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Growth and Physiological Responses of *Asplenium nidus* to Water Stress

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Abstract: Pot experiment conducted in the nursery of Faculty of Forestry, University Putra Malaysia during the periods from May to August, 20006 to investigate the effect of water stress on *Asplenium nidus* growth performance, transpiration rate and chlorophyll content in fronds. The plants were subjected to five water stress levels; control-watered every day, T_1-watered every three days, T_2-watered every seven days, T_3-watered every fourteen days and T_4-suspended without watering. Growth parameters were measured every week for a period of sixteen weeks. At the end of experiment, transpiration rate and chlorophyll content were measured as well as dry matter of plants. The results showed that levels of watering did not significantly affect all growth and physiological parameters of *Asplenium nidus* except T_4 which decreased all growth parameter and some physiological responses. T_1 had the highest dry matter production. The plants allocated more growth to the shoot under optimum conditions but more growth to the root under stress conditions. This plant was able to adapt and survive without watering for 16 weeks and demonstrate that it can survive in water limited condition.

Key words: Environmental stress, forest canopy, growth response, fern

INTRODUCTION

Epiphytes are importants components and contribute significantly to the biodiversity of tropical rainforests (Hsu *et al*., 2006). It plays an important role in nutrient cycling (Hietz *et al*., 2002) and provide important resources for many canopy animal (Basset *et al*., 2003). Epiphytes plants are able to grow and survive in the forest canopy due to various structures and mechanisms that make them able to withstand the extreme environmental stresses like droughts (Graham and Andrae, 2004).

Some epiphytes are able to withstand from the limited water supply by forming water storage organs, impounding tanks, or-similar to ground-dwelling the plants-by rooting exclusively in humus accumulations in crotches, in tree-holes, or in the axils of large leaves (Benzing, 1987). In order to survive in such stress-prone and limited water environment, epiphytes develop morphological adaptations to water shortage such as succulent leaves, thick cuticles, water shortage tanks, drought deciduousness and low stomatal conductance or engage in Crassulacean Acid Metabolism (CAM) (Winter, 1985; Benzing, 1990).

The ability of epiphytic plants surviving in extreme environment makes the plants versatile and some plants such as *Asplenium nidus* become popular as house and ornamental plants (Ainuddin, 2007). *Asplenium nidus* is a species of fern in the family Aspleniaceae, native to tropical Southeastern Asia and often known by its common name as Bird's Nest Fern.

Asplenium nidus has been used as medicinal plants and has been used in traditional medicine as a remedy for illnesses such as stomach ache, diueritic and also as a contraceptive. Bourdy and Walter (1992) conducted ethnobotanical studies in Vanuatu from 1985 to 1987. They found out that *Asplenium nidus* is being used as a contraceptive by eating two young coiled fronds just after menstruation in the morning.

The objectives of the study were to determine the effects of different level of water stress on some physiological growth performances and response of *Asplenium nidus* under water stress.

MATERIALS AND METHODS

The study was conducted in the nursery of the Faculty of Forestry, University of Putra Malaysia between May to August 2006. Uniform twenty five plants of *Asplenium nidus* were transplanted in pots (one plant in each pots) filled with potting mixture of coconut peat, loam and sand (3:1:1 v/v). Water stress treatments were

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initiated three weeks after transplanting (appearance of new fronds) conducting according Chang *et al.* (1995). The plants were subjected to five treatments with five replicates. The water regime treatments were well-watered (control) that were watered daily, moderate stress (T_1) watered once every three days, severe stress (T_2) watered once every seven days, highly severe drought (T_3) watered once every fourteen days and suspended without water or any treatment (T_4). Following are the definition of treatments:

Control : Watered everyday
T_1 : Watered once every three days
T_2 : Watered once every seven days
T_3 : Watered once every fourteen days
T_4 : No watering during the study

Data collection: Once water stress progress, measurement of growth parameters including number of fronds, fronds length and frond width, frond area per plant as well as shoot and root per weight were measured every weeks while transpiration rate and chlorophyll content in the fronds one week before harvest.

Total frond area of *Asplenium nidus* was measured using LI-3100 Frond Area Meter (Lincoln NE, USA) after dismantling the pots and after transpiration rate and chlorophyll rate were measured.

Transpiration rate of three plants per treatment was measured during the late morning (9.00 to 12.00 am) using A LICOR 1600 steady state porometer (Lincoln NE, USA).

Chlorophyll content measurements of three fronds were conducted during the late morning (9.00 to 12.00 am) using Chlorophyll Meter (Minolta model SPAD-502). Statistical analysis was performed using statistical software. The one-way Analysis of Variances (ANOVA) was used to compare the means of more than two different estimates of population variance from the data. Turkey's Test was employed to detect significant differences among and between the treatments at the 0.05 α level.

RESULTS

Growth parameters i.e. fronds number per plant, frond length, frond width and frond area as well as dry weight of plants were measured after sixteen weeks of treatments.

The results are discussed as with the help of Table 1. Total frond length of *Asplenium nidus* was the highest in T_3 (110.64 cm) followed by control (99.99 cm), then T_1 (98.06 cm), T_2 (79.44 cm) and the lowest T_4 (27.74 cm). Statistically analysis showed the total frond length for T_4 was statistically lowered compared to other treatment. This indicates that lack of water suppressed the growth of the plant but still survived despite 16 weeks without watering.

Mean total frond width was the highest in control (27.4 cm), followed by T_3 (27.02 cm), T_1 (26.78 cm), T_2 (21.28 cm) and T_4 (8.78 cm). Total frond width for T_4 was statistically lower compared to other treatments.

Mean total frond area was the highest in control (697.28 cm^2), followed by T_1 (644.27cm^2), T_3 (627.98cm^2), T_2 (510.44 cm^2) and T_4 (263.39cm^2). Total frond area for T_4 was statistically lower compared to other treatments.

Total number of fronds were the same for control (78) and T_1 (78) followed by T_3 (75), then T_2 (73) and the lowest T_4 (66). The analysis showed the total number of fronds for T_4 was statistically lowered compared to other treatment. This indicates that a lack of water will suppressed the growth of the plant but still survived despite sixteen weeks without watering.

Treatment T_1 had the highest total frond dry weight (12.6 g) followed by control (12.03 g), T_3 (11.28 g), T_2 (11.23 g) and lastly T_4 (5.04 g). Mean frond dry weight was statistically higher compared to T_4.

Table 1: Mean and standard deviation (in bracket) of growth parameters to various water stress treatments

	Water stress treatments				
Growth parameters	Control	T_1	T_2	T_3	T_4
Mean growth parameters					
Total frond length (cm)	99.88b (13.93)	98.06b (25.55)	79.44b (21.86)	110.64b (43.24)	27.74a (10.30)
Total frond width (cm)	27.42b (5.71)	26.78b (5.68)	21.28b (3.36)	27.02b (7.47)	8.78a (3.34)
Total frond area (cm^2)	697.28b (145.35)	644.27b (210.60)	510.44ab (172.52)	627.98b (222.48)	263.39a (44.32)
Total number of leaves	15.60a (1.14)	13.20a (1.64)	15.60a (0.55)	15.40a (1.34)	15.00a (3.67)
Mean dry weight (g)					
Frond	12.03ab (1.95)	12.60b (4.85)	11.23ab (4.54)	11.28ab (4.91)	5.04a (2.07)
Root	52.80b (20.96)	36.91ab (5.19)	29.69a (4.26)	38.99ab (9.73)	28.22a (2.47)
Root shoot ratio	4.50a (1.92)	3.22a (1.04)	3.18a (1.70)	4.07a (2.43)	6.73a (3.84)
Dry matter production (g)	64.83b (20.95)	49.51ab (9.38)	40.92a (5.35)	50.27ab (11.89)	33.26a (2.57)
Physiological parameters					
Transpiration rate ($\mu g/cm^2/s^1$)	38.04c (0.96)	41.19c (2.78)	33.25b (1.73)	31.48b (1.50)	9.54a (1.39)
Chlorophyll content (SPAD units)	203.12b (21.39)	221.60b (30.71)	176.76ab (33.41)	228.22b (58.17)	105.70a (34.42)

*Values followed by the same letter(s) are not statistically significant at the 0.05 alpha levels

Mean root dry weight for the treatment were 52.80, 38.99, 36.9, 29.69 and 28.22 g for control, T_3, T_1, T_2 and T_4 respectively. Mean root dry weight for control was the highest while T_4 was the lowest. Under water stress condition plants grew more to roots compare to shoots. *Asplenium nidus* has fiber root systems. Roots developing from the short stem grow into the decaying debris and with time, the entire mass becomes a sponge with soaks up an enormous amount of water after each period of rain. This mass of decaying matter thus supplies the fern with nutrients and water.

Root shoot ratio indicates the allocation root biomass with regard to shoot. Root shoot ratio was the highest is T_4 (6.73), followed by control (4.5), T_3 (4.07), T_1 (4.5) and T_2 (3.18). The severe water restriction had caused the plant to focus more on the root so that water can be absorbed by the plants.

The total dry matter production was the highest for control (64.83 g), (14.56 g) greater than T_4 (33.26 g) than followed by T_3 (50.27 g), T_1 (49.51 g) and T_2 (40.92 g). In terms of partitioning of total dry matter production during the treatments, plants grown allocated more to roots compared to the leaves at all levels of water stress. Throughout the water stress treatments, better and good foliage was observed in control treatment. This showed that *Asplenium nidus* were able to survive and growth under high moisture condition. *Asplenium nidus* despite without having water for 16 weeks still survives. However the production of total dry matter was greatly reduced in the severe water stressed plants.

Transpiration rate for control, T_1, T_2, T_3 and T_4 were 38.04, 41.19, 33.25, 31.48 and 9.54 $\mu g/cm^2/sec$, respectively. T_1 was statistically higher other then the other treatments except for control while transpiration rate for T_4 was significantly the lowest.

As regard to chlorophyll content, clearly indicate that withholding water for sixteen weeks had a lower content of chlorophyll (105.70 SPAD Unit) as compared to control plants. This decreasing was accompoined with some chlorosis symptoms, yellowish frond and soft, thinned fronds. On the other hand, irrigation of plants every 14 days (T_3) gave a highest content of chlorophyll followed by T_1 and T_2 as compared with control plants.

DISCUSSION

Asplenium nidus showed morphological and physiological responses to the different cyclical water stress. The plants under higher water stress showed lower frond length, frond width, total frond area and mean dry weight compared to control plants. The reduction in length is due to the reduction of cell expansion and elongation (Thomas *et al*., 1999) and also in the reduction in length of the growing zone (Durand, 1995). This finding is consistent with finding by Fedorenko *et al.* (1995) on *Medicago minima* var. minima.

Plants subjected to water stress will reallocate growth to the roots so that more roots will be available to absorb moisture. Furthermore, the shoots are also reduced in size hence causing an increase in the root/shoot ratio in the severe water stress plants. This result concurs with study conducted by Chang *et al.* (1995) on *Mimosa strigillosa* which indicate water stress plants had higher root to shoot ratio.

Asplenium nidus responded physiologically to the different water stress levels. As the water stress increases, the frond transpiration rate decreases due to closing stomata. (Zhang *et al*., 2009). Present investigation clearly indicates that severe water stress decreases the chlorophyll content, these results were agreement with finding of Naderikharaji *et al.* (2008).

Even though *Asplenium nidus* were affected by cyclic water stress, this study also showed that this plant can also survived long period of without watering such as in this study for sixteen weeks. This concur with Benzing (1990) which stated that epiphytic plants exhibit physiological and morphological traits that enable them to withstand intermittent drought. This characteristics may be due to different photosynthetic carbon flow pathway such as crassulacean acid metabolism. A study by Ong *et al.* (1986) showed that two epiphytic tropical ferns *Drymoglossum piloselloides* and *Pyrrosia longifolia* are obligate CAM plants which are able to withstand water stress. However, this need further study on the nature of photosynthetic pathway for *Asplenium nidus*.

CONCLUSION

This study showed *Asplenium nidus* growth parameter were affected by water stress but can survive for a long period without water due to increasing fibrous root system. This plant has a very high survival rate because it is one of the epiphytes species that can continue to grow under the highly stress condition.

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