

Impact of Different Water Levels on Growth, Plant Water Relations and Leaf Characteristics 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 leaves characteristics 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 total leaf area and specific leaf area (SLA) in Tongkat Ali decreased in response to the stress treatments especially from moderate stress (75%FC), to severe stress (25% FC), compared with the control. Therefore by decreasing the SLA plant can preserve the internal water led to increase the tolerance of plant in case of water deficit condition.

Keywords—*Eurycoma Longifolia Jack*; Leaf water potential; Relative water content; Total leaf area; Specific leaf area; Photosynthetic rate.

I. 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 T.A 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 below Fig.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, 1999–2001 (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 T.A is very crucial and also essential [4].

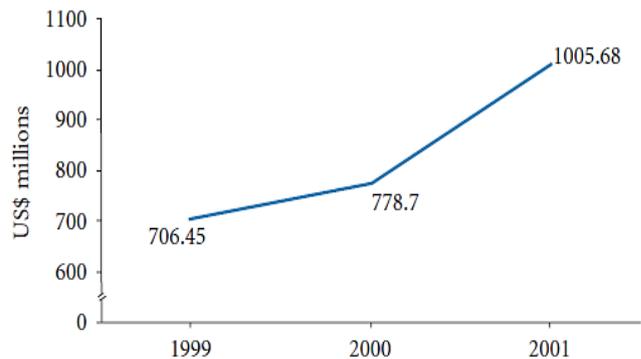


Fig. 1. Sales of herbal medicine of nine representative countries

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

II. METHOD AND MATERIALS

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 $\mu\text{mol}/\text{m}^2/\text{sec}$ 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 5 x 4 experiment was designed and conducted 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 laid out 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. Leaf water potential was verified using a pressure bomb.

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 Ψ_w was measured every two mounts after imposing drought stress. It was measured between 11:00 AM and 01:00 PM because [6] showed that Ψ_w was reasonably stable during this period [7]. Measurements of Ψ_w were made by pressure chamber. A pressure bomb or pressure chamber or Scholander bomb is an instrument with which it is possible to measure the estimated water potential of plant tissues. A leaf attached to a stem is placed inside a sealed chamber and pressurized gas is added to the chamber gradually as the pressure increases at some point sap will be forced out of the xylem and 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]. In this study, the leaves were separated into green and senesced leaves and leaf area measurements were made on the green leaves using leaf area meter (LICOR-3100). All of the measurements were doing between 08:00 and 10:00 am. The plant parts were then oven dried to a constant weight at 70 °C for a minimum of 48 h. SLA was calculated from the green leaf area as below equation: $SLA = \text{Leaf area}/\text{Weight of Leaf}$.

III. STATISTICAL ANALYSIS

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

IV. RESULT AND DISCUSSION

Fig. 2 and 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 (Fig 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].

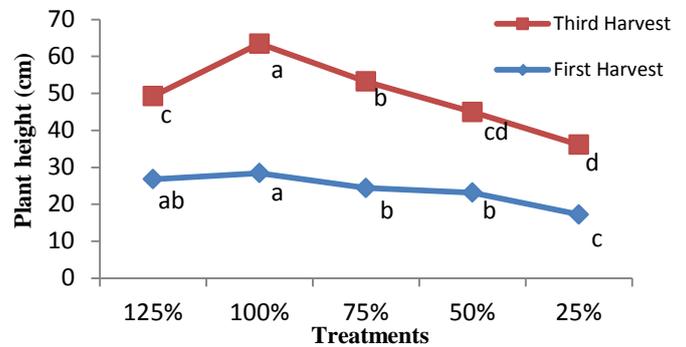


Fig. 2. Plant height (cm) as affected by different water Levels (125%FC, 100%FC, 75%FC, 50%FC, 25%FC). Letters represent differences between means

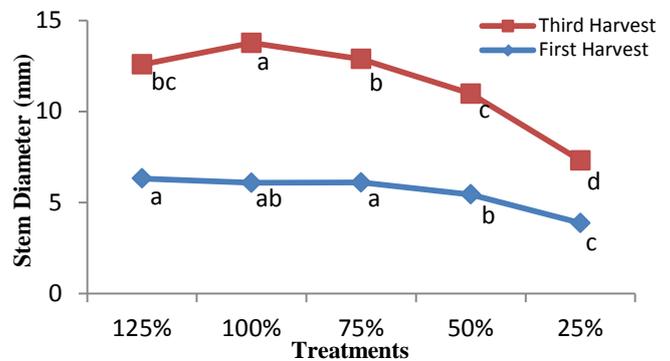


Fig. 3. Stem diameter (mm) as affected by different water Levels (125%FC, 100%FC, 75%FC, 50%FC, and 25% FC). Letters represent differences between means

Plant water relations are given in Fig 4 and 5. As follows from the Fig 4, leaf water potential (Ψ_w) 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 (Ψ_w) is considered to be a trustworthy parameter for measuring plant water stress response.

Current results revealed that Ψ_w is dramatically decreased with the increasing water deficit. These results are in agreement with those [16] who find similar decrease of Ψ_w 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.

Fig. 5 depicts relative water content (RWC). This is illustrated in this figure, increasing water stress led to decrease the relative water content in T.A. The author in [7] indicated that the declining Ψ_w and RWC were associated with lower stomatal conductance and photosynthetic rate and also Ψ_w 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.

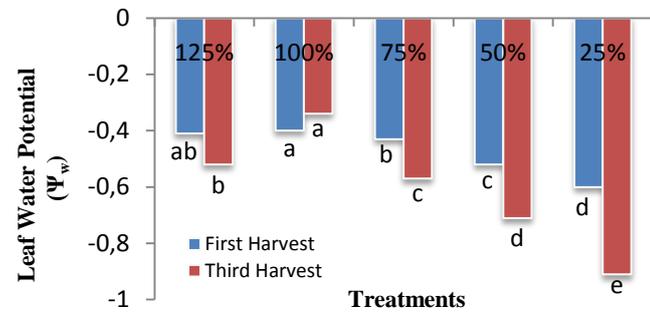


Fig. 4. Water Potential (MPa) as affected by different water Levels (125%FC, 100%FC, 75%FC, 50%FC, 25%FC). Aitches represent differences between means

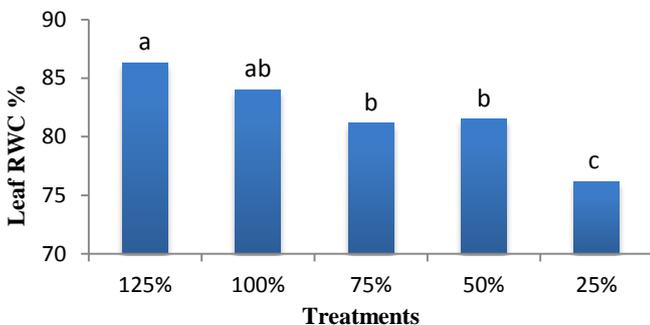


Fig. 5. Leaf Relative Water Content (RWC %) as affected by different water Levels (125%FC, 100%FC, 75%FC, 50%FC, 25%FC). Aitches represent differences between means

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.

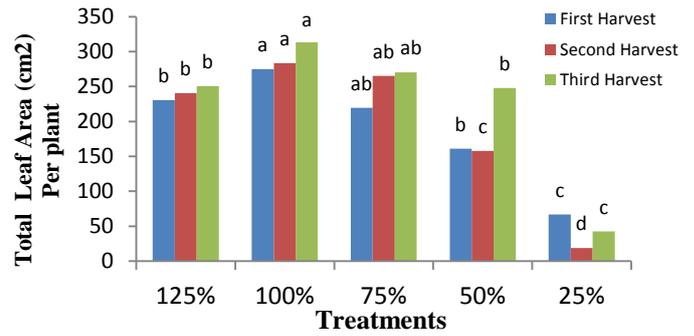


Fig. 6. Total Leaf Area as affected by different water Levels (125%FC, 100%FC, 75%FC, 50%FC, 25% FC). Letters represent differences between means. Each Harvest was analyzed separately, each column with same letter in each harvest show non-significant differences.

As follows from the figure shown above, with the increase in drought stress from 100 to 25% field capacity, LAI were reduced steadily significantly. The present result is in agreement with the findings of [21] in soybean, [22] in white clover, [23] in tobacco and [24] in maize where a decrease in Total Leaf Area was reported in stressed plants.

Under water deficit stress, leaf area was sharply reduced due a combination of leaf growth reduction and abscission [19] actually in agreement with the current finding.

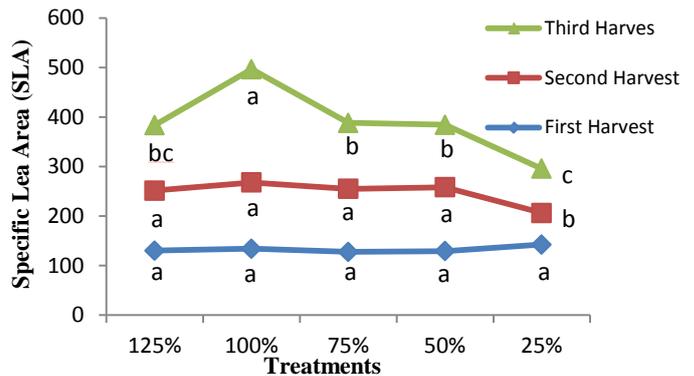


Fig. 7. Specific Leaf Area as affected by different water Levels (125%FC, 100%FC, 75%FC, 50%FC, 25%FC). Aitches represent differences between means. Each Harvest was analyzed separately, each column with same letter in each harvest show non-significant differences.

As can be seen from above fig, with the increase in drought stress from 100 to 25% field capacity, the lowest amount of SLA as for 25% FC with thicker leaves and the highest amount belong to 100% FC.

Specific Leaf Area (SLA) decreased in water deficit [25].

A reduction in the SLA is generally observed for grain legumes under water stress [26]; [27]; [28]. possibly indicating thicker leaves which aids in leaf water conservation & water loss reduction from the evaporative surfaces because of the lower surface/volume ratio.

V. CONCLUSION

To domestication of *E.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 leaf characteristics, the total leaf area was found to be higher in 100% FC and also 75% FC followed by 50% FC, 125% FC and lowest in 25% FC. Also in terms of SLA it does not have any significant difference between 125% FC, control and 75% FC as mild stress and even 50% FC as high stress especially in first and second harvest, meanwhile the lowest belong to 25% FC in second and third harvest.

Hence based on the results of this research the best, economical and worst water proportion for treatment and demonstrate of this superior herb are 100% FC, 75% FC and 25% FC respectively. It is worth to say that in terms of above plant characteristic, it doesn't 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 author's recommend to farm and domestication of Tongkat Ali.

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