



## PHYSIOLOGICAL RESPONSES OF MANGO (*Mangifera Indica*) ROOTSTOCK SEEDLINGS TO WATER STRESS

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### ABSTRACT

Water stress affects plant growth and crop productivity in the vast semi-arid and arid regions of Kenya. The present study was aimed at investigating the effect of different watering regimes on the growth of young mango rootstock seedlings. Six months old mango (*Mangifera indica*) rootstock seedlings were grown in polythene pots in a green house at Maseno University, Kenya and were subjected to four watering regimes namely watering daily, twice in a week, once in a week and once in two weeks. Treatments were in a completely randomized design replicated six times. The physiological parameters determined were stomatal conductance, transpiration rate, CO<sub>2</sub> assimilation rate and intercellular CO<sub>2</sub> concentration, all determined by infra red gas analyser and leaf chlorophyll content determined by standard methods. The results showed that increasing water stress reduced the physiological parameters particularly at the later days of plant growth, due to stomatal and non-stomatol factors. Leaf chlorophyll content however, chlorophyll content was slightly increased since the chrophyll pigments may have been resistant to dehydration. It was concluded that increase in water stress reduces the gas exchange parameters of mango rootstock seedlings but slightly increased chlorophyll content.

**Keywords:** mango, rootstock, gas exchange, stress, physiological irrigation.

### INTRODUCTION

Environmental factors, such as soil moisture content can have a profound effect on plant metabolism and development. Repeated water deficits can reduce the sensitivity of stomata to low water potentials. The acclimation of photosynthesis to these conditions in cotton (*Gossypium hirsutum* L.) has been attributed to altered stomatal response to water potential (Mathews and Boyer, 1984). When drying, soil causes water absorption to lag behind transpiration loss. Water deficit develops in the plant which, if unrelieved will result in permanent wilting and finally in dehydration and death. This deficit is typically characterized by decreases in water content, turgor and total water potential, partial or complete closure of the stomata and reduction in the rate of cell enlargement and growth (Kramer, 1983). Crop water deficit or stress is the result of interactions between factors in the rhizosphere, the plant and the atmosphere in relation to the amount of moisture available to plants. It is therefore vital to understand whether a plant simply responds or adapts to effects of water stress.

Water Stress in the dry land areas may lead to wilting of seedlings immediately after germination. For plants at development stage, it may give rise to undeveloped seeds. Likewise premature fall of fruits may be observed in the fruit trees. A plant will suffer water stress when its water potential decreases as a result of falling water content (Mansfield and Wilson, 1981; Prioul *et al.*, 1984).

Water stress has a greater effect during certain phases of plant cycle than others. Seedling establishment and floral development are often very sensitive stages in a plant's growth. Seedling drought resistance is important for the early establishment of plants under stress conditions. Exposure to moisture stress at the seedling stage confers some degree of "hardening" against current

and later drought periods (Ashley, 1993). However the degree of hardening will vary within varieties and species.

On the other hand, plants with efficient water supply system behave as lavish water spenders and do not limit the rate of transpiration. Sensitivity of stomata to water stress varies with age, plant species and cultivars (Turner, 1980). Ackerson and Krieng, (1981) observed a converse relationship between transpiration rates and water deficit in maize. When the soil water content decreases, there is a decline in the plant water potential and the plant experiences a water deficit. Therefore, the amount of water transpired reflects the amount of water in the plant and the evaporative demands of the atmosphere. Reduced transpiration is an important physiological indicator of water stress (Xu *et al.*, 1995). Leaf transpiration includes both stomatal transpiration and cuticular transpiration. Under water deficit conditions, transpiration rate is controlled by stomatal conductance which is mainly determined by tissue water status at a given vapour pressure deficit between the leaf surface and the air. Cuticular transpiration on the other hand is affected by characteristics of the leaf surface such as thickness of the waxy layer and morphological structure (Xu *et al.*, 1995).

In mature leaves, osmotic adjustment sustains photosynthesis by maintaining leaf water content at reduced water potentials. In drier areas, the carbohydrates gained from this increased adjustment causes deeper rooting and consequently greater water use. Generally, plants experiencing declining soil water levels ultimately have stomatal closure and reduced transpiration. As a consequence of a reduction in transpiration, foliage temperatures may increase above the ambient temperatures of the surrounding air. The elevated temperatures may limit the dry matter accumulation



because of reduced transpiration, reduced photosynthesis and cellular damage (Burke and Hatfield, 1987).

Leaf temperatures normally reflect on the behaviour of the stomata, which control transpiration. Stomatal closure of the sun-lit leaves result in increased leaf temperature if factors like wind speed and vapour pressure remain relatively constant. Stomatal resistance increases with soil moisture stress resulting in an increased leaf temperature. In cotton (*G. hirsutum* L.), reduced transpiration results in a warmer canopy.

Chlorophyll and Carotenoids absorb radiant energy, which is used for photosynthesis. In many observed cases chlorophyll content declines under stress conditions. Potato leaves show a significant decline in chlorophyll content with increasing water stress (Nadler and Bruvia, 1998). Chlorophyll content in plants decreases with increased mesophyll resistance commonly observed in dry areas (Levitt, 1980)

The main objective of this study was to investigate the physiological responses of *M. Indica* rootstock seedlings to different levels of water stress with a view to recommending the appropriate watering levels in the dry areas.

## MATERIALS AND METHODS

### Experimental Site

The experiment was set up in the greenhouse at Maseno University which is situated of a latitude 0<sup>01</sup>'N-0<sup>02</sup>'S and longitude 34<sup>025</sup>'E-34<sup>047</sup>'S. Maseno is approximately 1500m above sea level. It receives an annual mean precipitation of 1750 mm with a bimodal pattern of distribution. The mean temperature is 28.7<sup>0</sup>C with a relative humidity of 40%. The soils, which are classified as acrisols are deep reddish brown, friable clay with the pH ranging from 4.5-5.5, soil organic carbon and phosphorus contents are 1.8% and 4.5mg/kg, respectively (Netondo, 1999). The minimum and maximum temperatures inside the green house were 26±6 <sup>0</sup>C and 35±6<sup>0</sup>C, respectively with a relative humidity of 38±5%.

### Treatment and experimental designs

Overripe mango (*Ngowe* cultivar) fruits were collected from Luanda market about 5km from Maseno, Kenya. The seeds were extracted and then planted in a nursery under a shade. Six months after germination, the seedlings were transplanted into 20 litre polythene pots measuring approximately 20cm in diameter and 30cm in height. The composition of the soil mixture used as the rooting medium in the pots was in the ratio of 1 sand: 2

loam: 3 composite manure. The pots were perforated at the bottom to allow for proper drainage of water in order to avoid water logging and were placed on a table in the greenhouse. All the agronomic practices including weeding, pest control and fertilizer application were observed apart from watering which was controlled.

The experimental set up was a completely randomized design (CRD) consisting of four treatments and six replications. Treatments commenced four weeks after transferring the seedlings to the greenhouse. The four treatments were: Watering daily (W), Watering twice in a week (X), Watering once in a week (Y) and watering once in two weeks (Z).

Analysis of data was done using SAS statistical package to obtain analysis of variance and separation of means using L.S.D. ( $P \leq 0.05$ ).

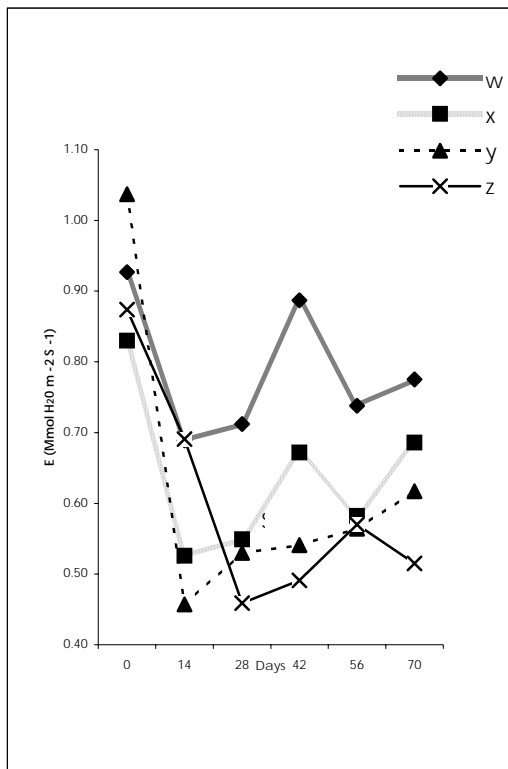
### Measurement of parameters

Measurement of parameters was taken for a period of 4 months during the duration of the experiment from December to March 2004. The parameters determined were CO<sub>2</sub> assimilation, transpiration rate, stomatal conductance, leaf temperature, internal CO<sub>2</sub> concentration. These measurements were taken on the most recently emerged; full expanded and well exposed leaves under bright light using an infrared gas analyzer (CIRAS 1-PPSystem, Stortfield, Hitchin, Herts, U.K.): Chlorophyll content was also determined using standard methods.

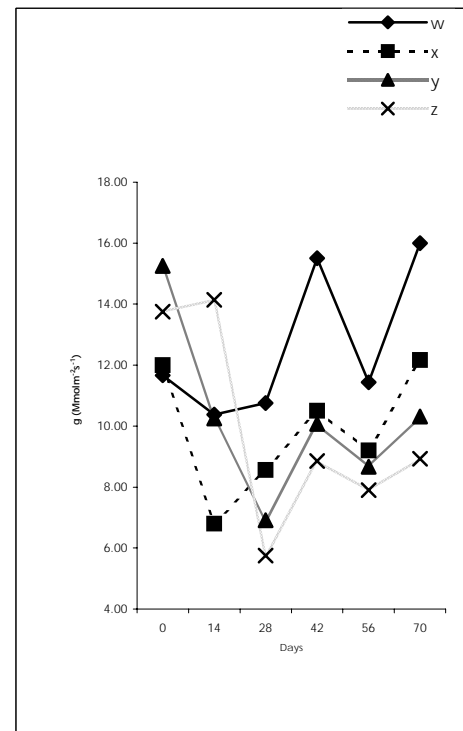
## RESULTS

### Transpiration

The rate of transpiration was higher in the well-watered plants compared to the extremely stressed plants on all the days, except on D0 and D14 (Figure-1). The lowest rates of transpiration in all treatments were observed on D14 when the temperatures in the green house were lowest (24<sup>0</sup>C) and the humidity was higher (40%) than observed on all other days of data collection. On D0, there was no significant difference between the treatments. However on D14 there was a significant difference ( $P \leq 0.05$ ) between the treatments. At D56 a decline in the rate of transpiration was observed under extreme water stress (treatment Z) as compared to W, X and Y where an increase in transpiration was observed. Highly significant differences ( $P \leq 0.001$ ) were observed between the treatments at D28, D42, D56 and D70 (Figure-1).



**Figure-1.** The effect of water stress on the rate of transpiration (E) in mango rootstock seedlings. Values represent means of six replications.



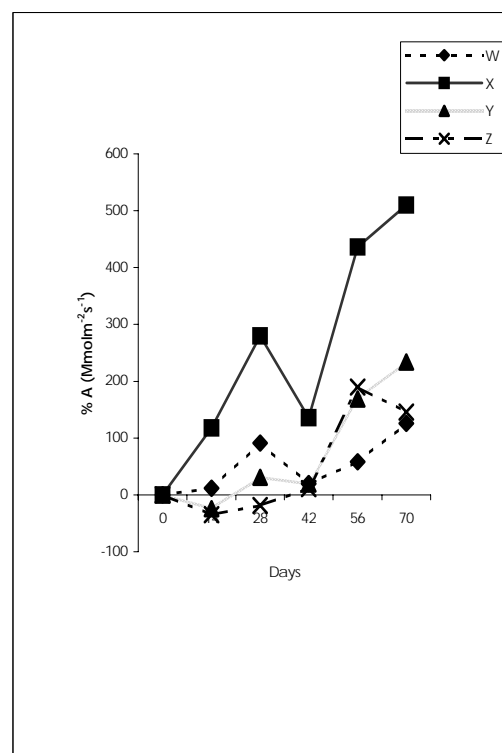
**Figure-2.** The effect of water stress on leaf stomatal conductance (g) of mango rootstock seedlings grown at Maseno University, Kenya. Values represent means of six replications.

### Stomatal conductance

The trend in stomatal conductance is almost similar to that of transpiration. The stomatal conductance was highest in the well-watered plants (W) and lowest in the extremely water stressed plants (Z) on all days except on D0 and D28. At D0 there was no significant difference between treatments. However, a highly significant difference ( $P \leq 0.001$ ) occurred on D14 and D42. The same case was observed between D56 and D70 (Figure-2).

### CO<sub>2</sub> assimilation

There was a steady increase in CO<sub>2</sub> assimilation with time except on D42 where a decline occurred (Figure-3). In the highly stressed plants (Z) had a decline was observed from D56 to D70. Significant differences ( $P \leq 0.05$ ) occurred between particular treatments on certain days. Plants that were highly stressed (treatment Z) apparently had a higher photosynthetic rate than W and Y, which received more water implying a possible resistance of the photosynthetic apparatus to water stress.

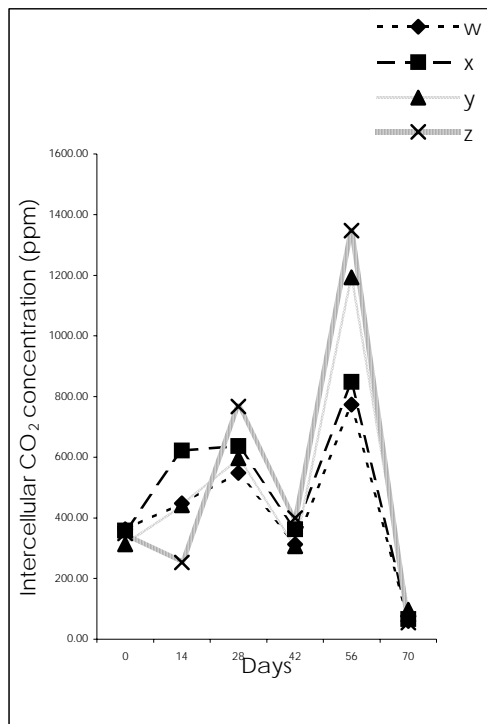


**Figure-3.** The effect of water stress on the rate of CO<sub>2</sub> assimilation (A) on the leaves of mango rootstock seedlings. Values represent means of six replications.



### Interacellular CO<sub>2</sub> concentration (Ci)

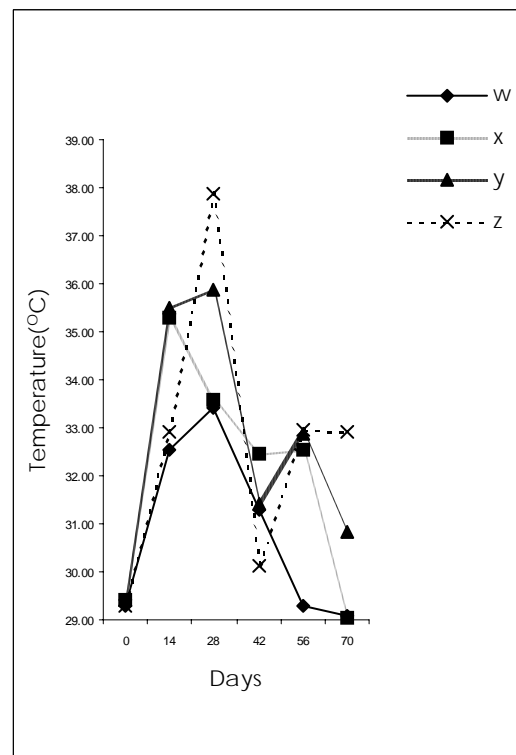
The results for the effect of water stress on intercellular CO<sub>2</sub> concentration are shown in Figure-4. There was an initial increase in Ci among the treatments from D0 to D28 except in treatment 2 when a decline was first observed at D14 then the Ci concentration rose up. A significant difference ( $P \leq 0.05$ ) was observed between the treatments at D28. Generally Ci concentration was higher in the highly stressed plants (Z) as compared to W, X and Y. A highly significant difference ( $P \leq 0.001$ ) was also observed among treatments at D56 where the more stressed plants (Y and Z) had a higher Ci than W and X (Figure-4).



**Figure-4.** The effect of water stress on intercellular CO<sub>2</sub> concentration (Ci) on mango rootstock seedlings. Values represent means of six replications.

### Leaf temperature

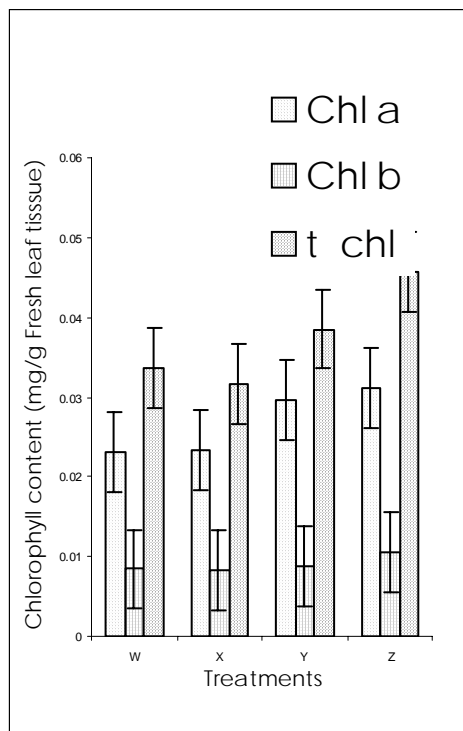
The results for leaf temperature are shown in Figure-5. The general trend of the graph shows a higher leaf temperature in treatment Y and Z which were the most stressed as compared to W and X. A highly significant difference ( $P \leq 0.001$ ) was observed between treatments on all the days except at D0.



**Figure-5.** Effect of water stress on leaf temperature of mango rootstock seedlings grown at Maseno, University, Kenya. Values represent means of six replications.

### Chlorophyll content

There was a steady rise in chlorophyll *a* and total chlorophyll content with increase in water stress as shown in Figure-6. Values for chlorophyll *b* however remained almost constant in all treatments except in Z when there was a slight increase. Total chlorophyll showed a general increase with stress, especially between treatment Y and Z.



**Figure-6.** The effect of water stress on chlorophyll extracted from fresh leaves of mango rootstock seedlings. Values represent means of six replications.

## DISCUSSION

### Effect of water stress on gas exchange parameters

Transpiration decreased significantly in the plants under extreme stress as compared to the control. Similar results have been reported in beans (Gardner, 1972; Ouma, 1988), in soya beans (Sionit *et al.*, 1984), in tomato (Xu *et al.*, 1995) and in wheat (Morgan, 1983; El Hafid *et al.*, 1998). Transpiration is a process controlled by the closing and opening of the stomata. Water absorbed by the roots is lost to the atmosphere via the leaves depending on the prevailing environmental conditions. The transpirational pathway of water flow in woody plants is the xylem in which the conducting elements are non-living, heavily thickened and lignified tracheids and xylem vessels (Jones, 1992).

The trend taken by transpiration is quite similar to that of  $\text{CO}_2$  assimilation. This means that a correlation occurred between transpiration and photosynthesis. Similar results were reported in sunflower by Robertson *et al.* (1985). Under water stress, cells lose their turgidity causing stomatal closure. This limits the rate of  $\text{CO}_2$  diffusion through the stomata causing a decline in the photosynthetic rate. Apart from moisture stress, the rate of transpiration is also affected by other environmental factors such as temperature, radiation and relative humidity. The fluctuations observed in transpiration on specific days like at D42 (Figure-2) are due to daily changes in temperature and relative humidity. Higher air temperatures increase the rate of transpiration. The effect

of this is probably to enhance the cooling of leaves by evaporation as was also discussed by Burke *et al.* (1990). Leaf temperature however increased with decline in transpiration. Transpiration cools the plant by loss of latent heat of vaporization. Wilting of some seedlings under extreme stress was observed after D56. The plants were unable to absorb capillary water which was scarce and tightly held by the soil particles due to low soil water potential. The reduction in transpiration rate in plants under water stress may also be attributed to morphological changes such as increased cell wall thickness and cell wall lignification (Netondo, 1999). Reduced transpiration is an important physiological effect of stress.

Stomatal conductance took a similar trend like that obtained in transpiration. Stomatal conductance in the water stressed plants was generally lower as compared to the well-watered plants even though the difference was not significant. The reduction in the leaf water potential may have led to the development of a water deficit in the leaves causing guard cells to lose turgor and hence the stomatal pores to reduce. In addition the increased stomatal resistance may have led to reduced water transport in the leaves further causing a decrease in the stomatal conductance. Reduction in stomatal conductance decreases transpiration and also limits photosynthesis (Tezera *et al.*, 2002), as also demonstrated in the present study. In certain plants, stomatal conductance declines even before severe water stress sets in thereby avoiding desiccation during drought. This has been observed in *Quercus Ilex* (Fortelli *et al.*, 1986). Leaves of plants exposed to higher water deficits have higher ABA concentration. This may have also contributed to regulation of closing and opening of the stomata in the leaves of the mango rootstock seedlings.

Net photosynthesis ( $\text{CO}_2$  assimilation) seemed to increase in all treatments except the rate was higher in the well-watered plants as compared to the stressed ones. However, no significant difference was observed (Figure-2). Carbon dioxide assimilation is affected by both stomatal and non-stomatal factors. In the present study, it appears that the photosynthetic apparatus may have been resistant to dehydration since there was no decline in net  $\text{CO}_2$  assimilation under extreme stress. The lower rate of increase in the  $\text{CO}_2$  assimilation under water stress may be attributed to reduced stomatal conductance. However, in order for stomatal closure to have an effect on both transpiration and  $\text{CO}_2$  assimilation,  $\text{CO}_2$  assimilation must at least be limited by the rate of  $\text{CO}_2$  diffusion through the stomata (Boyer, 1976)

There was higher water use efficiency (WUE) in the stressed plants as compared to the well watered. Water use efficiency is defined as the ratio of leaf photosynthesis to transpiration (A/E) measured simultaneously (El Hafid *et al.*, 1998) or the carbon gained during photosynthesis in relation to the water lost during transpiration (Hsiao, 1993). In paw paw plants (*Carica papaya*), similar results were obtained (Clemente and Marler, 1996). Water use efficiency of drought stressed paw paw was reported to be 10% higher than that of well-watered plants (Clemente



and Marler, 1996). This behaviour is a major adaptive significance for plants growing in dry areas. However, there is a consequence of maximizing WUE by minimizing water-loss; carbon gain is in fact not maximized. Water conservation at the expense of carbon acquisition is probably an adaptation for mangoes to water limited areas.

Another important factor, which may have a profound effect on CO<sub>2</sub> assimilation in the plants, is the internal CO<sub>2</sub> concentration (C<sub>i</sub>). In this study though, C<sub>i</sub> seemed not to be affected by water stress. Therefore the low increase in CO<sub>2</sub> assimilation under water stress, without a corresponding decline in C<sub>i</sub>, could be due to non-stomatal effects on the photosynthetic processes, possibly an increase in the mesophyll resistance. Similar results have also been observed in wheat (Kecheva *et al.*, 1994). A reduction in C<sub>i</sub> can be very detrimental to the photosynthetic process especially in the presence of enzyme Rubisco, which has a high affinity for oxygen (O<sub>2</sub>) when the intercellular CO<sub>2</sub> concentration is low. Therefore under low C<sub>i</sub>, Photosynthesis is limited by enzyme Rubisco. For many species, C<sub>i</sub> tends to remain constant over a range of environmental conditions, including water stress (Wong *et al.*, 1979; Pearcy, 1981).

The chlorophyll content is another factor that affects the photosynthetic process in green plants. In this study however chlorophyll *a* was more resistant to dehydration, it increased slightly with water stress, as compared to chlorophyll *b*, which was constant. The slight increase in total chlorophyll under water stress suggests that the chlorophyll pigments in these leaves were somewhat resistant to dehydration. Another possible explanation may be that chlorophyll synthesis was induced by water stress, especially chlorophyll *a*.

## CONCLUSIONS

Clear results show that the rate of transpiration reduced with increasing water stress. Apart from water stress, transpiration is also controlled by other atmospheric factors such as temperature humidity and air movements (wind). These factors are however controlled in the greenhouse. As transpiration reduced, leaf temperature increased. This confirms that the transpiration process cools the leaf surface by reducing the latent heat of vaporization. Drying and shedding of lower leaves, observed under extreme stress in this study alone is a mechanism for water conservation. Other mechanisms involved in mango seedlings water conservation in drought prone areas need to be established.

The rate of CO<sub>2</sub> assimilation was significantly reduced by water stress. Other factors that also reduced under extreme water stress were the intercellular CO<sub>2</sub> concentration and the chlorophyll content. A reduction in transpiration coupled with an increase in CO<sub>2</sub> assimilation implies that photosynthesis was largely controlled by non-stomatal factors. Continued CO<sub>2</sub> assimilation under water stress is only possible in the drought tolerant plants. This implies that the photosynthetic apparatus is resistant to drought. Increase in chlorophyll *a* may be due to the

continued synthesis of this pigment even under stress conditions, however, further research is required to reach this kind of conclusion.

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