

## Physiological and Morphological Responses of Four Tree Species Used in Dry Zone of Myanmar and Ubame Oak to Progressive Drought Stress

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**Summary :** Four tree species currently being used in dry zone greening of Myanmar and Ubame oak were grown in green house condition using two types of soil viz. clay and loam. Rapid drought stress conditions were imposed to the seedlings by withholding water entirely. The growth, soil water content, electron transport rate and transpiration rate measurements were conducted for 100 days. The obtained parametric values were used to compare the drought tolerance of the species, and also to study their responses to drought stress. Ubame oak could avoid water deficit due to its slow growth rate and low transpiration rate per leaf area. Among the species used in dry zone, *Eucalyptus camaldulensis*, *Acacia catechu* and *Acacia auriculiformis* exhibited the same pattern of physiological responses to drought stress and could survive, while *Cassia siamea* failed to survive 100 days of drought stress. *A. catechu* with moderate growth rate in growing period showed physiological and morphological adaptations to overcome prolonged drought stress.

**Key words :** Drought stress, Drought tolerance, Electron transport rate, Transpiration rate

### Introduction

Although 50 % of the total land area of Myanmar is still covered with vast and diverse forests, its central part is barren and no forest cover, threatening desertification. This central dry zone occupies 10 % of the total land area of the country, and is a vast semi-arid low land surrounded by higher mountain ranges. Dry zone area gets the average annual rainfall of about 720 mm, compared to that of 2,300 mm for the whole country. Some places receive less than 500 mm of annual rainfall (Myint 1995). The rainfall is nearly zero from December to the end of March. Although the average mean temperature is about 27 °C, the temperature often rises to above 40 °C

in the hot season. Various environmental degradations are now observed in this region as a result of deforestation, population pressure, fuel wood demand, over-cultivation and overgrazing.

At present, the dry zone area of Myanmar is being rehabilitated by two different methods: plantation establishment and conservation of remaining natural vegetation. For the plantations, the planted seedlings have to overcome four months period of severe drought every year. This drought condition contributes one of the major barriers to successful plantations, especially in newly established ones. For successful establishment of plantations, the practice of irrigation, use of drought tolerant species and manipulation

of soil conditions for effective water retention (eg. optimum soil volume, use of input materials to improve soil water retention) are important considerations (Oo and Nakao 2002).

Two exotic species (*Acacia auriculiformis* A. Cunn. ex. Benth. and *Eucalyptus camaldulensis* Dehn) and two indigenous species (*Acacia catechu* Willd. and *Cassia siamea* Lam.) shared 80 % of the dry zone plantation area. Urame oak (*Quercus phylliraeoides* A. Gray.) naturally occurs at coastal areas in southern part of Japan (from Okinawa to western part of Honshu island) and is planted for charcoal and as ornamental tree. Judging from its natural distribution and leaf morphology (small, hard and bright leaves), it is said to be tolerant to drought.

This experiment aims to compare the drought tolerance of five tree species and their water use, to study the effect of soil types on their growth, to study the seedling responses to drought stress and to identify drought tolerant species suitable for dry zone greening programs in Myanmar.

## Materials and Methods

In February 2002, the seeds of *Acacia auriculiformis*, *Eucalyptus camaldulensis*, *Acacia catechu* and *Cassia siamea*, collected from central dry zone of Myanmar and *Quercus phylliraeoides*, collected from Miyazaki University campus were germinated in green house of Miyazaki University. At the start of June, eight seedlings from each species were transplanted to 13.5 liter pots (15.5 cm in diameter and 71.5 cm in height) filled with two types of soils (loam and clay). Slow release fertilizers were applied to all the pots to ensure that the seedlings suffered no nutrient deficit during experiment period. Every pot was irrigated until August 4 by pouring water of about 90 mm per month, which is the average monthly rainfall during the growing season of dry zone in Myanmar. From August 4, rapid drought stress conditions were imposed to half the number of seedlings by withholding water

entirely. All the physiological measurements and growth measurements were conducted from August 4 to November 13 (100 days).

Mean daily temperature from August to November in greenhouse was 29.5 °C, and mean daily maximum and minimum temperatures were 40.7 °C and 23.5 °C respectively. Average relative humidity was 74.7 %.

The seedling height (H) and stem diameter (D) of all the seedlings were measured every month. The square of the stem diameter (D<sup>2</sup>) multiplied by the stem height was used as a surrogate for total seedling biomass (Elliot and Vose 1994). The leaf area of every seedling was also measured monthly by using LI-3000A Portable Area Meter (LI-COR, inc., USA) without damaging any leaf.

ECH<sub>2</sub>O Dielectric Aquameters (Decagon Devices, Inc., USA) were inserted at 50 cm depth of the pots to measure soil water content. The water potential was determined by soil water content versus soil water potential curve, which was obtained by the centrifuging procedure.

Electron Transport Rate (ETR) measurements were conducted using Portable photosynthesis yield analyzer, MINI-PAM (Heinz Walz, Germany). The photosynthetically active photon flux density (PPFD) was determined at the site of the fluorescence measurement by the machine. The ETR was calculated using the following formulae (Schreiber *et al.* 1995 ; Mohammed *et al.* 1995) :

$$ETR = 0.84 \times 0.5 \times PPFD \times (\text{Fluorescence yield})$$

$$\text{Fluorescence yield} = (F_m' - F_s) / F_m'$$

Where ETR = electron transport rate ( $\mu\text{mol electrons m}^{-2}\text{s}^{-1}$ ),  $F_m'$  = maximum fluorescence attained on application of the pulse,  $F_s$  = fluorescence at the steady state. In this experiment, the light curves were drawn from PPFD and ETR of the measured leaves, and calculated ETR at 1000 PPFD were used to compare the photosynthetic ability of the seedlings as described by Schreiber *et al.* (1995).

Transpiration Rate (TR) measurements were

made by using LI-1600 Steady State Porometer (LI-COR, inc., USA). Two representative leaves from each seedling were measured every week for both ETR and TR measurements. The selected leaves showed no symptom of senescence. Both measurements were done in the same days under natural light until all the leaves of the seedling became permanently wilted.

## Results and Discussion

### Growth responses

The drought stress reduced the growth parameters of the seedlings. The leaf area and growth increment of each species under stressed condition were much lower than those of controlled condition (Fig.1 and 2). Under the drought stressed condition, effect of soil type on

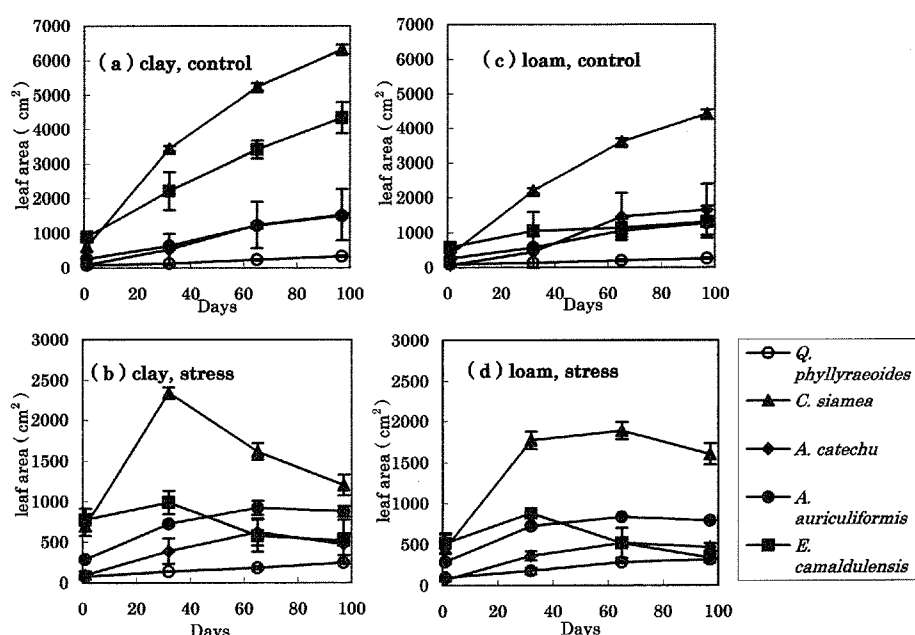


Fig. 1. Leaf area expansion of five species under water controlled and stressed conditions in two soil types.

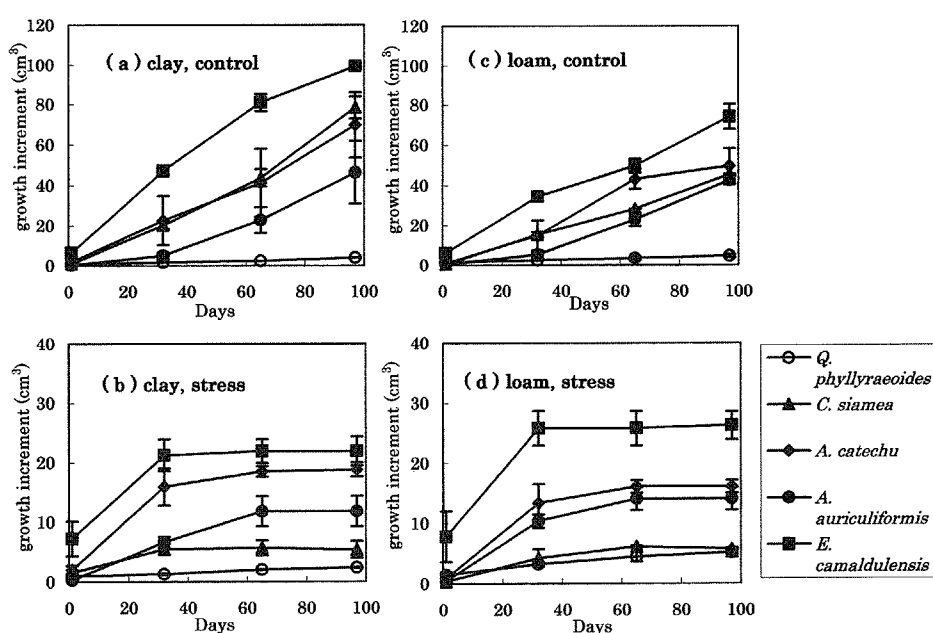


Fig. 2. Growth increment of five species under water controlled and stressed conditions in two soil types.

leaf area and growth increment was not observed.

*C. siamea* exhibited largest leaf area in all conditions (Fig. 1), but highest growth increments were observed in *E. camaldulensis* (Fig. 2).

*Q. phylllyraeoides* had lowest leaf area and growth increment under all conditions. *A. catechu* showed some morphological responses to drought stress by folding up of the leaflets even in the day time. It was inferred that the folding of the leaflets reduced the exposed leaf area, which in turn reduced the water loss from the leaves.

Under the drought stress condition, leaf abscission and growth cease were observed in *C. siamea* and *E. camaldulensis* after 30 days (Fig. 1 and 2). *A. catechu* and *A. auriculiformis* showed leaf abscission and growth cease after 60 days of the drought stress. However, *Q. phylllyraeoides* continued the leaf area expansion and growth increment up to 100 days of drought stress though the amount was too small.

Leaf area was known to be sensitive to soil water availability (Gholz 1982) and the seedlings responded the drought stress with decreased number of leaves per plant (Arndt *et al.* 2001) and smaller leaf area (Muraoka *et al.* 2002), and hence reducing the growth. Early leaf abscission and growth cease of *C. siamea* and *E. camaldulensis* indicated that these two species suffered water deficit in their growth metabolisms earlier than other species.

### Soil desiccation rate

In both clay and loam soils, the lowest soil water potentials were observed in *E. camaldulensis* and the highest in *Q. phylllyraeoides* (Fig. 3). Change of soil water potential at 50 cm depth of the pots reflected the water use of the species. *E. camaldulensis*, with highest growth increment, exploited more water from the soil at all the time of the experiment. *C. siamea*, with largest leaf area, used more water in first 30 days of the drought condition. But, after the cease of growth increment and leaf area expansion, soil desiccation rate of this species became slower. *A. catechu* and *A. auriculiformis* showed lowest values in the first 30 days, whereas, the soil water potentials became lower as they increased their growth during 60 days of the experiment. *Q. phylllyraeoides*, with lowest growth increment and smallest leaf area used less water from the soil. For each species, the soil water potentials in clay soil were lower than those in the loam soil. It could be due to the higher evaporation rate of clay soil surface, as the evaporation from surface soils can increase with increase in the percentage of fine-textured materials (Fisher and Binkley 2000).

### Physiological process responses

Chlorophyll fluorescence analysis has been used successfully with trees in many practical forestry applications. These include investigations on the effects of high and low temperature,

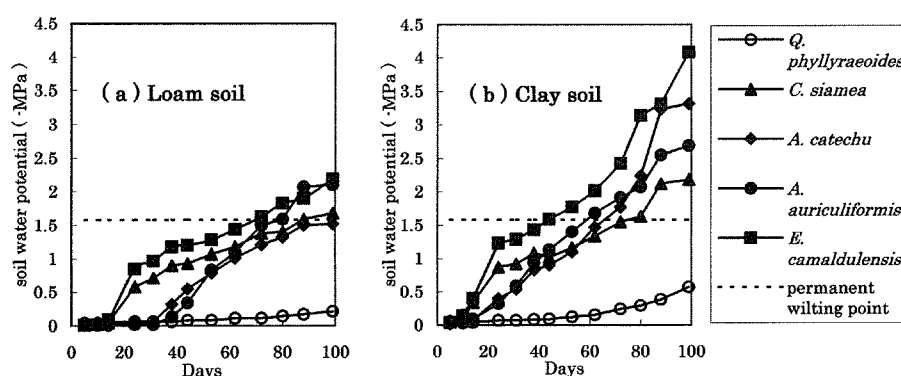


Fig. 3. Soil desiccation rates of five species at 50 cm depth in (a) loam soil and (b) clay soil under 100 days of drought stress.

drought stress, nutrient deficiency, disease, herbicides, and air pollution (Mohammed *et al.* 1995; Binder *et al.* 1997). In this experiment, chlorophyll fluorescence measurements, associated with transpiration rate measurements were used to assess the physiological process responses of the seedlings to drought stress.

**Electron Transport Rate:** The seedlings responded the drought stress with lower light curves as water deficit became severe (Fig. 4). In all dry zone species, ETR values were lowest at 100 days of the drought stress. ETR values of *C. siamea* were zero at 100 days of experiment, indicating that its photosynthetic functions were seriously damaged by the drought stress (Fig. 4b).

ETR at 1000 PPFD of *Q. phyllireoides* were nearly the same from beginning to the end of the experiment (Fig. 5e). *E. camaldulensis*, *A. auriculiformis* and *A. catechu* responded the drought stress with the same pattern. ETR between controlled and stressed seedlings were not different up to 40

days of the experiment. But after 40 days, ETR appeared to decline permanently as a result of severe water deficit (Fig. 5a, 5c and 5d). In *C. siamea*, early declinations of ETR were observed first in clay soil seedlings and then in loam soil (Fig. 5b). It indicated that photosynthetic ability of this species was damaged by drought stress earlier than other species. Some small peaks of ETR values could be observed in *A. catechu* even after 60 days of the drought stress (Fig. 5d). It might be because of its adaptation to severe drought stress. Its roots could effectively absorb available water from the deeper part of the soil and provide some amount of water for seedling physiological processes.

Some authors already reported lower net photosynthesis values (Arndt *et al.* 2001; Fang *et al.* 1996) and decreased ETR values (Li and Kakubari 2001; Higuchi *et al.* 2001) under drought stressed conditions. In this experiment, the results of ETR measurement were consistent

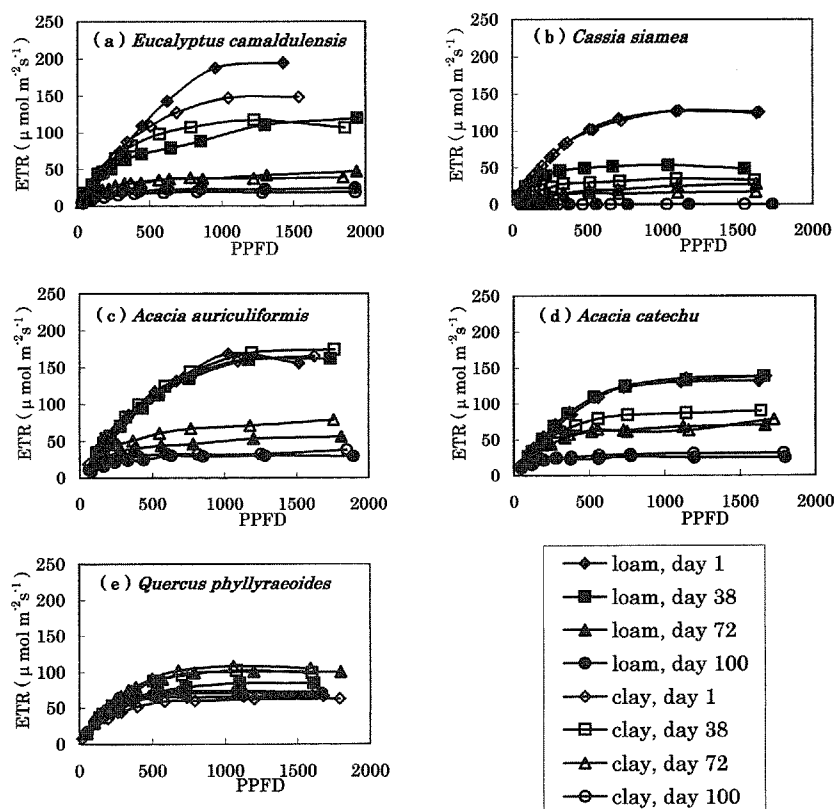


Fig. 4. Light curves of the stressed seedlings in two soil types after 1 day, 38 days, 72 days and 100 days under drought stress.

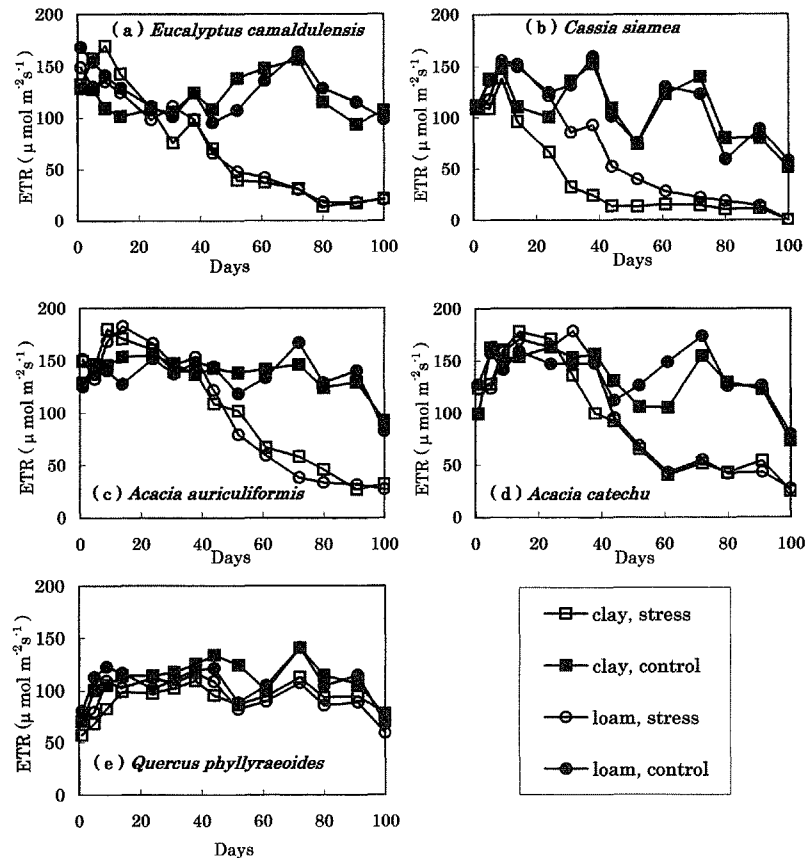


Fig. 5. Comparison of electron transport rates (at 1000 PPFD) of the seedlings under water controlled and stressed conditions in two soil types.

with the above reports. ETR between controlled and stressed seedlings were not different at the early stage of drought stress. But they became significantly lower and declined to nearly zero as water deficit became severe.

The permanent decline of ETR values in stressed condition could be considered due to a decrease of water molecules in the leaves. Limited availability of water molecules in leaves made the electron transport process lower and formation of NADPH and ATP were seriously damaged as water stress became severe. The water stress affected photosynthetic capacity by reduction of electron transport in PSII of chloroplasts (Potter and Boyer 1973), inactivation of the primary photochemistry of the PSII reaction centre complex, and reduction of ATP (Powles and Critchley 1980).

**Transpiration Rate:** Drought stress reduces the transpiration rates of the seedlings (Seiler and

Johnson 1985; Edwards and Dixon 1995). When the seedlings suffer water deficit in their growth metabolism, they open stomata only partially to reduce water loss from the leaf surface. In this experiment, TR in all species declined sharply just after 20 days of drought stress (Fig. 6). TR of controlled seedlings showed gradual declination with some peaks but never reached to zero, while those of stressed seedlings finally declined to zero. In *E. camaldulensis* and *C. siamea*, clay soil seedlings showed faster TR declination than loam soil seedlings. The early decline of TR values could also be considered because of carbon allocation of the seedling metabolism. At early stage of drought stress, carbon was remobilized from leaves and preferentially redistributed to stems and roots, and leaf senescence reduced whole plant transpiration and enforced dormancy.

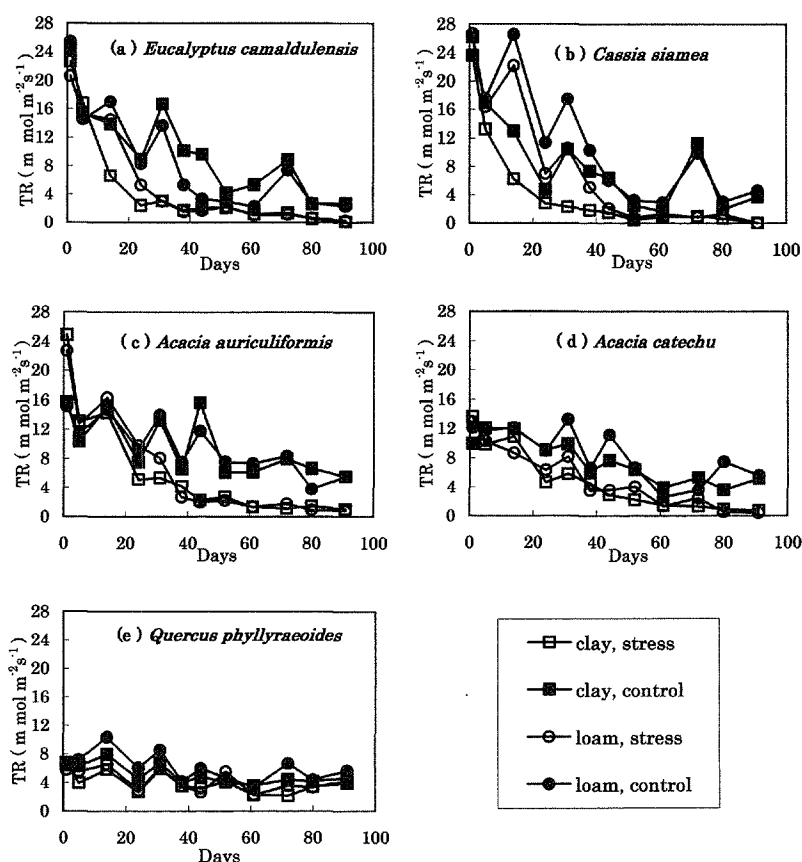


Fig. 6. Comparison of transpiration rates (TR) of the seedlings under water controlled and stressed conditions in two soil types.

### Species responses to drought stress

*Q. phylliraeoides* with slow growth rate and low transpiration rate per leaf area was able to avoid water deficit induced by drought stress. Its physiological and morphological processes were functioning well after 100 days of drought stress. Its soil water potential never reached to permanent wilting point. However, its drought tolerant ability in low soil water potentials still have to be studied.

*Acacia catechu*, having a moderate growth rate, small leaf area and low transpiration rate per leaf area, exhibited some morphological changes (e.g. folding up the leaves to reduce leaf exposed area). It also showed some physiological adaptations (e.g. recovering ETR values to some extent after 60 days of drought stress) and had a relatively low water demand. This species could be considered having a potential to overcome prolonged drought stress.

*E. camaldulensis*, with highest growth and higher transpiration rate, exploited much water from the soil. Its early leaf abscission could be considered because of carbon allocation from the leaves to other parts of the seedling for further use (eg. sprouting when the water budget became favorable). It still exhibited low ETR at the end of experiment and could survive 100 days of the drought stress.

*A. auriculiformis*, with lower growth rate and smaller leaf area, showed a lower water demand for the first part of drought stress. Its physiological processes were also functioning with small values and survived 100 days of the drought stress.

*C. siamea*, with largest leaf area and higher transpiration rate per leaf area, showed leaf abscission and growth increment ceased earlier. It consumed more water for its growth in first stage. Its physiological processes were observed to

decline earlier than other species, indicating that it wouldn't survive 100 days of drought stress. Early leaf abscission and growth cease couldn't be regarded as adaptations to drought stress, because its electron transport functions were also damaging at the same time.

In controlled condition, all the seedlings showed better growth increment in the clay soil than those in the loam soil. But in stressed condition, clay soil seedlings showed sharp decline of ETR and TR values earlier than those in loam soil. It showed that the poor capability of the seedlings to uptake water from the clay soil could reduce the drought tolerance ability. In addition, the high water loss due to the surface evaporation from the clay soil could also reduce the amount of available water for the seedlings.

## Conclusion

Water stress is one of the most important stress factors to plant growth in arid and semi-arid areas. To overcome prolonged water stress and hostile environments, the planted species must possess ability to maximize water uptake (eg. tapping ground water by deep roots), to minimize water loss (small leaf area, low transpiration rate, stomatal closure), and to adapt physiologically and morphologically to water deficit. It was evident that prolonged drought stress could seriously damage the seedling growth and physiological processes. Water loss from the soil surface by evaporation was not the only cause of the water deficit in prolonged drought stress. In this experiment, *Q. phylliraeoides* could attain enough water from the deeper part of the soil for its growth, and its water budget never reached to critical state in 100 days of the drought stress. Water loss from the seedlings through their metabolism (transpiration, respiration) also accelerated the water deficit in drought stress condition. *A. catechu* with low transpiration rate and smaller leaf area could minimize the water loss from the seedling and the soil, hence showing effective tolerance to prolonged

drought stress. The species with high transpiration rate, larger leaf area and high growth rate need more water to support their growth conditions eg. by increasing the size or depth of the pits and/or by irrigation methods.

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## ミャンマーの乾燥地域に植林される4樹種とウバメガシの乾燥ストレスに対する生理的及び形態的反応

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### 要 約

ミャンマーの乾燥地域の緑化にはどのような樹種が適しているかを明らかにすることを目的に研究を行った。

ミャンマーの乾燥地域で植栽される4樹種(郷土種2樹種 *Acacia catechu*, *Cassia siamea*, 導入種2樹種 *Acacia auriculiformis*, *Eucalyptus camaldulensis*)とウバメガシ(*Quercus phylliraeoides*)を2種類の土壌(埴土および壤土)でポットに植付け、ガラス室で生育させた。活着後、完全に灌水を止めて苗木を急激な乾燥ストレス条件下に置き、100日間の成長、土壌水分変化、生理的反応を測定し、乾燥ストレスに対する反応から5樹種の耐乾性を比較した。乾燥初期の生理的反応は埴土で生育している苗木に見られた。ウバメガシは成長を抑え単位葉面積当りの蒸散速度を小さくすることで水不足を回避していた。生育期中程度の成長速度を示す *Acacia catechu* は長引く乾燥ストレスに耐えるような生理的及び形態的適応を示した。

**キーワード:** 乾燥ストレス, 耐乾性, 電子伝達速度, 蒸散速度