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PROLINE ACCUMULATION IN THE LEAVES OF WATER STRESSED OIL PALM (*Elaeis guineensis* Jacq.) SEEDLINGS

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An increase in stomatal resistance of oil palm seedlings was related to increasing water deficit indicated by a reduction in leaf water potential. Proline began to accumulate after stomatal resistance reached a peak (215.6±16.8 s.cm^{-1}). This led to a recovery in leaf water potential followed by a similar reduction in stomatal resistance. The proline level eventually fell to the control level after watering was resumed. Water stress was also found to reduce dry matter production and its partitioning to the root system of oil palm seedlings.

INTRODUCTION

Water stress is a major limiting factor in oil palm productivity as is clearly shown in West Africa where the seasonal yield is much influenced by the long dry seasons (Broekmans, 1957; Sparnaaij *et al.*, 1963 and 1965). Lack of rain is associated with the initiation of male inflorescences and abortion of female inflorescences (Corley, 1976; Turner, 1976). Midday stomata closure - a criterion of crop water stress - was observed during a drought in Malaysia by Corley (1973), whereas in rainy conditions the stomata stay open all day (Squire, 1983, unpublished data). Although oil palm in Malaysia generally does not experience severe drought, they still yield seasonally. This may be due to the effects of transient water deficits on photosynthesis, either through stomatal closure or chloroplast activity or both.

Measuring stomatal closure alone may not be adequate to detect water stress in oil palm under Malaysian conditions. Therefore, other more sensitive indicators of internal water stress should also be considered, such as the content of the stress-related amino acid proline (Le Rudelier *et al.*, 1984). Increased proline levels in response to water deficits or increased osmotic pressure have been reported in higher

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plants by Palfi *et al.*, (1973). It accumulates in all organs of water-stressed plants although accumulation is higher and more rapid in leaves (Barnett and Naylor, 1965; Singh *et al.*, 1973). This study investigates proline levels in leaves of oil palm seedlings suffering from water stress.

MATERIALS AND METHODS

Fifteen one-year-old DxP seedlings grown in polybags of soil (21 cm dia. x 30 cm height) were kept unwatered in a rainproof shelter for 10 weeks. Watering was only applied after the 10th week to study their recovery from stress and continued until final destructive sampling for dry weight estimation at 16 weeks.

The same number of control seedlings was placed outside the shelter and watered daily. Foliar proline, stomatal resistance, osmotic and water potentials were measured from the same five plants weekly.

Five plants from each treatment, randomly chosen, were sampled at 10 and 16 weeks for dry weight analysis. The sacrificed seedlings were separated into shoots and roots, oven dried at 70°C for three to four days and weighed.

Proline assay

The method used was that of Bates (1973). One gramme of fresh leaf tissue from a fully-expanded frond was homogenised and soaked overnight in 10 ml of 25% ethanol with 5% acetic acid at 4°C. The mixture was then filtered. A reaction mixture made up of 0.5 ml filtered extract, 4 ml 50% acetic acid and 2 ml ninhydrin reagent was put into a glass-stoppered test tube and heated for one hour in a water-bath at 100°C. The solution was left to cool. Toluene (4 ml) was then added, the test tube vigorously shaken and the contents left to separate into aqueous and toluene phases. The colour of the toluene phase was measured at 515 nm on a spectrophotometer (Perkin Elmer, Lamda 3A). Each sample was analysed in triplicate with the proline contents established by colour reference to standard mixtures.

Stomatal resistance, osmotic and water potential measurements

Weekly recordings were taken on fully expanded leaves under sunny conditions starting from 1100 hours. Stomata resistance was measured using an automatic porometer (Delta-T Devices, Mark 3). Total plant water potential was determined from fully expanded leaves using a pressure chamber (Scholander *et al.*, 1965). Osmotic potential or osmotic pressure was measured by dew-point hygrometry on sap expressed from leaf tissues killed by freezing (Campbell *et al.*, 1973).

RESULTS

Stomatal resistance in water stressed seedlings began to increase after the fourth week of experiment and reached a peak of 215.6±16.8 $s.cm^{-1}$ at 7 weeks before declining to 20.6±9.3 $s.cm^{-1}$ at 10 weeks. It recovered to the control level ($\leq$ 5.0 $s.cm^{-1}$) when watering was resumed after the tenth week *(Figure 1a)*.

Figure 1b shows foliar proline started to accumulate after the seventh week and reached a maximum of 278.5±56.0 $\mu g.g^{-1}$ fresh weight at the 10th week. Proline started to accumulate after stomatal resistance reached its peak. As the proline level increased, stomatal resistance began to fall to a low value. On watering, the proline level also decreased and within four weeks, was at the control level ($\leq$15.0 $\mu g.g^{-1}$ fresh weight).

Leaf water potential in the water-stressed seedlings started to decrease from -0.85±0.03 MPa at Week 4 to -2.19±0.08 MPa at Week 8 as shown in *Figure 2(a)*. Then it rose as proline began to accumulate. A similar trend was observed for the osmotic potential *(Figure 2b)*.

The total dry weight of water-stressed seedlings at Week 10 was significantly lower (at $p \leq 0.05$) by 31 per cent than that of the control *(Figure 3a)*. It remained constant at 100 grammes even after four weeks of watering. The root-to-shoot ratio values were not statistically significant but a 20% reduction from control was observed in water-stressed seedlings at the 10th week. It then recovered the same value as control six weeks after watering *(Figure 3b)*.

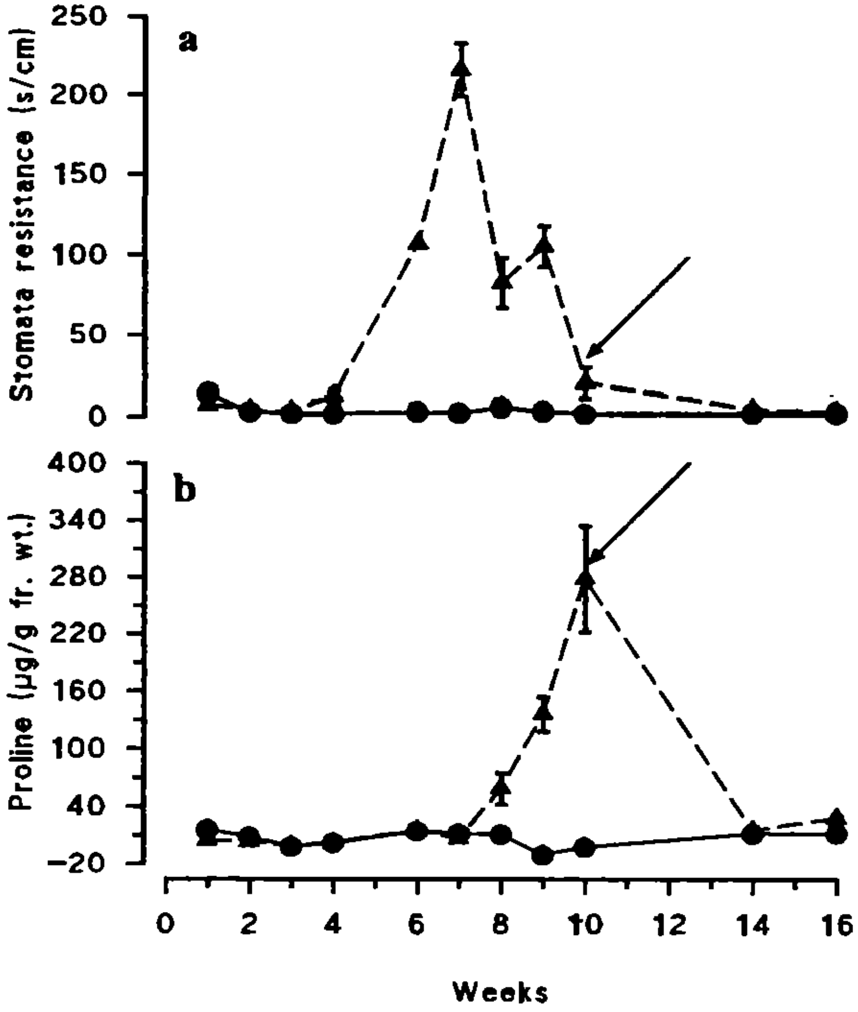

Figure 1. Stomatal resistance and proline level from expanded leaves of oil palm seedlings (● control and ▲ water-stressed). Arrows show when watering was resumed for water-stressed plants. Points are mean ± standard error of five plants.

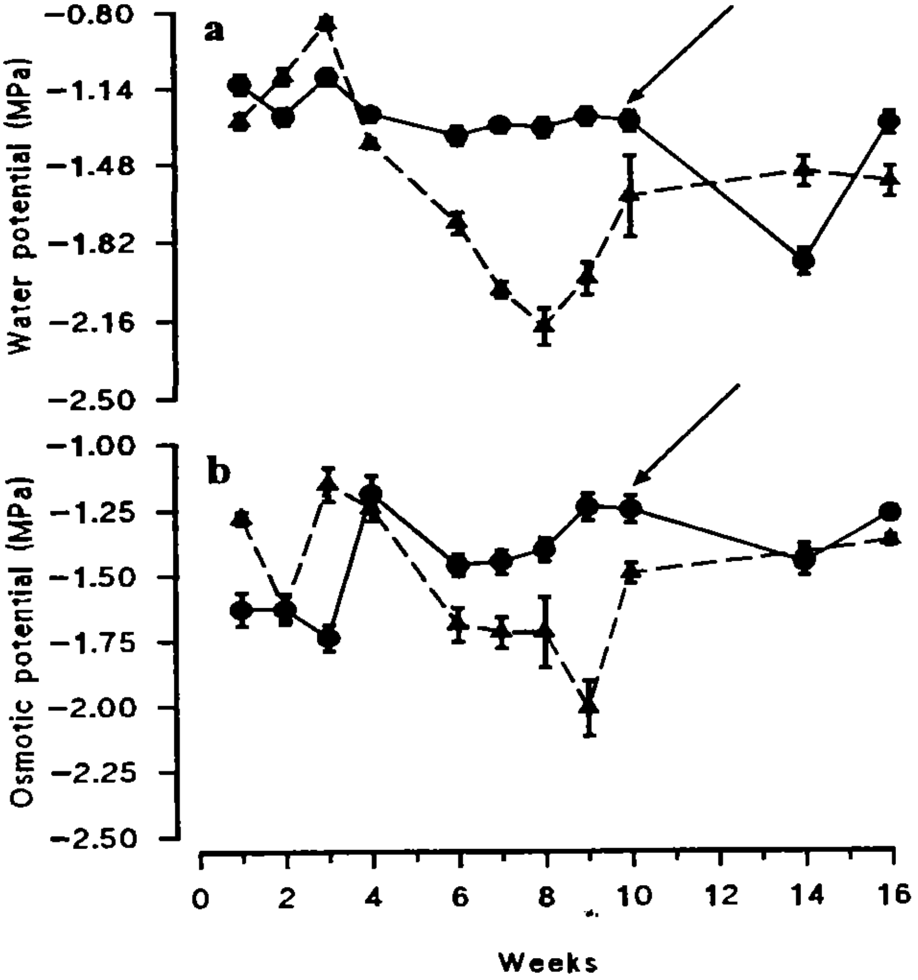

Figure 2. Water potential and cell osmotic potential measured from expanded leaves of oil palm seedlings (● control and ▲ water-stressed). Arrows show when watering was resumed for water-stressed plants. Points are mean ± standard error of five plants.

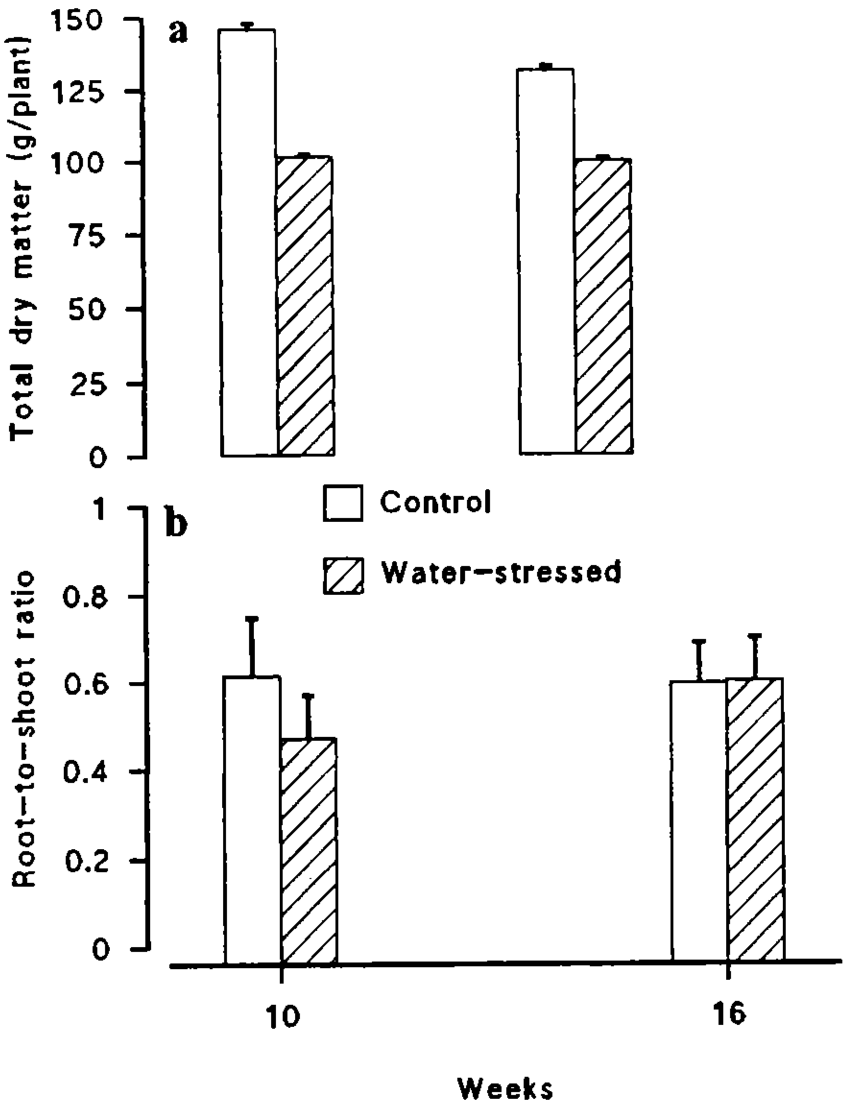

Figure 3. The total plant dry matter production and root-to-shoot ratio measured at 10 and 16 weeks from control and water-stressed oil palm seedlings. Watering was resumed for water-stressed plants after the 10th week. Bars represent mean ± standard error of five plants.

DISCUSSION

The increased stomatal resistance was due to the decreasing water status of the stressed oil palm seedlings. Peak stomatal resistance occurred at a very low leaf water potential level. This clearly showed that the stomata were closing in response to the increased plant water deficit. Thus, stomatal closure is one mechanism that allows plants to regulate moisture loss and increase their tolerance to water stress.

The results from this experiment also showed that stress-induced stomatal closure began to decrease while the plants were still greatly stressed. This occurred when the proline level started to rise. Stomatal resistance was significantly reduced when the proline level was increased by a factor of 10.

Proline reduces the symplastic osmotic potential leading to an increase in leaf water potential. Part of the decrease in osmotic potential in other crops have been shown to be due to increases in the concentrations of cell solutes, particularly amino acids, organic acids and sugars (Acevedo *et al.,* 1979; Meyer and Boyer, 1981). This allows cell turgor to be maintained with a low water potential and is a mechanism for increasing drought tolerance in plants. Turgor potential is important for cell enlargement, stomatal opening and maintenance of form in most plants. Therefore, proline in stressed oil palm seedlings allows them to maintain high turgor and water potential. This would explain how their stomata can remain open even during water stress.

When the water stress was removed, the high proline level gradually fell back to normal. The proline accumulated may be a storage reserve used for growth in recovery after the stress.

Photosynthesis was seriously affected by water stress as suggested by the low dry matter production in the stressed seedlings. This could be through either increased stomatal closure reducing gaseous exchange or a reduction in chloroplast activity lowering the efficiency of carbon fixation. The latter may be more limiting than stomatal closure at low water potentials (Boyer, 1971 and Boyer, 1976; Ackerson *et al.,* 1977).

The seedlings also adapted to the stress through a reduction in root-to-shoot ratio. Under the limited source situation, less assimilates were partitioned to the roots. However, this was rectified when seedling growth recovered after watering was resumed as the root-to-shoot ratio became the same as that for the control.

CONCLUSION

The results from this preliminary study suggested that proline accumulation in the leaves of water-stressed oil palm seedlings plays an important role in maintaining their turgor. This enables them to maintain a high leaf water potential and prevents their stomata from closing. Stomatal closure measurements was not enough to detect water stress in oil palm seedlings. Further work is needed to study the water status of mature oil palms and the relationship between proline accumulation, stomatal closure and gaseous exchange.

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