

## Leaf Growth and Stomatal Sensitivity after Water Stress Relief and its Relation to Xylem Sap Abscisic Acid

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### ABSTRAK

Pengaruh tanaman cili (*Capsicum annuum* L. var Bell Boy) yang di beri pemulihan tegasan air yang di berikan secara perlahan telah di kaji. Pokok di beri tegasan air selama 5 dan 10 hari kemudian di beri pengairan semula. Pertumbuhan daun, kation air, konduksi stomata dan kandungan asid absisik (ABA) di dalam xilem di bandingkan di antara pokok yang didedahkan kepada tegasan air dan tanpa tegasan air. Potensi air daun telah pulih dengan cepat apabila pokok yang didedahkan kepada tegasan air di beri pengairan semula tetapi konduksi stomata tidak pulih sehingga 48 jam selepas di beri pengairan. Kepekatan ABA di dalam xilem menyamai kepekatan ABA pada pokok yang disiram air berterusan selepas 6 jam di beri pengairan. Selepas diberi pengairan, pertumbuhan pokok diberi rawatan tegasan air selama 11 hari adalah cepat dan melebihi pertumbuhan pokok yang didedahkan kepada pengairan berterusan. Pokok yang berada pada tegasan air berterusan menunjukkan pengurangan pertumbuhan daun, konduksi stomata dan pertambahan kepekatan ABA didalam xilem.

### ABSTRACT

Responses of pepper seedlings to rewatering after being subjected to 5 and 11 days of gradual water stress were investigated. Leaf growth, water relations, stomata conductance and xylem sap ABA in these plants were compared with plants grown under continuous well watered and stressed conditions. Leaf water potential returned to the control values immediately after rewatering but the stomatal conductance of stressed plants did not recover until 48h after rewatering. The concentration of ABA in the xylem sap in the pre-treated stress plants returned to similar values to the control 6h after rewatering. After rewatering, leaf growth of plants pre-treated with 11d of water stress was rapid and exceeded growth of continuously well watered plants. The plants grown under continuous water deficit show reductions in leaf growth, stomata conductance and increase in xylem sap ABA.

### INTRODUCTION

When plants were exposed to drought, their growth rate is impaired due to several physiological and biochemical processes being disturbed. Over the past decade, the role of ABA induced leaf growth and stomatal closure has been investigated and reviewed extensively (Passioura *et al.* 1993; Schulze 1994). Researches have been concentrated on the responses of duration of water deficit on leaf growth and

stomatal closure in relation to ABA, but little information is available on the ability of plants to recover upon stress relief. Under field conditions in the tropics, plants were normally subjected to a period of gradual water stress and on several occasions, this period of water stress is followed by period of rainfall. Gates (1955) indicated that plants which were exposed to a brief period of water stress and then rewatered, showed a better stomatal regulation, and hence

productivity. This supported the earlier findings by Moroton and Watson (1948) who found renewed growth and development upon rewatering in sugar beet, which often proceeded at a more rapid rate than in the continuously watered plants. There were reports on the stomata responses that upon rewatering are associated with ABA metabolism. Doerffling *et al.* (1977) reported that a delayed recovery in stomatal was due to elevated ABA persisting several days after relief of stress. Similarly, Correria and Pereira (1994) showed a 100-fold increase in apoplasmic ABA concentration with soil drying but did not return to pre-stress values immediately following rewatering. In contrast, several other investigators reported that the delay in stomatal recovery is not associated with the residual ABA following rewatering (Loveys and Kreidemann, 1973; Cornish and Zeevart, 1985). The discrepancy could be contributed to the experimental procedures, genotypic differences or plants parts in which ABA have been detected. In pepper plants, Aloni *et al.* (1991) indicated that leaf water potential returned to the control values 24 h after rewatering but photosynthetic rate of stressed plants did not recover and was dependent on the extent to which the water potential had decreased during stress. To our knowledge, the roles of ABA influencing leaf growth and stomata closure upon stress relief in pepper plants has not yet been investigated. In this paper, we report the plant responses upon stress relief and examine the role of ABA influencing leaf growth and stomatal response.

## MATERIAL AND METHODS

### *Experimental Procedures*

Seed of pepper (*Capsicum annuum* cv Bell Boy) were germinated in seed trays under greenhouse conditions. After 7 d, seedlings were transferred to pots with a diameter of 90mm containing a mixture of John Innes compost No 2 and grit. At the 5-6 leaf stage seedlings were transferred to the polyvinyl-chloride (PVC) tubes which were filled with a similar compost mixture. The PVC tubes of 105mm inside diameter and 300mm length accommodated 3.2 litres of compost mixture. The plants in the column were grown in the controlled environment cabinet with temperature at 22-25° C (day) or 18° C (night), relative humidity of 48%-56% and photoperiod of 14h with PFD average of 320  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . The plants grown with daily watering in the

growth cabinet for acclimation. The plants were divided into four groups in which two groups were watered daily or left unwatered. The other two groups consisted of plants that were left unwatered for 5 and 11 days, and then rewatered. The plants randomly arranged in the growth cabinet in four replicates. Leaves were tagged prior to treatments for determination of leaf length increment. Water relations, stomatal conductance and leaf growth were measured at 3, 6, 24, 48, 72, and 96 h after rewatering. The pre-stress values were also recorded prior rewatering. Xylem sap was collected from each plant by extracting extrudate from cut stem portions. A pressure of 0.3 – 0.5 M $\beta$ a above the balance pressure was applied for the collection of extrudate. The first 5mm of extrudate were excluded from the samples. The measurements of leaf water potential and stomatal conductance were carried out on the same leaf. Xylem sap ABA was determined from the extrudate from the cut stem portion of the same plant.

The mean volumetric soil water content was measured in the soil column prior to watering the plants. Three random samples were taken for each treatment and after oven-drying at 80°C for 48h, soil water content was calculated. Leaf water potential was measured on the youngest fully expanded leaf using a pressure chamber on each sampling day. These leaves were then inserted into the 10ml syringe and immediately placed in liquid nitrogen before storage in a deep freezer at -20°C for 5 d before determination of osmotic potential. Two fractions of leaf extrudates were collected from each leaf and osmotic potential of extrudates was determined using a vapor pressure osmometer. Turgor potential was calculated from the difference between water potential and solute potential.

Measurements of stomatal conductance were made on the abaxial surface of the youngest fully expanded leaves with a diffusion porometer (AP-4, Delta-T Devices Ltd, Cambridge).

Leaf length and breadth increments were determined on the leaves that were tagged before treatments began. The leaf length and breadth increments were calculated at each sampling date by measuring the differences between the length on the sampling date and the initial measurement.

Concentration of xylem sap ABA was determined using a radioimmunoassay (RIA) (Quarrie *et al.* 1988).

**RESULTS**

Mean moisture content from different portion of soil column supporting plants was  $0.34\text{g cm}^{-3}$ ,  $0.25\text{g cm}^{-3}$  and  $0.18\text{g cm}^{-3}$  under well watered, 5 and 11 d of water stress, respectively. This reduction in soil moisture content had reduced leaf length by 2 and 4.4 cm in plants which were subjected to 5 and 11 of water stress, respectively, compared to the well watered conditions (Fig. 1). Restoration of water to the plants that were pre-stressed resulted in a resumption of leaf growth. The recovery seemed dependent on the duration of plants being left unwatered. These was an over recovery in leaf growth on the plants that were subjected to 11 d on water stress 24 h after rewatering (Fig. 2).

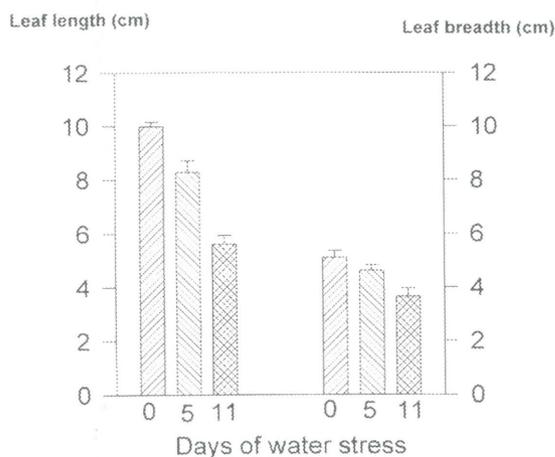


Fig. 1. Leaf length and breadth of pepper plants exposed to duration of water stress. Bars represent S.E. Each point represent means of replicates.

Fig. 3 shows the change in water relations as water was restored to the soil column of water stressed plants. Leaf water potential had already declined to  $-0.65\text{ M}\beta\text{a}$  and  $-1.09\text{ M}\beta\text{a}$  when plants were left unwatered for 5 and 11, respectively. Upon restoration of water, leaf water potential in plants that were subjected to pre-stress treatments, increase immediately and maintained at a similar value to the control throughout the experimental period. Osmotic potential of plants subjected to water stress for 11 d, maintained a lower osmotic potential but increased progressively after rewatering. As expected, plants that were subjected to continuous water stress maintained a continuous

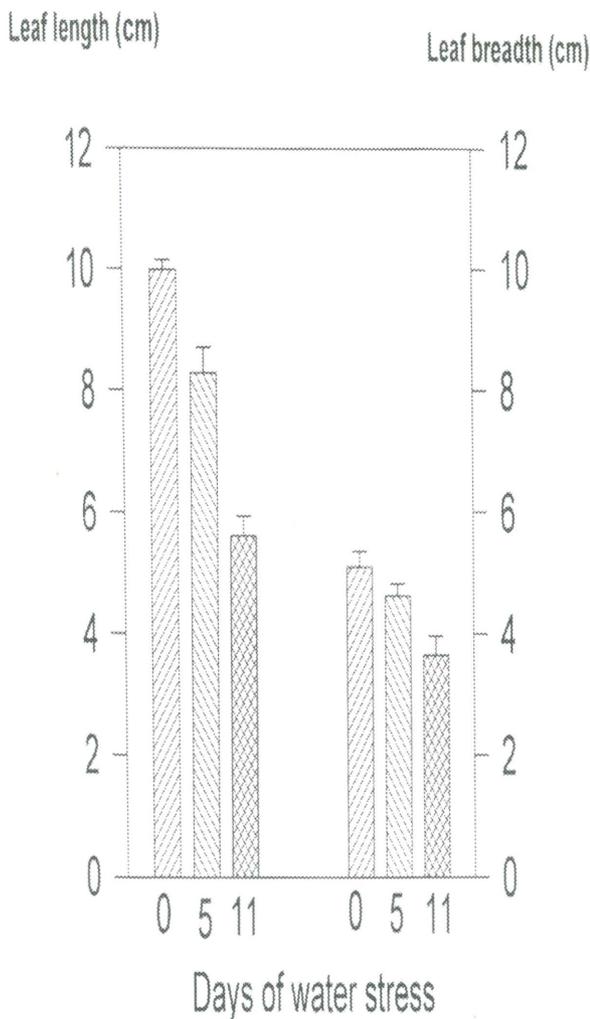


Fig. 2. Cumulative leaf and breadth of pepper plants after rewatering. ● = well watered, ■ = water stress for 5 days and rewatered: □ = water stress for 11 days and rewatered: ○ = continuous water stress. Bars represent S.E. of 6 replicates.

lower osmotic potential. These changes in leaf water potential and osmotic potential in plants subjected to various treatments resulted in changes in turgor potential in which plants left unwatered for 11 d and rewatered, maintained higher turgor than the other treatments.

Stomatal conductance declined progressively with the cessation of watering to the plants, reaching to less than  $100\text{mm}^0\text{l m}^{-2}\text{s}^{-1}$  after 4 – 5 d of water stress. Once plants were rewatered, stomatal conductance began to recover, but not until 48h to reach a similar values to the control. Similar to the effects on leaf length increment,

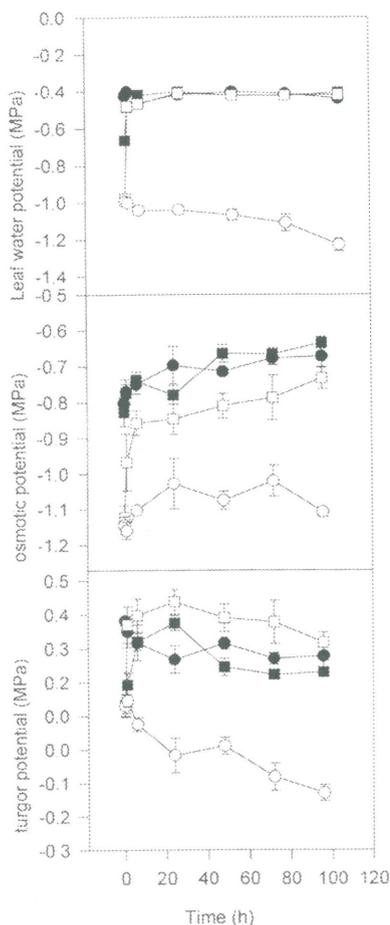


Fig. 3. Changes in water relations upon stress relief of pepper plants. ● = well watered, ■ = rewatered after 5 days of water stress; □ = rewatered after 11 days of water stress; ○ = continuous water stress. Bars represent ± SE of 4 replicates.

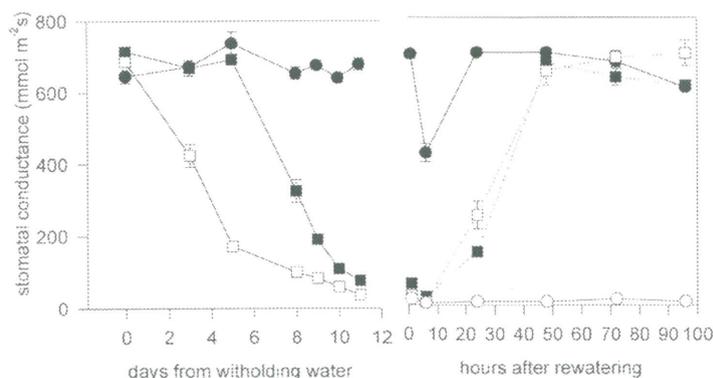


Fig. 4. Stomatal conductance of pepper plants exposed to duration of water stress and the effect on rewatering (●) well watered : (■) rewatered after 5 days of water stress; (□) rewatered after 11 days of water stress; (○) continuously stress. Bars represent S. E. Each point represent means of 6 replicates. Measurements after rewatering at 6 hours were carried out at 6pm where plants had already being exposed to 12 hours of light period and relative humidity had declined to 39% RH.

there were was a tendency that the plants stressed for 11 d to recover at a greater rate than control plants 96h after rewatering (Fig. 4).

Xylem sap ABA in pepper plants when subjected to water stress for 5 and 11 d, increased from less than 40umol m<sup>-3</sup> to over 160umol m<sup>-3</sup>. Xylem sap ABA decreased rapidly in these plants after restoration of water to a similar values to the control 6h after rewatering. Xylem sap ABA in continuous water stress plants increased as water stress proceeded. (Fig. 5).

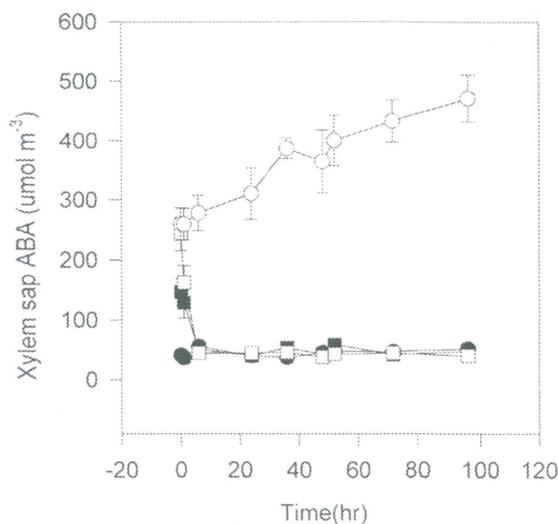


Fig. 5. Xylem sap ABA of pepper plants. ● = well watered : ■ = rewatered after 5 days of water stress : □ = rewatered after 11 days of water stress : ○ = continuous water stress. Bars represent ± SE of 4 replicates.

## DISCUSSION

The reduction in leaf growth is considered as the most sensitive response to water stress in most plant species (Hsiao, 1973; Dale, 1988). The causes of this reduction could be either hydraulic or non hydraulic. Under progressive development of water deficit, the reduction in leaf growth has been correlated with high levels of ABA in leaves or xylem sap (Zhang and Davies, 1990; Passioura, 1988). In our earlier investigation, we showed that early reduction in leaf growth of pepper species occurred without any detectable changes in leaf water potential but coincided with a slight increase in xylem sap ABA (Mohd Razi and Davies 1997). In this experiment, we show a recovery of leaf growth and stomata conductance upon stress relief. The recovery was greater than the control plants especially when plants were rewatered after 11 d of water stress. Plants that were pre-stressed for a period of 5 d and then rewatered resume growth at a similar rate as the control. The resumption of leaf growth and stomatal conductance at a similar of greater rate than the continuous well watered plants upon stress relief is consistent with those observed in earlier study on several plant species (Morton and Watson 1948; Kramer 1950; Gates 1955). Kleinedorst (1975) indicated that an inhibition in leaf growth in maize plants to be due to retardation in cell elongation rather than in cell division, and temporary accelerated leaf growth occurred on discontinuing the stress condition. This supports earlier suggestions by Hsiao and Acevedo (1974) who indicated that the reduction in growth during a mild and short water stress could be offset completely by a rapid transitory phase of growth following release of stress. Aloni, Daie and Karni (1991) investigated the recovery of young transplant pepper plants grown in a rapid soil drying, however, showed a contradictory results on the leaf growth response upon stress relief. They reported that plants which were stressed for 24 and 48h and then rewatered, resume leaf elongation but did not attain similar values as the control even after 10 d and of rewatering due to the disturbance of assimilate partitioning.

The stomatal conductance did not attain a similar values to the well watered plants until 48h after rewatering. This is consistent with observation by Correia and Perreira (1994) upon rewatering of lupins plants exposed to 5 d of

stress, but their data did not show a complete recovery as their experiment was terminated before 48h after rewatering. As watering proceed to the pre-stress plants, stomatal conductance attained a similar of greater value to the control similar to those observed on leaf growth. The days to a complete recovery of stomatal conductance on plants that were previously stressed and rewatered varied according to species e.g: 205 d for tobacco; Fischer, Hsiao and Hagan (1970); 14 d for conifer (Jackson *et al.* 1995).

When plants were rewatered after a period of water stress, stomatal conductance recovers more slowly than leaf water status. Correia and Pereira (1994) indicated that extent of recovery of stomatal conductance upon stress relief is associated with the changes in concentration of ABA in leaf apoplast. They, however, were not able to show a consistent response in a later report (Correia and Pereira 1995). Our results suggest that one of the possibilities in an early recovery of leaf growth and stomatal conductance during stress relief is due to immediate decline in xylem sap ABA. As watering progressed, this role of ABA diminished particularly in plants which were subjected to 11 d of water stress before rewatering. In this case, we could suggest that the recovery of leaf growth due to higher osmoregulation in plants previously subjected to water stress and then rewatered. Fisher *et al.* (1970) suggested that the stomatal opening potential of a given leaf is related to ageing during which there was an increase in the number of leaves separating the leaf from the apex. Since post-stress leaves were closer to the apex representing a physiological younger state contribute to the better stomatal opening compared to the control. Cornish and Zeevart (1985) rewatered the wilted *Xanthium* plants and found that the stomatal reopening did not coincide with the decline of bulk leaf ABA but as a result of elevated levels of ABA remained in the apoplast after the bulk leaf contents had returned to their pre-stress values. We, however, would not be able to suggest the role of ABA in rewatered plants since stomatal conductance was at least 60% lower than the continuous well watered plant 24 h after rewatering when ABA has already declined to a similar values to the control. We could argue that with a gradual development of soil water deficit, it is possible that when plants were watered, ABA could be

more diluted and the concentration would be similar to the well watered plants. There are many reports suggesting that under condition of slight decline in soil moisture content, stomatal closure precedes any detectable increase in xylem sap ABA (Trejo and Davies, 1991; Jackson *et al* 1995). It has been suggested ABA does not impinge directly on stomata immediately after roots were subjected to soil drying. This has been suggested earlier by Weyers and Hillman (1979) indicating that although stomatal reopening occurred when net efflux of ABA was allowed, it is not possible to suggest that uptake of ABA as a single factor was directly related to the events of stomatal closure. The events leading to abscisic acid-evoked stomata closure has been discussed by Blatt and Thiel (1993) who indicated that the progression of events from ABA stimulus is not linear and least pass through Ca and pH intermediate.

Our attempt to explain the recovery of stomata conductance and leaf the basis of the decline in ABA after restoration of water to drying soil, was inconclusive. Relief from water stress results in a decline in ABA concentration to a pre-stress values within a few hours. The stomata, however, require at least 48h to achieve values similar as the continuous well watered plants. We speculated that there may be also involvement of hydraulic factors which regulate cell metabolism in the process of recovery to water stress. The over recovery of leaf growth and stomata response upon restoration of water to the drying soil presumably is associated with the physiological 'younger' condition following turgor recovery.

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