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Impact of Different Water Levels on Growth, Plant Water Relations and Photosynthesis Parameters in Seedling of Tongkat Ali (*EURYCOMA LONGIFOLIA JACK*)

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Abstract:
The effects of water availability on growth, water relations and related plant physiological and biochemical activities of young Tongkat Ali (Eurycoma longifolia jack) plants were investigated. Plant vegetative growth was inhibited with reduced water availability. Leaf water potential and relative water content were reduced with increasing soil water stress. The rate of photosynthesis in Tongkat Ali leaves decreased in response to the stress treatments especially from moderate stress (75%FC), to severe stress (25% FC), compared with the control. Stomatal conductance of Tongkat Ali also steadily decreased with increasing the water stress Due to low water potentials and respectively reduce photosynthesis rate of Tongkat Ali therefore increasing stomatal diffusive resistance in the water deficit led to decline of transpiration rate in young seedling of *EURYCOMA LONGIFOLIA JACK*.

Keywords:
Eurycoma Longifolia Jack; Leaf Water Potential; Relative Water Content; Rate of Transpiration; Stomatal Conductance; Photosynthetic Rate

1. INTRODUCTION

Eurycoma longifolia Jack (Tongkat Ali, Genus: Eurycoma; Family, Simaroubaceae) is one of the most popular tropical herbal plants, indigenous to South-East Asian countries like Malaysia, Indonesia, and Vietnam. Some of the plant species are also found in certain patches in regions of Cambodia, Myanmar and in Thailand. This plant is locally known as Tongkat Ali wherein Ali means walking stick assigned due to the presence of long twisted roots. The plant extract, especially roots, are exclusively used (traditionally) for enhancing testosterone levels in men and also Tongkat Ali as same as another herbs is widely used as basic ingredient whether in traditional treatment or in modern medication.

Recently, there has been an increased attention in understanding the mechanism of plant acclimation to environmental stresses [1–3]. Plants respond to an adverse ecosystem by modifying their morphology, physiology, and biochemistry [2].

On the other hand **Figure 1** depicts that, between 1999 and 2001 alone, the sales value of herbal medicines in this group of countries increased by more than 40% growth in the sales of herbal medicines in a group of nine representative countries, 19992001 (Bhutan, Canada, the Czech Republic, Iran, Madagascar, Malaysia, Pakistan, Sudan, and Sweden).

Also the measurements for collecting Tongkat Ali from wild tropical forest for the commercial productions of traditional medicines at one stage will endanger and at least extinct. This herb has received a lot of attention among scientists, herbalists and the pharmacy industry in Malaysia due to It properties, therefore domestication of slow growing plant like Tongkat Ali is very crucial and also indispensable [4].

Studies were therefore undertaken to examine the growth, water relations and photosynthesis parameters in seedling of Tongkat Ali in three different of water levels.

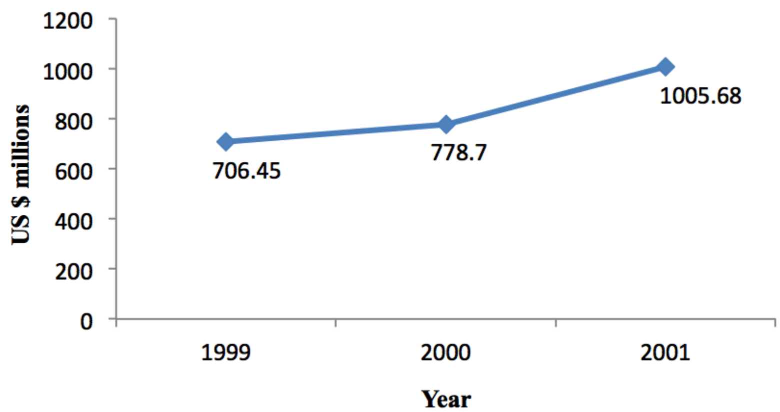

Figure 1. Sales of herbal medicine of nine representative countries.

2. METHODOLOGY

Experiments were conducted in the Greenhouse Unit in Ladang 10 at University Putra of Malaysia. The average daily air temperature in the greenhouse was 28°C to 30°C, with a relative humidity of 80-90% and also with 700-800 μmol/m^2/sec as irradiance intensity. Longitude 101°44' N and latitude 2°58' S, 68 m above sea level with mean atmospheric pressure 1.013 KPa. Six-month-old uniform seedling of Tongkat Ali, were planted in with poly bags. Each poly bag contained 6 kg of soil mix (top soil: Sand: Coco Peat as (organic manure), 3:2: 1).

A randomized complete block design (RCBD) 5×4 experiment was designed and undertaken for 6 months to determine the growth, water relations and photosynthesis parameters of Eurycoma Longifolia, under five levels of water treatments based on field capacity (125%FC; flooding water), (100%FC; well watered or control), (75%FC, moderate water stress), (50%FC; high water stress) and (25%FC; severe water stress).

It should be noted before conducting the experiment, soil analyzing was done and field capacity was measured by analytic laboratory of agricultural faculty of U.P.M. to measure the plant height and also stem diameter use by ruler and digital clipper respectively every 2 weeks.

Three to four young, healthy and completely expanded leaves were chosen from each treatment for the determination of leaf water potential, stomatal conductance and leaf photosynthesis rate. Leaf water potential was verified using a pressure bomb. In this study, the net photosynthetic rate (A) also stomatal conductance and transpiration rate were determined by a calibrated portable infrared photosynthesis system LI-6400 (LI-COR, Lincoln, NE, USA).All of the measurements were doing between 08:00 and 10:00 am.

To measure relative water content, separating the leaves of plant then, immediately after cutting them, the leaves were wrapped in aluminum foil, put in a plastic bag and kept in a cold room. Fresh weight was determined two hours after cutting. Turgid weight was determined by soaking the leaves in distilled water at room temperature (approximately 20°C to 24°C) for 16-18 hours; then, they were quickly and carefully dried by soft tissue; next, their fresh weight was determined by digital scale; and finally, their relative water content was calculated by a modification of the method of [5]:

$$RWC\% = \frac{FW - DW}{TW - DW} \times 100$$

Where, RWC, FW, DW and TW are relative water content, fresh weight, dry weight and turgid weight, respectively.

In this experiment water potential of leaves of Tongkat Ali also assessment. Water potential Ψw was measured every two mounts after imposing drought stress. It was measured between 11:00 AM and 01:00 PM because [6] showed that Ψw was reasonably stable during this period [7]. Measurements of Ψw were made by pressure chamber. Apressure bomborpressure chamberorScholander bombis an instrument with which it is possible to measure the estimatedwater potentialofplanttissues. Aleafattached to astemis placed inside a sealed chamber and pressurized gas is added to the chamber gradually as the pressure increases at some pointsapwill be forced out of thexylemand will be visible at the cut end of the stem. The pressure that is required to do so is equal and opposite to the water potential of the leaf [8].

3. STATISTICAL ANALYSIS

Data were exposed to analysis of variance (ANOVA), and means were compared using LSDs range test at P = 0.01. All calculations were performed with the help of the SAS software, version 9.2.

4. RESULT AND DISCUSSION

Figure 2 and **Figure 3** indicates the effect of water stress on plant height and stem diameter respectively. With the increase in water stress from 100 to 25% field capacity, stem length were reduced significantly.

The results showed application drought stress had marked effect on morphology characters such as plant high and herbage mass [9]. Similar responses have been reported in several other fruit trees including apple [10], peach [11] and papaya [12]. Under water stress conditions, plants lose their turgor and consequently cell expansion and growth are reduced the result in agreement with the finding of [13].

With the increase in drought stress from 100 to 25% field capacity, stem diameter were reduced steadily significantly (**Figure 3**). Under water stress conditions, plants lose their turgor and thus cell expansion and growth are reduced [13]. In [14], explained the reduction of stem diameter due to retardation in cell elongation instead of cell division.

Stem diameter due to an overall reduction in vascular tissues as an affected by water scarcity was reduce this finding is in agreement with the result of [15].

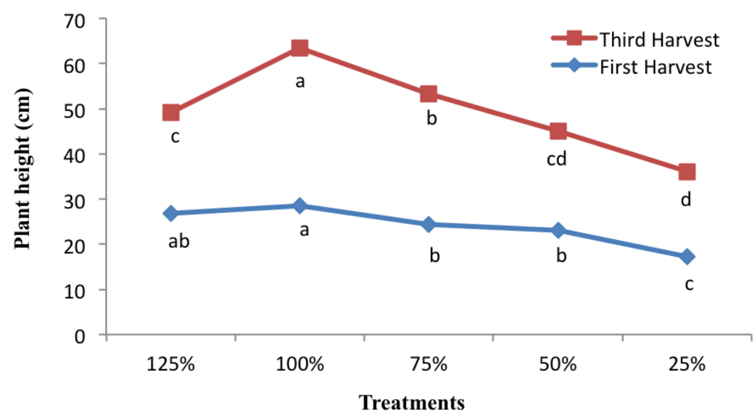

Figure 2. Plant height (cm) as affected by different water Levels (125%FC, 100%FC, 75%FC, 50%FC, 25%FC). Letters represent differences between means.

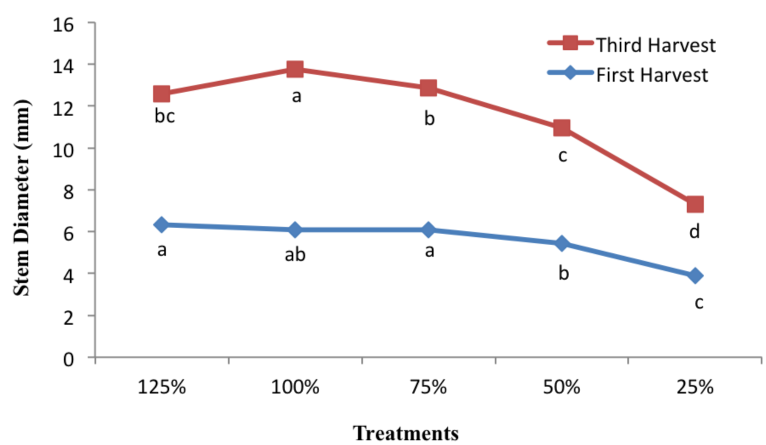

Figure 3. Stem diameter (mm) as affected by different water Levels (125%FC, 100%FC, 75%FC, 50%FC, and 25% FC). Letters represent differences between means.

Plant water relations are given in **Figure 4** and **Figure 5**. As follows from the **Figure 4**, leaf water potential (Ψw) under water stress conditions was significantly lower in 25% FC; it started to decrease under milder drought stress (75% FC and 50% FC). The researcher in [7], argue that water potential (Ψw) is considered to be a trustworthy parameter for measuring plant water stress response.

Current results disclosed that Ψw is dramatically decreased with the increasing water deficit. These results are in agreement with those [16] who find similar decrease of Ψw in alfalfa as a result of water scarcity. The changes in plant water potential might be attributed to a change in osmotic potential [7]. The results obtained by [17, 18], suggest that Water stress decreases the leaf water potential of the plants and it is considered a reliable parameter for quantifying plant water stress reaction.

Figure 5 depicts relative water content (RWC). This is illustrated in this figure, increasing water stress led to decrease the relative water content in Tongkat Ali. The author in [7] indicated that the declining Ψw and RWC were associated with lower stomatal conductance and photosynthetic rate and also Ψw measured under water stress conditions was significantly lower in water stressed plants. Evacuation of soil water from the root zone affects the plant water situation, thus affecting physiological and biochemical activities in the plant [19]. Decrease in RWC in plants under drought stress may depend on plant vigor decline and have been observed in many plants. Previous study conducted by [20], Is in agreement with this finding.

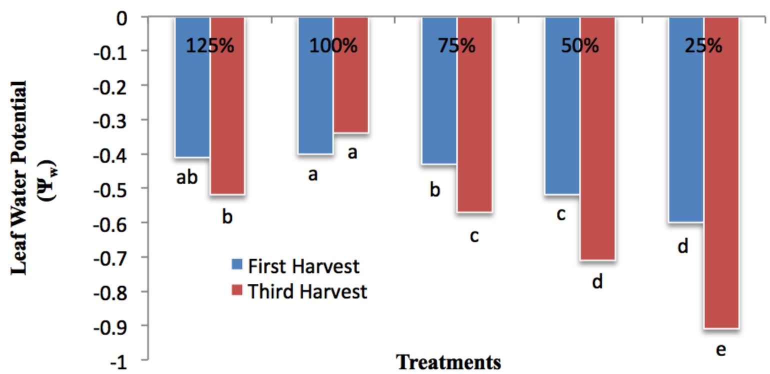

Figure 4. Water Potential (MPa) as affected by different water Levels (125%FC, 100%FC, 75%FC, 50%FC, 25%FC). Aitches represent differences between means.

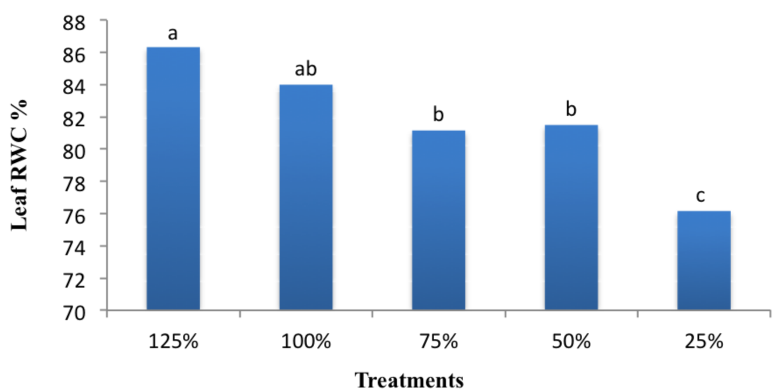

Figure 5. Leaf Relative Water Content (RWC %) as affected by different water Levels (125%FC, 100%FC, 75%FC, 50%FC, 25%FC). Aitches represent differences between means.

From the results, it can be concluded that water stress significantly altered the inner water status by decreasing RWC and also water potential. In severe stress the lowest amount of water relation belong to 25% FC due to stomatal closure.

Water deficit led to a striking ($P < 0.01$) inhibition in photosynthetic rate. The rate of photosynthesis in Tongkat Ali leaves decreased in response to the water treatments especially from moderate stress (75%FC), to severe stress (25% FC) (**Figure 6**). Many scientific researches show that in completely turgid leaves the net photosynthesis rate is higher than water stressed leaves [21]. Water stress substantially alters plant metabolism, decreasing plant growth and photosynthesis [22]. Critical threshold values of leaf water potential causing stomatal closure and reduction in photosynthetic rate [23]. for example: -0.6MPa

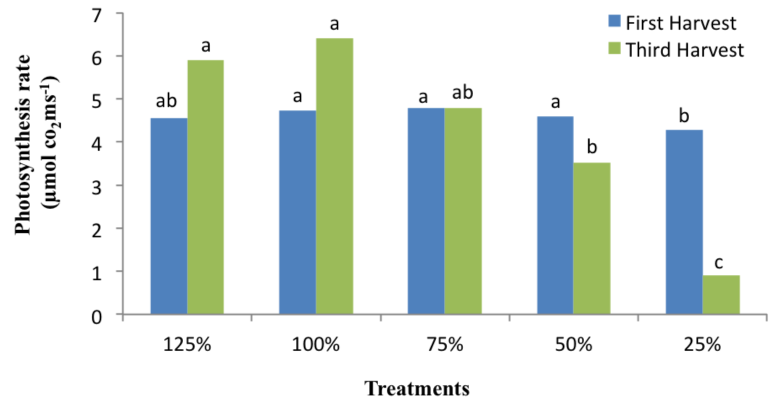

Figure 6. Photosynthesis rate (measured by LICOR 6400) at the 1st & 3rd harvest as affected by different water Levels (125%FC, 100%FC, 75%FC, 50%FC, 25%FC). Aitches represent differences between means.

for kiwifruit [24], -1.0 to -1.5MPa for macadamia [25] and -2.0 to -2.5MPa for more stress tolerant species such as sorghum [26].

Water scarcity led to a significant ($P < 0.01$) inhibition in stomatal conductance. Stomatal conductance of Tongkat Ali steadily decreased with increasing the water stress (**Figure 7**). Critical threshold values of leaf water potential causing stomatal closure and reduction in photosynthetic rate have been reported for a wide range of crops [14]. for example: -0.6 MPa for kiwifruit [15], -1.0 to -1.5MPa for macadamia [16] and -2.0 to -2.5MPa for more stress tolerant species such as sorghum [17].

Figure 7 indicates the effect of water stress on stomatal conductance of young seedling of Tongkat Ali. With the increase in water stress from 100 to 25% field capacity, stomatal conductance was reduced significantly. The results showed Stomata were highly sensitive to changes in soil water deficit [7]. The influence of water stress on stomatal response and photosynthesis rate has been extensively reported [18].

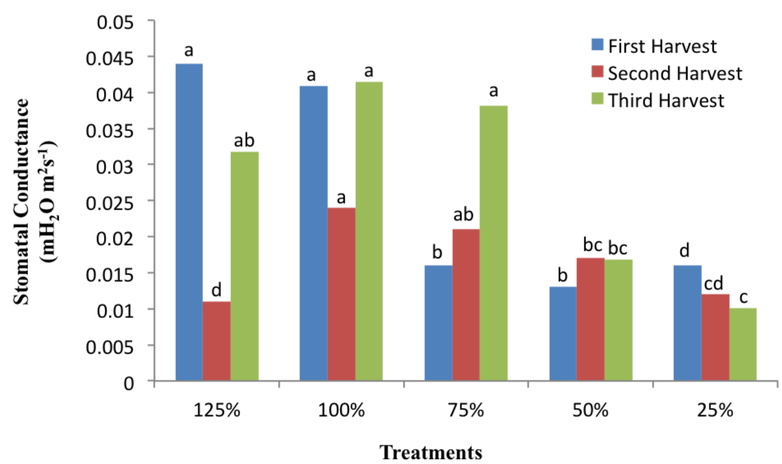

Figure 7. Stomatal conductance (measured by LICOR 6400) at the 1st, 2nd & 3rd harvest as affected by different water Levels (125%FC, 100%FC, 75%FC, 50%FC, 25%FC). Aitches represent differences between means.

The second reason that has a vital role to decrease the stomatal conductance under critical situation such as water scarcity is abscisic acid. Abscisic acid also affects the closing of stomata by influencing the movement of potassium ions out of guard cells (**Figure 8**).

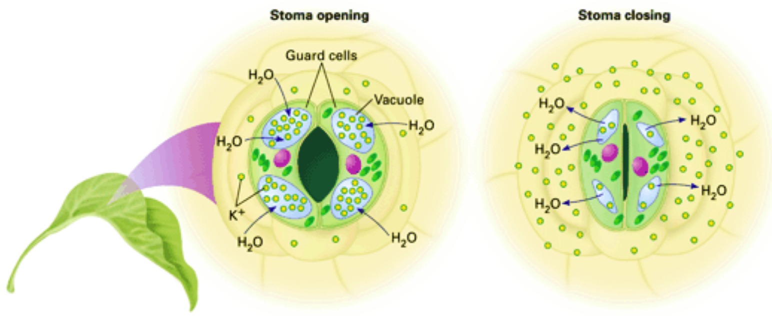

Figure 8. The effect of abscisic acid (ABA) to stomatal closure by influencing the movement of potassium ions out of guard cells.

From the result, it can be concluded that stomata normally adjust to water stress by closing in response to declining leaf water potential. Furthermore stomatal closure led to decline the rate of transpiration in plant respectively.

As follows from the figure shown below (**Figure 9**), with the increase in drought stress from 100% to 25% field capacity, Transpiration rate was reduced alternatively.

Transpiration rate revealed a decreasing trend throughout the growth period of the crop with increasing in leaf temperature and stomatal diffusive resistance in the water deficit [27].

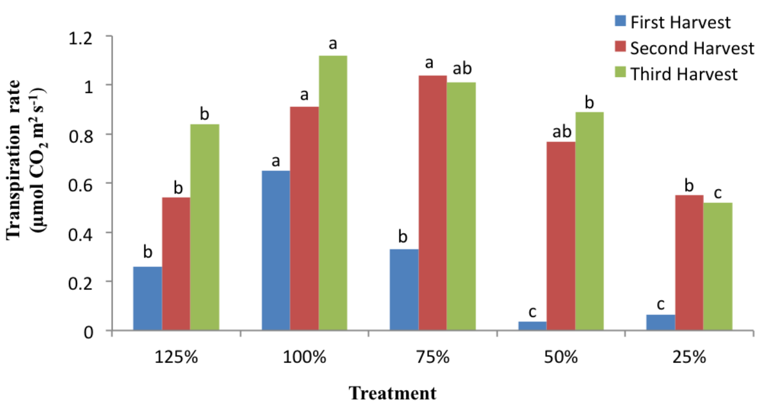

Figure 9. Transpiration Rate (measured by LICOR 6400) at the 1st, 2nd & 3rd harvest as affected by different water Levels (125%FC, 100%FC, 75%FC, 50%FC, 25%FC). Aitches represent differences between means.

In case of transpiration rate, the accumulation of the plant hormone Abscisic acid (ABA) under water deficit around guard cell can led to stomata closure, consequently can cause the reduction of transpiration rate in plant [28].

5. CONCLUSION

To domestication of Eurycoma longifolia, recognizing and also prognosticating water requirement of this superior herb has paramount role. Therefore five water treatments based on field capacity was conducted and measured the morphological and physiological traits of Tongkat Ali as below.

1. Stem diameter and shoot height reduced significantly with increasing the water scarcity and the results show that in 100% FC the diameter and height of shoot of the seedling is in high range and also the shortest and thinnest one belong to severe stress (25% FC).

2. Plant water relation consists of relative water content and leaf water potential. The lowest leaf water potential is belong to control treatment (100% FC) and mild stress (75% FC) respectively. It is important to say that in case of leaf relative water content there is not any significant difference between 100% FC and 75% FC but in terms of 125% FC and control, the 100% FC is the author recommendation.

3. In case of photosynthesis parameters, the photosynthesis rate was found to be higher in 100% FC and also 125% FC followed by 775% FC, 50% FC and lowest in 25% FC. Also in terms of stomatal conductance and transpiration rate it does not have any significant difference between 125% FC, control and 75% FC as mild stress and the lowest belong to 25% FC.

Hence based on the results of this research the best, economical and worst water proportion for treatment and domesticate of this superior herb are 100% FC, 75% FC and 25% FC respectively. It is worth to say that in terms of photosynthesis characteristic, it doesnt have any significant differences between 100% FC and 75% FC, therefore for conserving the water and also reduce the runoff water 75% FC is the authors recommend to farm this exclusive herb and Tongkat Alis domestication.

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