

## Water Consumption of Greenhouse Lychee Trees under Partial Rootzone Drying

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### ABSTRACT

Litchi (*litchi chinensis*, Sonn.) is one of the predominant fruit crops in upland areas of Northern Thailand, where it is produced during the dry season under irrigation. As water is an increasingly scarce resource on the hillsides, strategies for more efficient water use are fundamental for a sustainable increase in agricultural production. Partial rootzone drying (PRD) is an innovative irrigation technique which is presumably based on the induction of changes in the plant hormonal balance. By targeted withdrawal of irrigation water a hormonal change and chemical signals are produced, which make the plant reduce its stomatal aperture and thereby decrease water consumption.

To test the response of litchi to PRD, 30 trees were exposed to different irrigation treatments under controlled conditions. The trees were grouped in three groups with the following irrigation treatments: a.) full irrigation, b.) PRD with 50% of full irrigation, changing the irrigated sides every two weeks c.) no irrigation. The trees were watered in a three days interval. Water consumption was measured gravimetrically. Soil moisture was controlled with TDR probes. Twice a day the stomatal resistance has been measured with a portable porometer. At three dates leaf samples have been analyzed on their amount of abscisic acid (ABA) content.

It was possible to influence the hormonal balance of litchi trees in the long term by PRD, reaching a rather constant level of ABA and, thereby, alter water consumption. PRD has to be applied to bearing litchi trees in order to complete these findings by the yield component.

**Keywords:** Irrigation, drought stress, *Litchi chinensis*, abscisic acid, ABA, PRD

## 1. INTRODUCTION

In upland areas of Northern Thailand, litchi is one of the predominant fruit crops. The flowering of the trees presumably depends on a change in the hormonal balance induced through low temperatures which are only reached during the cold dry season in December and January (Chatrakul, 2005). Fruit development takes place during the hot dry season (January until June). Therefore, high litchi yields can only be obtained with irrigation. As water is an increasingly scarce resource on the hillsides (Neef *et al.*, 2004), strategies for more efficient water use are fundamental for a sustainable increase in agricultural production. An important objective of irrigation demand management is to reduce water demand through selection of low demand crop varieties or crop patterns, and adopting deficit irrigation, i.e. deliberately allowing crop stress due to under-irrigation, which is essentially an agronomic and economic decision (Pereira, 2005). While an irrigation which avoids drought stress is appropriate to increase yields per area, slightly deficient irrigation can maximize the yield per unit of water (Ewemoje, 2007). Including economic models, such as presented by El Amani *et al.* (2001) in irrigation scheduling of horticultural crops can be a way to increase water use efficiency (WUE).

Deficit irrigation is a strategy to increase (WUE) by an altered allocation of water resources. As it is mainly an allocation strategy, it deliberately takes lower crop yields into account. Furthermore, low application depths wetting only a shallow soil surface layer and not favoring deep rooting, increases the risks for crop failure (Rodrigues *et al.*, 2001). Unlike that, partial rootzone drying (PRD) is an innovative irrigation technique which is thought to reduce plants' water consumption based on the induction of changes in the plants' hormonal balance and chemical signaling of roots in the drying soil (Davies *et al.*, 2000, 2002). To stimulate these responses, under PRD one side of the root system is not irrigated and falls dry, while the other is well watered. In the drying part of the roots a high amount of abscisic acid (ABA) is produced which makes the plant reduce its water consumption. On the irrigated side the root system can take up water to maintain fruit growth, while vegetative growth is reduced due to the stress reaction of the plant (Dry *et al.*, 1996, 2000). Thus, fruit yield is widely unaffected or only slightly reduced and WUE can be increased massively. This has been described for a variety of tree crops, such as grape-wine (Dry *et al.*, 2000), apple (van Hooijdonk *et al.*, 2004), pear (Kang *et al.*, 2002), citrus (Hutton, 2000), mango (Spreer *et al.*, 2007), raspberries (Grant *et al.*, 2004) and olives (Wahbi *et al.*, 2005). However, the exact functioning of PRD is not well understood and different plant species react in different ways to the spatial limitation of water under PRD. No study on the hormonal response of litchi to PRD has been published so far. Longan (*Dimocarpus longan*, L.) was the closest related tree species that has been studied (Satieperakul *et al.*, 2006). It was reported that WUE of PRD irrigated longan trees, considering quantity of yield and the quality of the fruits was significantly higher as compared to unstressed trees. The economic viability of PRD as a practical alternative for farmers in Thailand has been demonstrated in the same study. Another study on PRD irrigation in mango, carried out in the same area, showed that the specific cost for irrigation per fruit tree are only slightly higher than for conventional micro-irrigation systems (Spreer and Koeller, 2005).

The hypothesis for the present study was that litchi trees – similar to other tree species – would react on the alternate irrigation with an increased production of ABA and consequently close the stomata to lower the water consumption. Through the changing of the irrigated and non-irrigated side this high level of ABA would be maintained to obtain constantly a lower

water consumption. To prove this hypothesis would support further field experiments with bearing litchi trees in order to calculate WUE.

## 2. MATERIAL AND METHODS

### 2.1. Plant Material

30 litchi trees (*litchi chinensis*, var. Hong Huay) were planted in split root technique half a year previous to the start of the experiment, using two rectangular based pots glued together (2 x 5.8 L). As substrate a combination of sand (25%), TKS II (25%) and Filder loam (50%) was selected. At the time of the experiment the trees were two years old and in their juvenile phase. Thus, they did not bear fruits why, consequently, no WUE could be calculated in this experiment. The trees have been cut to a uniform height of 80 cm previous to the experiment and were kept under controlled conditions of 25 - 28° C and RH 70% in a greenhouse at the University of Hohenheim, Stuttgart, Germany.

### 2.2. Irrigation Treatments

The water demand of the trees was determined previous to the experiment. The trees were kept well watered and water consumption was measured by weighing the entire pots using an electronic balance prior to watering. Measurements were taken in a three-day interval. The pots were covered by a plastic sheet in order to reduce evaporation to a negligible level. The average water consumption over two weeks was 140 g/day. This was assumed to be the optimal water supply for the trees. No correction factor due to climate was foreseen, for the constant environmental conditions in the greenhouse.

The experiment started on July 19<sup>th</sup> 2004. The 30 trees were split in three groups and subjected to the following irrigation treatments: a) full irrigation (FI): 70 g water per pot = 140 g day<sup>-1</sup> tree<sup>-1</sup>; b) partial rootzone drying (PRD): 70 g water in one pot, no irrigation in the other pot, the wet and dry side were changed every two weeks; c) no irrigation (NI). This treatment was stopped for each tree at the moment when it showed visual effects of drought stress (e. g. leave rolling, necrosis). Irrigation was performed every three days manually to guarantee the exact amount of water. Due to an extraordinarily hot summer with outside temperatures exceeding 35° C it was not possible to maintain the temperature below 30° C in the greenhouse and the water demand of the litchi trees increased. So, on August 1<sup>st</sup> 2004, the irrigation rate was adjusted to be 160 g day<sup>-1</sup> tree<sup>-1</sup> for FI and 80 g day<sup>-1</sup> tree<sup>-1</sup> for PRD. The experiment was stopped on August 28<sup>th</sup> 2004 and all trees subsequently watered as in FI. Water consumption measurements continued until September 20<sup>th</sup> 2004.

### 2.3. Measurements

The water consumption of all trees was determined gravimetrically by weighing the pots. If measurements were taken on the same day as the irrigation it was measured prior to watering. Measurements took place in different intervals between one and four days. Subsequently the average daily water consumption during the measurement interval was calculated. Irrigation was performed manually in a three day interval. Weight increase due to plant growth was neglected, as only a little amount of trees flushed during the time of experiment and the overall weight of the young leaves was minor as compared to the transpired water.

The stomatal resistance was measured by use of a portable porometer (AP4, Delta Devices, Cambridge, UK). As stomatal resistance changes in the course of the day and especially high values in the afternoon are a good indicator for plant drought-stress, measurements took place

twice per day in the morning starting from 9:00 am and in the afternoon starting from 4:00 pm. For each plant three leaves of the last matured flush were randomly selected and marked, so that measurements were always carried out on the same leaves.

Soil moisture was controlled by Time Domain Reflectometry (TDR) using a cable tester (Tectronix 502C, Tectronix, Beaverton, USA) and a TDR three-rod probe (rod Ø: 1 mm, rod length: 8 cm) by a single insertion in each pot in a three-day interval.

At three dates the concentration of ABA was measured: The first samples were taken on July 29<sup>th</sup>; the second two weeks later, on August 12<sup>th</sup>, when most of the trees in the NI group showed visual effects of drought stress; the last on August 26<sup>th</sup>, when there were no trees left in the NI treatment. Two leaves of the last matured flush were taken from the same branch and frozen in liquid nitrogen. The leaves of two trees of the same treatment were added to one sample. Then the samples were lyophilized and homogenized together with solid CO<sub>2</sub>. For each sample 0.3 g of dry mass were extracted over night in 50 ml of cold methanol (80%). The solvent was evaporated at 40° C under vacuum. Then the samples were resolved in 12 ml of 0.01M ammonium acetate (pH 7.5) and frozen in centrifuge beakers over night to

crack the membranes of small cell organelles. Then the extracts were centrifuged at 60 000 g for 20 minutes and subsequently cleaned using a combination of polyvinylpyrrolidone (PVP), Sephadex (anion exchanger) and Sep-Pak C<sup>18</sup> columns. Sample fractions eluted from the Sep-Pak C<sup>18</sup> columns were split in triplicates and dried in a vacuum concentrator over night. Diazomethane was used for methylation and the quantitative analysis was carried out via radio immunoassay (RIA) by use of specific antibodies raised against ABA. Data presented figured in the lower aliquot.

### 3. RESULTS

#### 3.1. Water Consumption

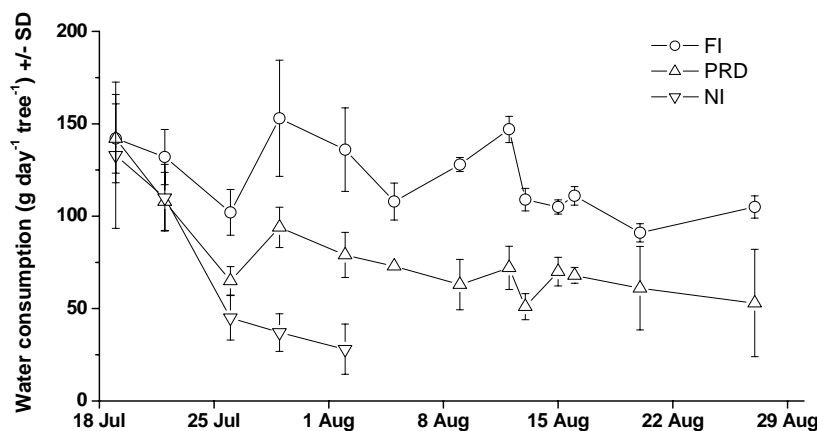


Figure 1: Average water consumption of trees with full irrigation (FI), partial rootzone drying (PRD) and without irrigation (NI).

Under conditions of drying soil, initially both treatments NI (drying out on both sides) and PRD (drying out on one side) reacted similarly with a decrease in water consumption, which stabilized under PRD to a stable 60% of the FI treatment, while the NI treatment had used up most of the water after 10 days and consequently reached the point close to zero consumption (fig.1).

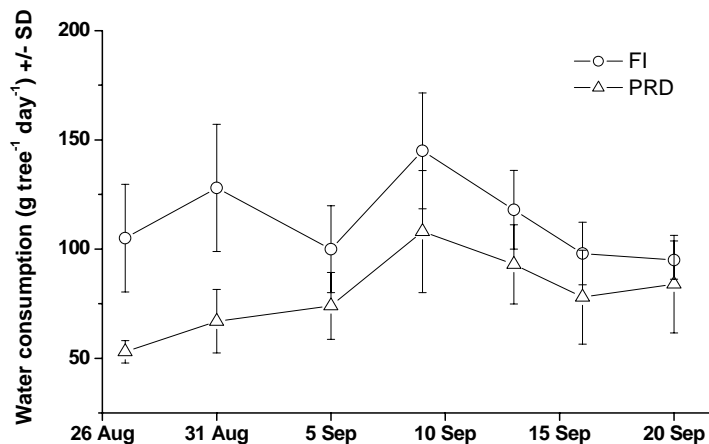


Figure 2: Average water consumption of trees with full irrigation (FI), partial rootzone drying (PRD) after re-watering of the PRD treated trees.

After re-watering the plants PRD trees increased their water consumption gradually. After three weeks there were no significant differences in water consumption, even though the average water consumption was still slightly lower for the re-watered PRD trees as compared to the control (fig. 2).

### 3.2. Stomatal Resistance

Under conditions of drying soil it was observed that the stomatal resistance under NI treatment increased sharply to a multiple of the FI treatment. The initially higher increase in stomatal resistance in the PRD stagnated and stabilized at about 200% of the FI treatment. During the time of increased temperature the PRD treated trees reacted more sensitively and even the morning values for their stomatal resistance increased (fig. 3). Following the same pattern, the differences in the afternoon values were even more pronounced (fig. 4).

### 3.3. Concentration of Abscissic Acid

On July 29<sup>th</sup>, ten days after start of the experiment the ABA concentration in the leaves of all treatments was not significantly different. The ABA concentration in dry matter (DM) of NI was with 82 ng/g only slightly higher than in PRD and FI with 78 ng/g each. On August 12<sup>th</sup>, the PRD treated plants had the highest ABA concentrations in the leaves (107 ng/g), topping even the NI trees (96 ng/g). On this date only four of the NI trees had been tested, as the rest had been taken out of the experiment due to visual damages of drought stress. On August 26<sup>th</sup> the levels in both FI and PRD had gone down, with higher values in the PRD treatment (fig. 5).

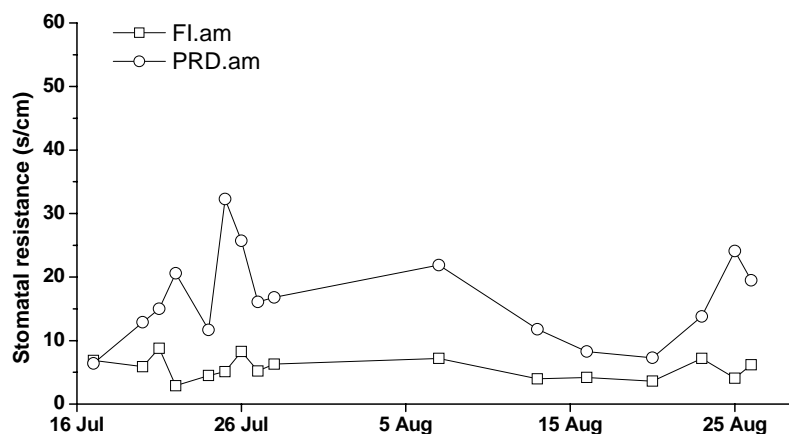


Figure 3: Stomatal resistance in the morning (9:00 – 10:00 am) for trees receiving full irrigation (FI) and subjected to partial rootzone drying (PRD).

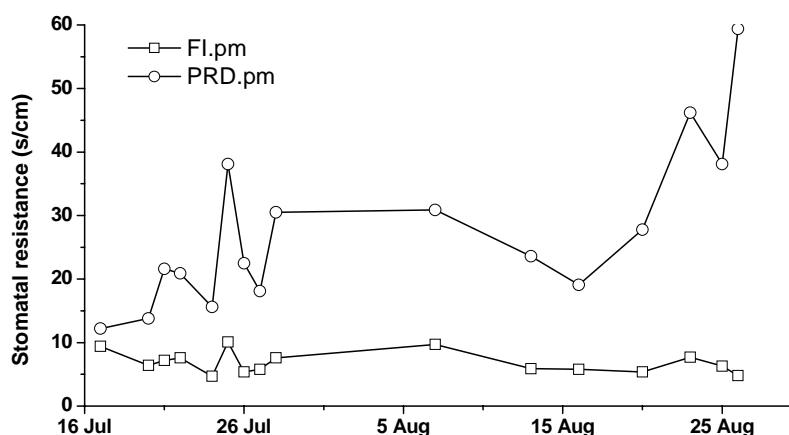


Figure 4: Stomatal resistance in the afternoon (3:00 – 4:00 pm) for trees receiving full irrigation (FI) and subjected to partial rootzone drying (PRD).

### 3.4. Plants with Visual Damages

In the NI treatment five trees showed visual damages and were eliminated from the experiment after July 29<sup>th</sup>, four trees after August 2<sup>nd</sup> and one tree on August 9<sup>th</sup>. On the 13<sup>th</sup> and the 16<sup>th</sup> of August respectively, two of the PRD trees were taken out of the experiment due to wilting. We observed that all young leaves in the PRD treated plants remained with a low turgor-pressure and did not reach full maturation before re-watering.

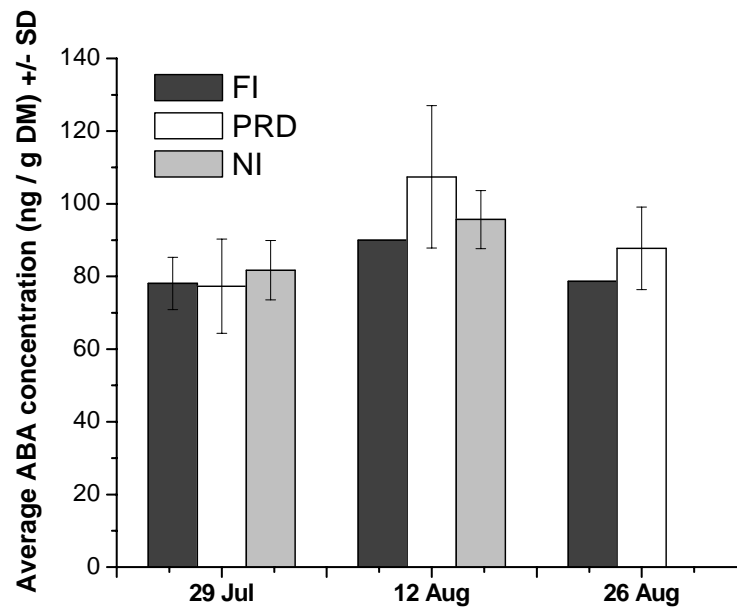


Figure 5: Absciscic acid (ABA) concentration in the leaves of trees with full irrigation (FI), partial rootzone drying (PRD) and no irrigation (NI).

#### 4. DISCUSSION

In this experiment it was observed that litchi trees subjected to PRD reacted in the same way as it is described for other fruit trees. Under limited water availability the stomatal resistance increased rapidly and the water consumption dropped at the same time. Both effects were less pronounced as in the treatment without irrigation. This finding is in agreement with results of previous studies, mainly on grape vines (Stoll *et al.*, 2000). The PRD trees stabilized at a higher level of ABA. Thereby, it was shown, that by application of PRD the hormonal level can be influenced over a longer period of time, keeping the ABA levels high and activating stress responses. The lower concentration of ABA in the analyzed leaves of non-irrigated plants may be the result of leaf shedding. Under extreme drought stress, ABA can be produced in the leaves, which consequently leads to abscission. The transport from the roots to the shoots would not play a major role under those conditions, as the total amount of the assimilates transport decreases considerably as a consequence of the low transpiration.

The stomata reacted much faster to the limited water supply than the ABA concentration in the leaves did. Thus, it can be assumed that the ABA level in the leaves of deficit irrigated trees does not determine the short term reaction, due to the momentum of the transport from root to shoot. However, it seems to be responsible for the water consumption of the plant in the long run. Therefore, it took the PRD treated trees nearly one month to fully recover and reach the same level of water consumption as the control trees. A further effect of the increased ABA level is the reduction of vegetative growth under PRD. The trees that had produced new leaves before being subjected to deficit irrigation stopped the maturation process and some did even shed the young leaves. This supports the assumption that litchis

under PRD mainly reduce their vegetative growth and possibly to a lower extend the generative growth.

## 5. CONCLUSIONS

It was observed that water consumption remained on a stable level below the maximal rate of consumption. This leads to the conclusion, that litchi trees react to alternate drought stress on two sides of the rootzone as described for other tree species. Based on the measurements of hormonal concentrations in the leaves, it can be concluded, that it is possible to influence the hormonal balance of litchi trees in a long term by PRD, reaching a rather constant level of ABA. During the time of controlled drought stress the growth of new biomass is hampered. The recovery to full water consumption after re-watering is apparently complete. However, the time delay of the recovery shows, that there are long-term effects that may arise from the PRD treatment. The comparatively high losses in the PRD treatment make further investigation on possible long-term effects necessary.

Considering the climate conditions in Northern Thailand with a pronounced drought period followed by a rainy season, the observed drought stress behavior of litchi trees submitted to PRD is adequate, as the observed stagnation in vegetative growth may promote fruit growth as a survival strategy of the plant. As this experiment was carried out with juvenile plants, no calculation of WUE could be carried out. In further experiments PRD has to be applied to bearing litchi trees in order to complete these findings by the yield component.

## 6. ACKNOWLEDGEMENTS

We are grateful to the Deutsche Forschungsgemeinschaft (DFG) for the financial support of this work in the frame of the Sonderforschungsbereich (SFB) 564 "Sustainable rural development in mountainous regions of Southeast Asia".

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