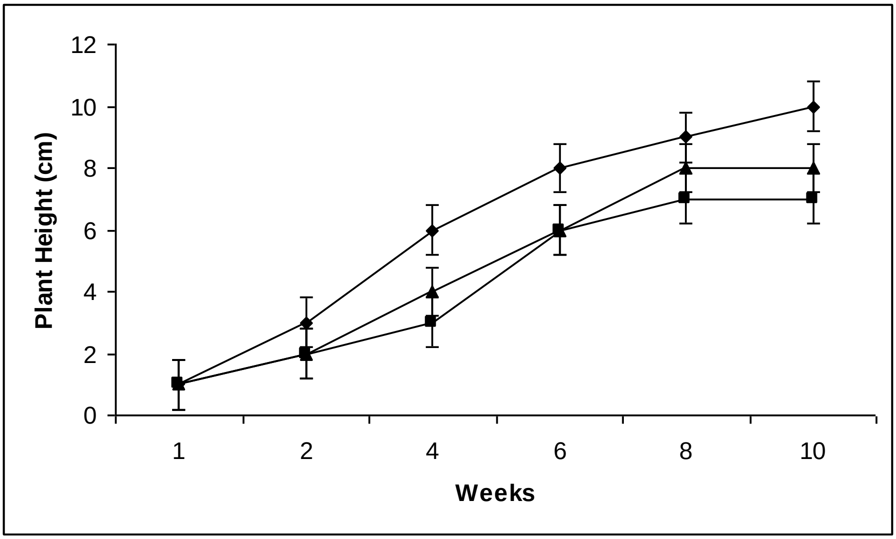

Figure 2. Effect of shading on the plant height of C. pallidinervia. Under tree canopy shading ( ), 50% shading ( ) and 75% shading ( ). Vertical bars are values of LSD = 0.05. Water level 0 cm.

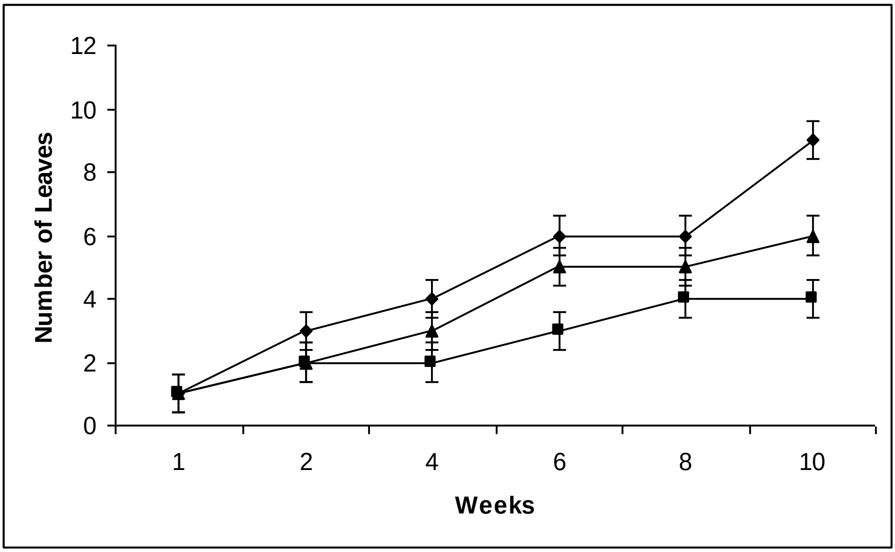

Figure 3. Effect of shading on leaf number of *C. pallidinervia*. Under tree canopy shading ( ) 50% shading ( ) 75% shading ( ). Vertical bars are values of LSD at 0.05.

Plants under tree canopy shading produced significantly more leaves compared to the other two light regimes (Figure 3). Plants under 75 % shading had the least number of leaves. However, there was no significant difference on the number of leaves for plants under 50 % and 75 % shade during the first 4 weeks. Plants under all light regimes were not significantly different in terms of leaf numbers in week 2. The expansion and maturity of individual leaves varied between shade regimes. By increasing level of shading, larger leaves with delay in their maturity was observed (Figure 4). Leaves from 75 % shade required 51 days to obtain full and maximum expansion where as 50 % shade and under canopy condition required 42 and 39 days respectively.

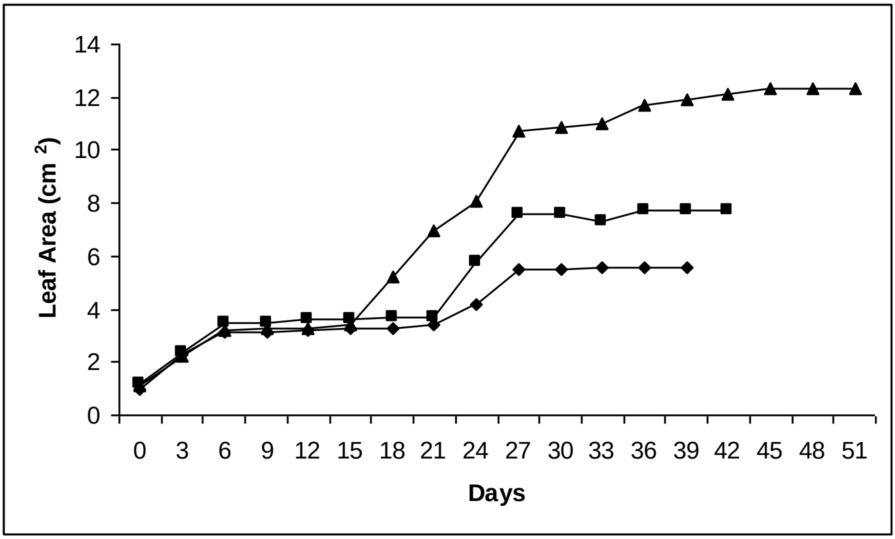

Figure 4. Effect of shading on individual leaf area of *C. pallidinervia*. Under tree canopy shading (—◆—), 50% shading (—■—) and 75% shading (—◆—).

Leaf weight ratio (LWR) of plants under 50 % shading was significantly lower than those under the tree canopy shading (Figure 5a). Petiole weight ratio (PWR) was not significantly differed between shade regimes (Figure 5b). Plants at 75 % shade had highest PWR. Rhizome weight ratio (RhWR) of plants under tree canopy shading was significantly lower than those under 50 % shading (Figure 5c). However, these plants had a significantly higher root weight ratio (RWR) than those from other light regimes (Figure 5d). The SLA of plants grown under 75 % shading was significantly higher than those from plants under tree canopy shading (Figure 5e). There was no significant difference of SLA between 50 % and 75 % shading and between 50 % shading and under tree canopy shading. Leaf area ratio (LAR) of plants under 50 % shading was significantly decreased compared to those under tree canopy and 75 % shading (Figure 5f).

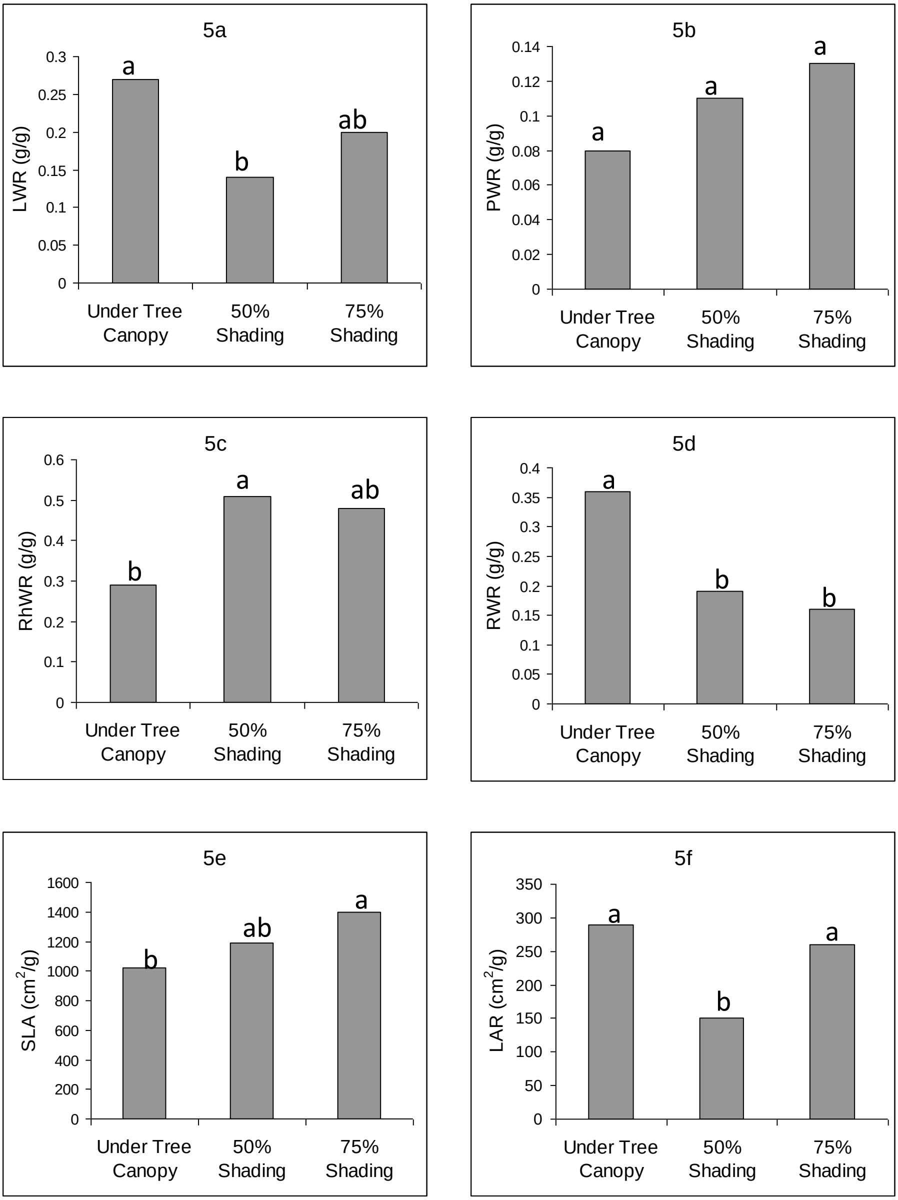

Figure 5. Effect of shading on vegetative growth, leaf area production and biomass allocation in *C. pallidernivia* (30th harvest) (5a = Leaf Weight Ratio (g/g), 5b = Petiole Weight Ratio (g/g), 5c = Rhizome Weight Ratio (g/g), 5d = Roots Weight Ratio (g/g), 5e = Specific Leaf Area (cm^2/g), 5f = Leaf Area Ratio (cm^2/g)). Values sharing the same letter are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test.

Both dry matter production (DMP) and leaf area duration (LAD) were significantly higher under tree canopy than those from 50 % and 75 % shading (Table 1). Plants under the 50 % shading regime showed a significantly higher value of net assimilation rate (NAR) than the other light regimes.

Table 1. Effect of shading on dry matter production (DMP), net assimilation rate (NAR) and leaf area duration (LAD) of *C. pallidinervia* during the 30^{th} to 60^{th} day interval after transplanting. Within each column, values sharing the same letter are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test.

Shading	DMP	NAR	LAD
Under tree canopy	0.20a	0.00014b	1588.92a
50%	0.14b	0.00039a	420.55c
75%	0.11b	0.00017b	737.80b

Water depth response

Plants grown in the water depth of 15 cm were significantly taller than the other two water depth regimes. However, plant height of plants in the water depth regimes of 0 cm and 7 cm was not significantly differed throughout the study (Figure 6). A similar trend was observed for leaf number from different water depths (Figure 7).

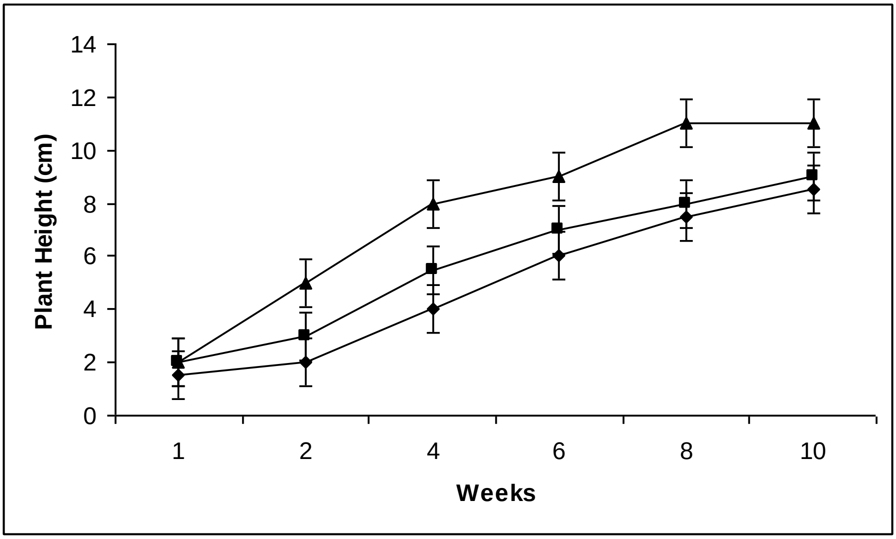

Figure 6. Effect of water depth on plant height in *C. pallidinervia*. 0 cm depth ( ), 7 cm depth ( ) and 15 cm depth ( ).Vertical bars are values of LSD = 0.05.

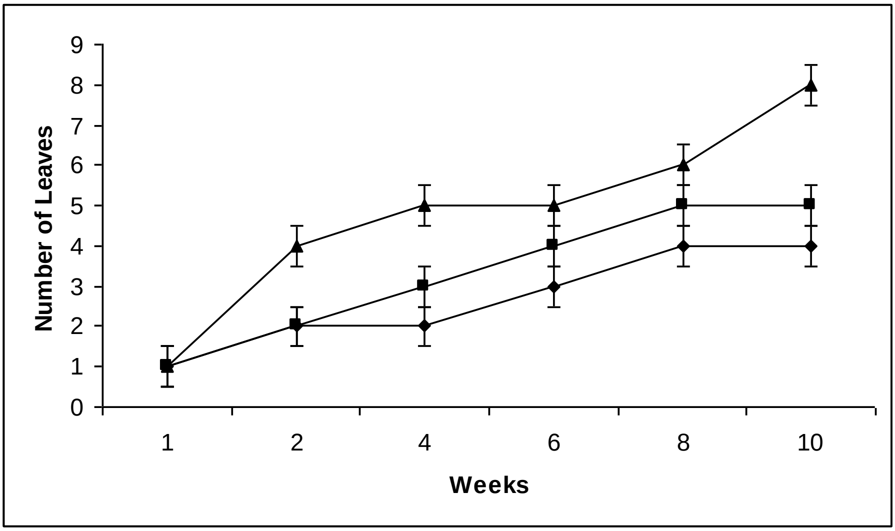

Figure 7. Effect of water depth on number of leaves in *C. pallidinervia*. 0 cm depth (—◆—), 7 cm depth (—■—) and 15 cm depth (—◆—).Vertical bars are values of LSD = 0.05.

The placement of plants at the depth of 15 cm had produced larger leaves and required a longer period to obtain maturity. It took 54 days to reach the maximum size. Both 0 cm and 7 cm water depth regimes had produced smaller leaves and required 36 and 42 days to obtain maximum size respectively (Figure 8).

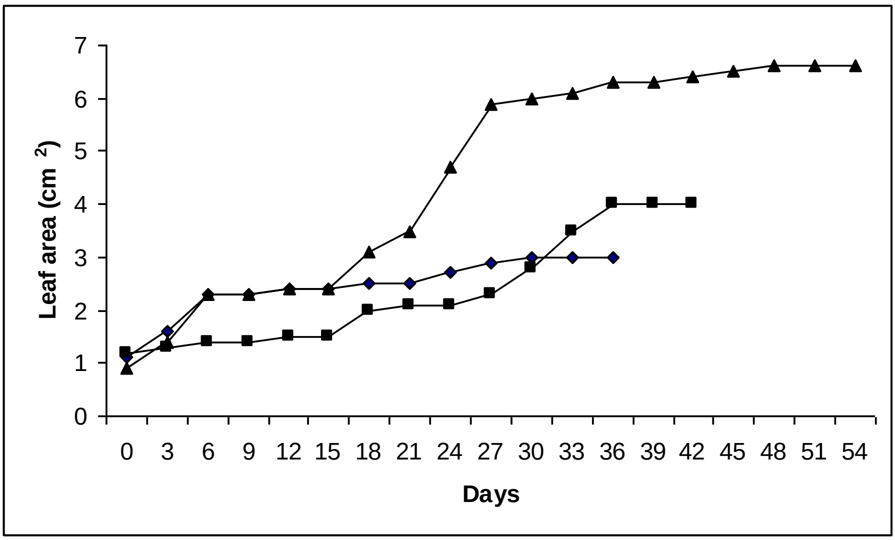

Figure 8. Effect of water depth on individual leaf area in *C. pallidinervia*. 0 cm depth (—◆—), 7 cm depth (—■—) and 15 cm depth (—◆—).

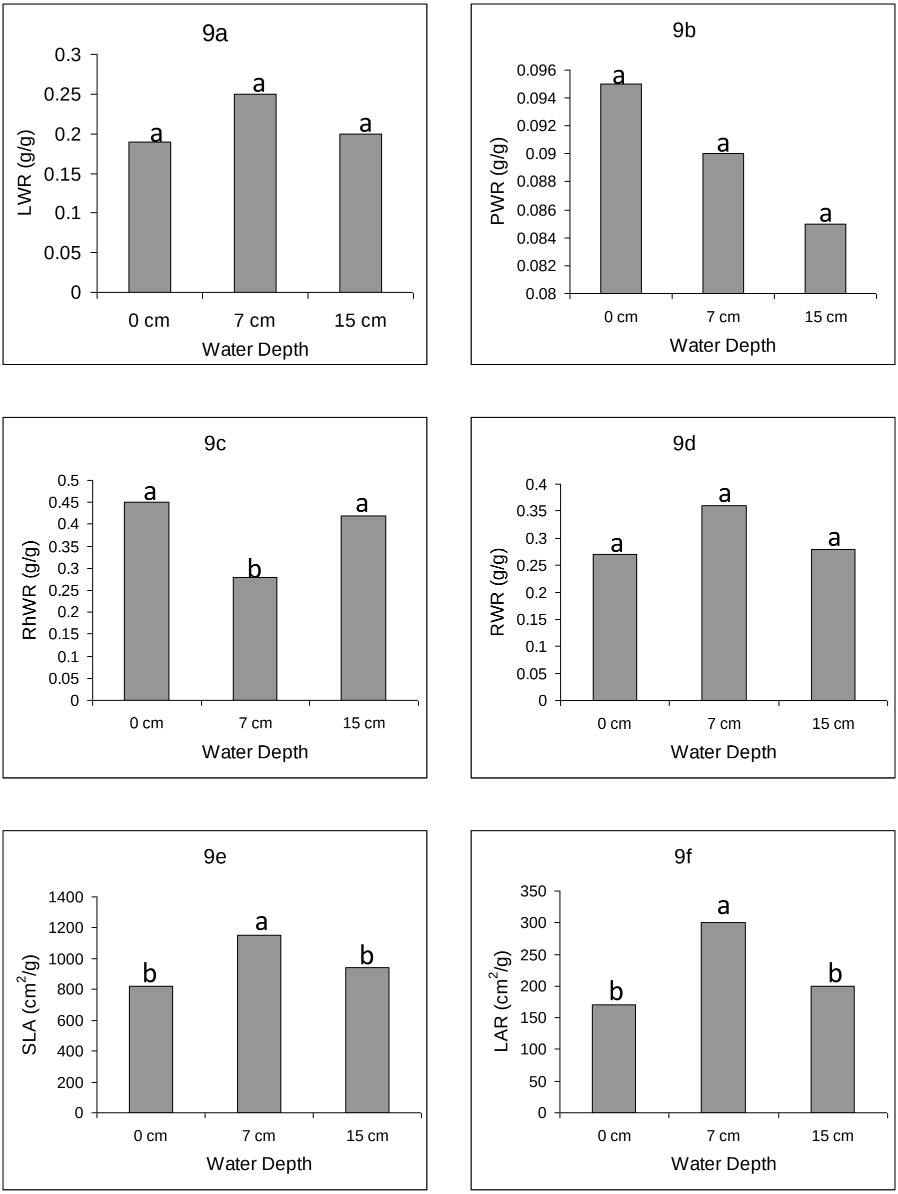

Figure 9. Effect of water depth on vegetative growth, leaf area production and biomass allocation in *C. pallidernivia* (30th harvest) (9a = Leaf Weight Ratio (g/g), 9b = Petiole Weight Ratio (g/g), 9c = Rhizome Weight Ratio (g/g), 9d = Roots Weight Ratio (g/g), 9e = Specific Leaf Area (cm^2/g), 9f = Leaf Area Ratio (cm^2/g)). Values sharing the same letter are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test.

There was no significant difference in the leaf weight ratio (LWR), petiole weight ratio (PWR) and root weight ratio (RWR) between water depths (Figure 9). Plants grown in the water depth of 7 cm had a significant decrease in rhizome weight ratio (RhWR) compared to the other two depth regimes. However, leaf area ratio (LAR) of those from 7 cm water depth was significantly higher than those from 0 cm and 15 cm water depths (Figure 9).

Dry matter production (DMP) was significantly different among the three different water depths (Table 2). The leaf area duration (LAD) of plants in the 7 cm water depth was significantly lower than the other depth regimes. Net assimilation rate (NAR) was not significantly differed among the water depth regimes.

Table 2. Effect of water depth on dry matter production (DMP), net assimilation rate (NAR) and leaf area duration (LAD) of *C. pallidinervia* during the 30^{th} to 60^{th} day interval after transplanting. Within each column, values sharing the same letter are not significantly different at P ≤ 0.05 according to Duncan's multiple range test.

Water Depth	DMP	NAR	LAD
0 cm	0.10440a	0.00006a	1731.23a
7 cm	0.05667c	0.00005a	1250.59b
15 cm	0.08755b	0.00005a	1792.82a

Placement of plants at 75 % shading resulted in significantly higher maximum quantum yield than those from 50 % shading (Figure 10). It had no significant difference to those under tree canopy shading and in field condition.

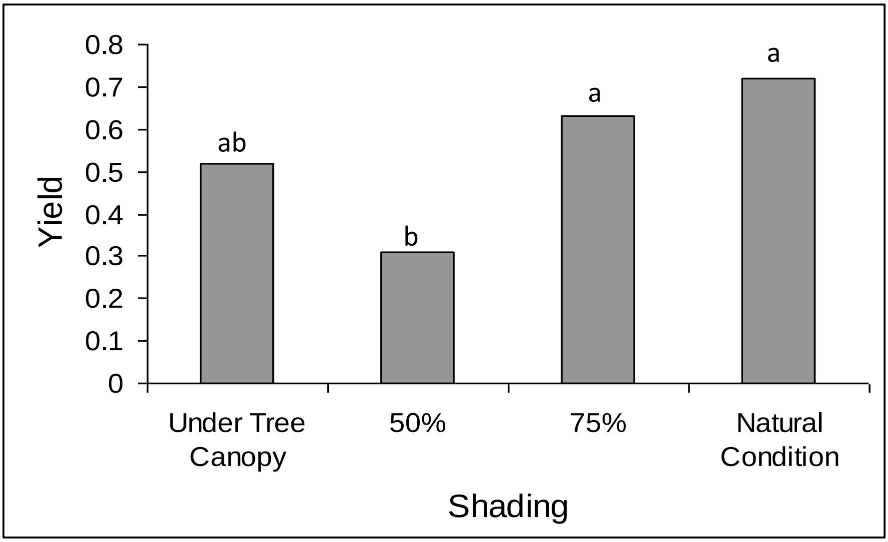

Figure 10. Effect of shading on maximal quantum yield of *C. pallidernivia*. Values sharing the same letter are not significantly different at P ≤ 0.05 according to Duncan's multiple range test.

Plants grown under 75 % shading had a higher photosynthetic production at 300 μmol quanta m^{-2} s^{-1} than those grown under the tree canopy and the 50 % shading regimes (Figure 11).

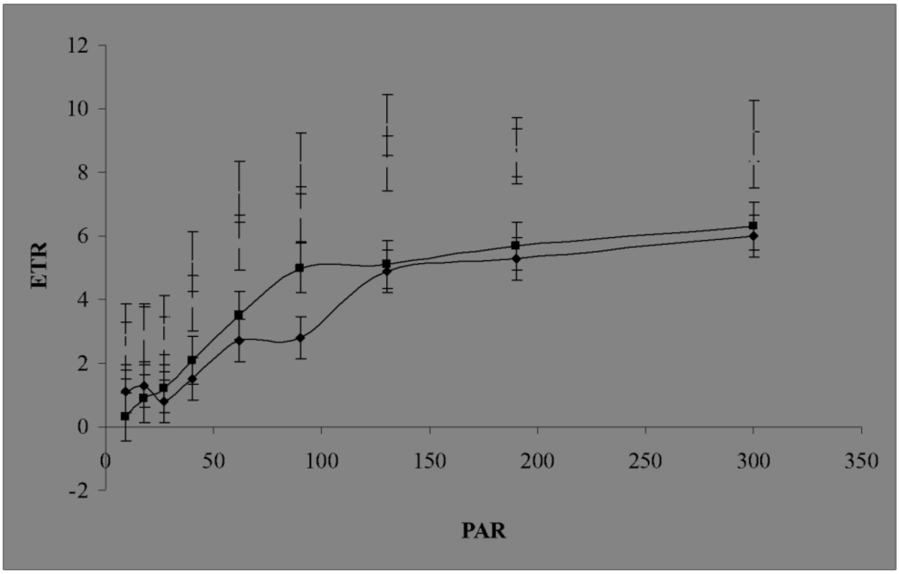

Figure 11. Effect of shading on light curve (electron transport rate (ETR, μmol. Protons $m^{-2}.s^{-1}$) vs. photosynthetic active radiation (PAR, μmol.$m^{-2}.s^{-1}$)) of *C. pallidinervia*. Tree canopy shading ( ), 50 % shading ( ), 75 % shading ( ) and natural habitat ( ). Vertical bars are LSD = 0.05.

The maximum quantum yield tended to decrease as water depth increased under greenhouse conditions (Figure 12). Plants grown in the water depth of 0 cm had a significantly higher maximum quantum yield than those in the 15 cm depth regime. However, it was not significantly different from those at the 7 cm depth regime. The rate at the water depth of 7 cm in natural condition was almost similar to those from the 0 cm depth in the green house condition. Plants grown in the water depth of 7 cm recorded higher photosynthetic production at 300 μmol quanta m^{-2} s^{-1} than those grown in the 0 cm and the 15 cm water depth regimes (Figure 13).

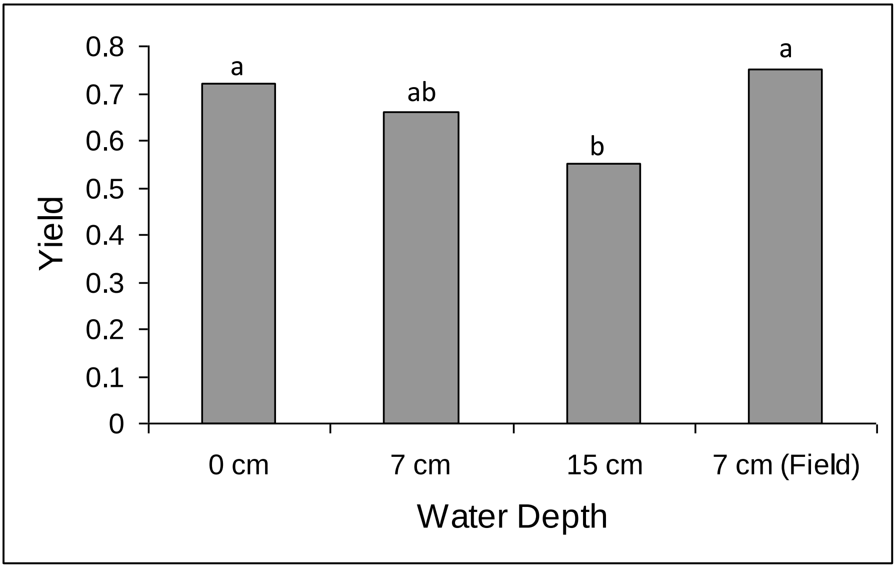

Figure 12. Effect of water depth on maximal quantum yield in *C. pallidernivia*. Values sharing the same letter are not significantly different at P ≤ 0.05 according to Duncan's multiple range test.

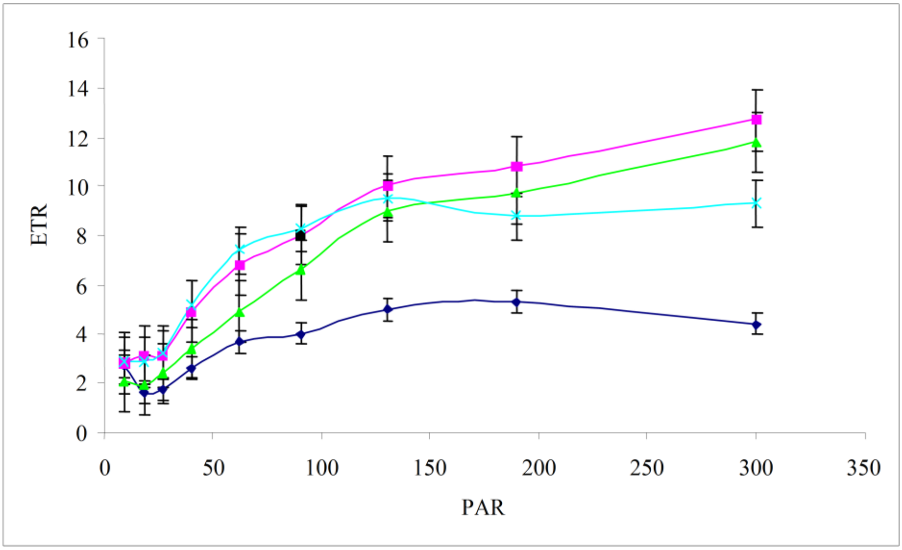

Figure 13. Effect of water depth on light curve (electron transport rate (ETR, μmol. Protons $m^{-2}.s^{-1}$) vs. photosynthetic active radiation (PAR, $\mu mol.m^{-2}.s^{-1}$)) in *C. pallidinervia*. 0 cm depth (—◆—), 7 cm depth (—■—), 15 cm depth (—◆—) and natural habitat (—✕—). Verical bars are LSD = 0.05.

Discussion

For *C. pallidinervia* plants grown under the tree canopy and the 75 % shading regimes, no significant difference in their height was recorded. Plants under those shading regimes were significantly higher than that under the 50 % shading regime. A similar pattern was observed for *C. zaidiana* being exposed to different light intensities (Ipor et al. 2009). This was due to the intensity of light received by plants under the tree canopy and the 75 % shading regimes being lower than the 50 % shading. Plants stretch themselves in order to reach for a sufficient light source.

According to Bjorkman (1968) and Ishimine et al., (1985), reduced light levels to a certain degree would result in an increased stem extension of *Solidago virgaurea* L. and height of *Paspalum urvillei* Steud. However, a further reduction of light suppressed plant growth. In addition, *Cryptocoryne* species are capable of growing well under a thick canopy (Jacobsen, 1985). In the study of water depth effect on *C. pallidinervia*, different water depths significantly affected the plant height. Plants grown at a depth of 15 cm of water were significantly taller than the 0 cm and 7 cm depth regimes. This might be due to the phototropism of the plants, where the plant grew taller to reach the light source on the surface of the water. Ipor et al., (2005b) observed that *C. striolata* Engler that grew in deeper water had longer leaves and therefore were taller plants.

C. pallidinervia plants grown under tree canopy shading had significantly more leaves than that under the 50 % and 75 % shading regimes. However, plants under the 75 % shading regime generally developed larger leaves. Higher light intensity condition promoted the plants to produce more leaves and the number decreased significantly with larger leaves under lower light condition (Ipor, 1992; Ipor & Price, 1991; Ipor & Tawan, 2002). In this study, it was observed that light penetration under the tree canopy shading was at a lower level compared to the 50 % shading regime and may sometimes be even at a lower level than the 75 % shading condition. On the contrary, based on light intensity measurements on those three light regimes, shading under the tree canopy allowed higher light penetration than 50 % and 75 % shading regimes at about 1400 hours during clear sunny days. This obviously affected the growth of the plants under the tree shading condition. Short exposure of plants to high light intensity may have triggered the plants in enhancing their physiological activities such as photosynthetic activities.

Plants grown under tree canopy condition were significantly higher in lateral shoot production on the tenth week. The delay in production of lateral shoots experienced by plants under the 75 % shading regime showed that the condition was not favourable for lateral shoot production. There was no significant difference on lateral shoots produced. According to Ipor & Naim (2003), the *Rottboellia exaltata* Lour. plants cultivated under 75 % shading regime had lower production of tillers. Kobayashi et al., (2001) in their study of *Plantago asiatica* L. reported the percentage of reproductive plants in shaded plant populations was less than that in exposed populations at all size classes. This was similar to the *C. pallidinervia* as plants under 75 % shading conditions produced fewer lateral shoots. However, plants under the tree canopy shading produced more lateral shoots. The periodic high light intensity in the tree canopy shading regime may have contributed to the higher lateral shoots production than those under the 50 % and 75 % shading regimes.

Based on the biomass allocation assessment of *C. pallidinervia*, the highest leaf weight ratio (LWR) was recorded from plants grown under the tree canopy shading which was significantly higher than those under the 50 % shading regime. There was no significant difference on petiole weight ratio (PWR) among the three light regimes. Partitioning of biomass to root and rhizome differed significantly among light regimes. Root weight ratio (RWR) of plants under tree canopy shading regime recorded significantly higher than that under the 50 % and 75 % shading regimes. Rhizome weight ratio (RhWR) of plants under the tree canopy shading regime was significantly lower than that under the 50 % shading regime, but not significantly different to that under the 75 % shading regime. Although there was no significant difference among light regimes on the specific leaf area (SLA), plants under 75 % shading recorded the highest value of SLA. The plants under a light-limited regime invested more to the production of light-harvesting apparatus rather than other components of plant biomass (Patterson, 1980). The tree canopy shade regime possessed significantly higher leaf area ratio (LAR) than that under 50 % shading.

The biomass allocation assessment in different water depths showed that there was no significant difference in leaf weight ratio (LWR), petiole weight ratio (PWR), root weight ratio (RWR) or specific leaf area (SLA) among the three different depths. Plants grown in the water depth of 7 cm were significantly differed on rhizome weight ratio (RhWR) compared to the other two depth regimes. Plants grown in the 7 cm water depth regime showed highest value in LWR, RWR, SLA and LAR.

DMP and LAD of *C. pallidinervia* were highest at the tree canopy shade. The values were significantly higher than that under 50 % and 75 % shading. DMP and LAD values among the three light regimes showed significant difference. The NAR value was recorded significantly higher at 50 % shade than the other two light regimes. For the assessment of DMP, NAR and LAD at three different water depths, it was shown that there was a significant difference among the three depths on the DMP values. However, the NAR values did not indicate a significant difference among the three different depths. Data showed that at a water depth of 7 cm, the value of LAD was significantly lower than the other two depths.

Plants under the 75 % shading regime resulted in a higher value of the maximal quantum yield. There was no significant difference on maximum quantum yield value of plants under the tree canopy shading and the 75 % shading. This may be due to the light intensity received by plants under both light regimes that were almost similar. Plants in water of 0 cm and 7 cm depths were significantly taller than those in 15 cm depth. A higher value of maximum quantum yield showed that plants had a higher photochemical efficiency of photosystem II (PSII) primary reaction centres (Krause & Weis, 1991).

Plants grown under the 75 % shading regime and the 7 cm water depth regime had higher electron transport rate (ETR) value at 300μmol quanta m^{-2} s^{-1}. Both samples indicated a higher photosynthetic production rate. Sample at the 75 % shading regime received a lower amount of light. This also happened to the sample from the 7 cm water depth regime, where the water was dark.

Conclusion

Based on the study, cultivation of *C. pallidinervia* under different light intensities showed different growth patterns. Placing the plants under the tree canopy regime resulted in higher number of leaves and lateral shoots than those under the 50 % and 75 % shade regimes. However, a lower light intensity would cause the plants to grow longer and produce larger leaves. The water depth levels of 0 cm, 7 cm and 15 cm did not affect the number of leaves produced, plant height growth and the number of lateral shoots produced. Different light intensities and water depths significantly affected the photosynthesis rate and biomass partitioning of *C. pallidinervia*. Similar pattern of and biomass allocation and growth response for *C. ferruginea* Engler was observed in different conditions of shading and water depth (Ipor et al. 2006).

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