Chapter 3

Chapter 3

Plant Screening : Drought Stress

3.1 Introduction

The role of water in maintaining turgidity for plant growth and survival is widely recognised (e.g. Matin *et al.*, 1989). Low plant water content has pronounced effect on photosynthesis and transpiration, amongst the processes first affected by a given plant water deficits through changes in water potential which in turn induce restriction of the stomatal opening (Hall and Hoffman, 1976; Sharkey and Seemann, 1989). However, others also believed that non-stomatal factors were important for the decline in photosynthesis (Boyer, 1971, 1976; Bunce, 1977; von Caemmerer and Farquhar, 1981; Kreig and Hutmacher, 1986; Gimenez *et al.*, 1992). A change of plant water status also affected cell enlargement which had a significant role in the development of apex and leaves (Hsiao, 1973).

Water stress is ubiquitous to life of terrestrial plants, even under conditions of frequent rainfall like Malaysia. In this chapter, water relations of the four species selected (Chapter 2) were examined in order to choose species which can tolerate water deficit as usually observed on slopes. Some physiological aspects of the shrubs during recovery phase will also be examined in this chapter.

3.2 Materials and Methods

3.2.1 Plant Culture

Four species selected were propagated at the Botanical Garden, University of Malaya, by stem cutting in polybags (13.5x16.5 cm) using light coloured sandy loam (Damansara Enterprise) for 20 replicates for each species. The plants were irrigated twice a day to assist growth with a range of PAR 365-1951 μE m^{-2} s^{-1}. Three months later (day 90), the plants were thinned in pots (22 cm in diameter; 13 cm in height) filled with the mixture of NPK fertiliser and the soil with the ratio of ratio 1:5. All the pots were placed in the glasshouse at the Department of Botany, University of Malaya (temperature 21-32°C, PAR 343-2100 μE m^{-2} s^{-1} and relative humidity of 60-71%). They were well watered every morning for 7 days (day 97) in order to adapt to new conditions.

3.2.2 Water Stress Treatment

Ten replicates from each species were well watered and the other ten pots underwent water-stress cycle for 40 days (day 98 to day 138). Unfortunately, some plants could not survive the stress treatment which reduced the replicates to as low as six pots in some species. Water-stress cycle was imposed by withholding water and rewatering when 75% of the leaves per plant showed sign of visible wilting. Later, the recovery period was observed for 30 days, between day 139 - 168. Water relation parameters were measured during the wilting and recovery phases.

3.2.3 Measurements

(a) Leaf Water Potential

Five leaves were taken randomly from each treatment at 8.00 a.m. Water potential was measured using pressure chamber technique (Scholander *et al.*, 1965). This technique was widely used in characterising plant water status (Turner *et al.*, 1978).

(b) Relative Water Content (RWC)

The method of Weatherly and Slatyer (1962) was used. Five fully expanded leaves were taken randomly from each treatment at 8.00 a.m. The leaves were weighed immediately to determine the fresh weight (FW), then were floated on distilled water (in petri dish) while being illuminated by fluorescent lamp at compensation light intensity over night. After this, the leaves were blotted gently with tissue paper and weighed to determine the turgid weight (TW). The pieces were oven dried (80°C) to constant weight.

(c) Photosynthesis, Transpiration Rate, Water Use Efficiency and Stomatal Conductance

Five fully expanded leaves were taken randomly from each treatment using equipment described earlier (2.2.2a) The measurements were taken between 12 - 2 p.m. with a range of PAR 1800-2100 $\mu E\ m^{-2}\ s^{-1}$. The instantaneous water use efficiency (WUE) was determined by:

$$WUE = \frac{\text{Photosynthesis rate (A)}}{\text{Transpiration rate (E)}}$$

(d) Leaf Area

The leaf area was measured as described earlier (2.2.2d).

3.3 Results and Discussion

3.3.1 Species Performance

All species in both treatments (except *H.mutabilis*) survived at the end of the experiment (Table 3.1). However, 12.5% and 55.6% of *H.mutabilis* in well watered and stress treatments, respectively, died. It appears that in water stress condition less than

Table 3.1: Species performance at the beginning and the end of the treatment (n=7-10) Highlighted species was much affected by the stress.

Species	**Survival rates (%)**	
	Well	**Stress**
J.betonica	100	100
H. mutabilis	87.5	44.4
L.camara	100	100
T.erecta	100	100

half of them survived. Obviously, in as far as survival rate is concerned, this species cannot tolerate water deficit conditions and therefore are not suitable as slope plant.

Despite the low survival rates, *H.mutabilis* seemed to adapt to water stress condition after the first water-stress cycle. It took two days before it wilted for the first time but it took longer time to experience the second wilting and so on (Table 3.2).

Survival rate of other species under the magnitude of water deficit in the experiment was 100 %.

3.3.2 Visual Observations

L.camara showed the best performance through visual observation of wilting frequency, 7x in 40 days (Table 3.2). *J.betonica*, *H.mutabilis* and *T.erecta* wilted 12, 8 and 8x, respectively. Grown in the size of pot in the experiment (1.5 litres), *L.camara* could withstand up to 6 days without water compared to *J.betonica*, 3 days, *T.erecta*, 4 days and *H.mutabilis*, 5 days, before showing any sign of wilting. *L.camara* also produced flower although the flowering rate was not as high as to the watered treatment (100% flowering rate - data were not shown).

The response of *T.erecta* was similar to *L.camara* for water-stress conditions. Moreover, it also showed low flowering rate towards the end of the treatment. Other species did not flower during the duration of the observation.

Unlike other species, *J.betonica* showed leaf rolling after about two weeks of treatment. Leaf rolling is known to be a strong manifestation of leaf response to water deficits. Rolling minimises water loss from the upper leaf surfaces and, consequently, imparts a higher degree of tolerance to water deficit (O'Toole and Cruz, 1980). Therefore, in the stressed *J.betonica*, partially rolled leaves may be associated with low stomatal sensitivity in maintaining high water potentials.

Table 3.2 : Visual observations of 4 species during the water-stress treatment within 40 days (n=7-10).

Days	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***	***11***	***12***	***13***	***14***	***15***	***16***	***17***	***18***	***19***	***20***
J.betonica			X			X			X			X				Xc			Xc	
H.mutabilis			X					X						X				X		
L.camara						X						X				X				
T.erecta					X					X					X					X

Days	***21***	***22***	***23***	***24***	***25***	***26***	***27***	***28***	***29***	***30***	***31***	***32***	***33***	***34***	***35***	***36***	***37***	***38***	***39***	***40***
J.betonica		Xc			Xc				Xc				Xc			Xc			Xc	
H.mutabilis			X						X				X						X	
L.camara	X_1					X_1						X_1							X_1	
T.erecta				X					X			X				X_2				

X = at least 60 % of the replicates showed the wilting sign

Xc= 90 % of the replicates showed partial rolling leaf

X_1= 25 % flowering rate

X_2= 10 % flowering rate

3.3.3 Leaf Water Potential

Leaf water potential of the stressed plants of all species were greatly affected by water deficits conditions (Table 3.3). Except *T.erecta*, it was interestingly observed that water potential of water-stressed plants never decreased to the initial value of stressed condition (after 10 days of treatment). In this case, rapid stomatal response may become a drought resistance mechanism to conserve water for later use. This observation is similar to the reports of other studies which concluded that higher total leaf water potential indicated increased drought resistance (e.g. Matin *et al.*, 1989).

3.3.4 Relationship of Stomatal Conductance and Leaf Water Potential

Higher positive correlation between these two parameters have been observed in *J.betonica*, *L.camara* and *T.erecta* and low positive correlation in *H.mutabilis* (Fig. 3.1). The results agree with most other studies, that stomatal conductance decreased as leaf water potential is reduced (e.g. reviews by Chaves, 1991; Cornic, 1994). However, *H.mutabilis* showed a weak positive correlation. Results suggest the various degrees of stomatal closure observed amongst the species in response to water stress may depend on age, growth conditions, pre-stressed history and magnitude of stress (Beadle *et al.*, 1978).

3.3.5 Influence of Leaf Water Potential on Photosynthesis Rate

Photosynthesis rates was linearly related to leaf water potential in all species studied (Fig. 3.2). This relationship indicates that photosynthesis rates of four species were very sensitive to water deficits conditions which may be due to stomatal closure (Fig. 3.1). Studies have also found that leaf water deficits effect chloroplast biochemistry that would contribute to depressions in photosynthetic performance (Kaiser, 1987 and Schulze, 1986).

Table 3.3 : Leaf water potential of the species throughout the experiment (W=well-watered, S=water-stressed) with n=7-10.

DAYS	**10th**		**20th**		**30th**		**40th**	
LEAF WATER POTENTIAL (-MPa)	**W**	**S**	**W**	**S**	**W**	**S**	**W**	**S**
J.betonica	0.12±0.01	0.44±0.01	0.08±0.02	0.28±0.04	0.09±0.02	0.23±0.02	0.07±0.01	0.18±0.03
H.mutabilis	0.11±0.04	0.24±0.03	0.09±0.01	0.25±0.09	0.07±0.02	0.13±0.01	0.05±0.02	0.12±0.02
L.camara	0.10±0.04	0.20±0.03	0.09±0.06	0.17±0.06	0.03±0.004	0.16±0.03	0.04±0.01	0.06±0.002
T.erecta	0.19±0.03	0.30±0.0	0.26±0.02	0.37±0.01	0.23±0.03	0.35±0.01	0.18±0.01	0.31±0.02

Fig. 3.1 : Relationship between stomatal conductance and leaf water potential. Each point represents the mean of 7-10 determinations.

Fig.3.1: Relationship between stomatal conductance and leaf water potential

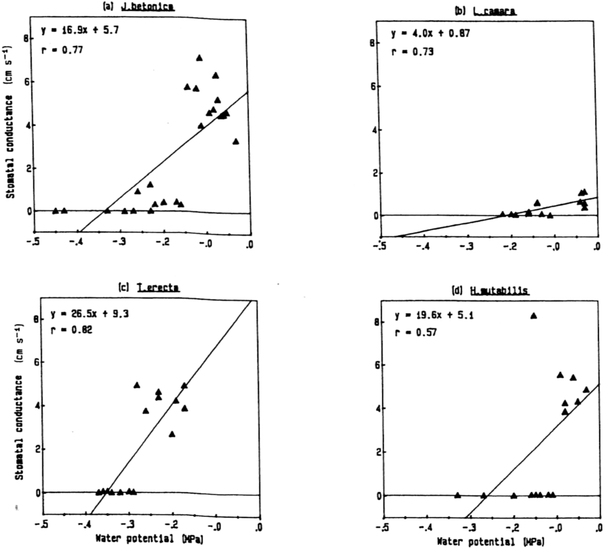
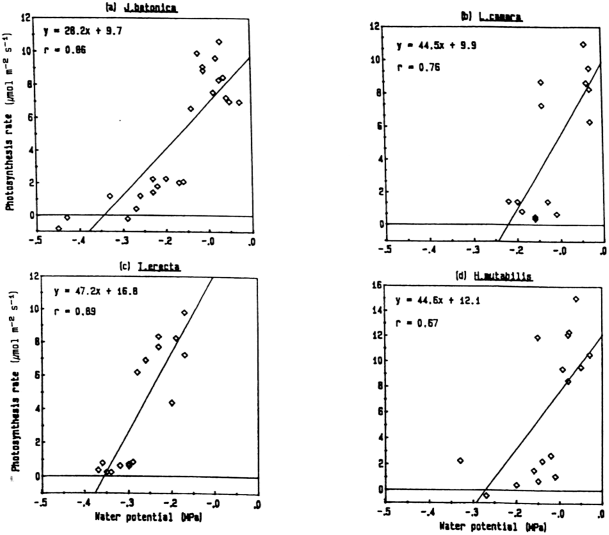


Fig. 3.2 : Relationship between photosynthesis rate and leaf water potential. Each point represents the mean of 7-10 determinations.

Fig.3.2: Relationship between photosynthesis rate and leaf water potential



3.3.6 Photosynthesis Rates and Stomatal Conductance

Photosynthesis and stomatal conductance of all species appeared to be affected by water stress conditions. All of the species showed strong relationship between the parameters (Fig. 3.3). The results imply that a decrease in net photosynthesis always goes hand-in-hand with a decrease in stomatal conductance.

The results also showed that when stomatal conductance was extremely low due to water deficits, photosynthesis rates approached zero. This may be due to the stomatal contribution to the protective mechanism against water shortage. Decreases in photosynthesis under water stress conditions, are generally considered to be the result of stomatal closure and a subsequent decrease in the CO_2 partial pressure in both intercellular spaces and the chloroplast (Tourneux and Peltier, 1995).

3.3.7 Transpiration Rates and Stomatal Conductance

All species studied showed positive correlation between these parameters (Fig. 3.4). The results indicate that transpiration rate was dependant on stomatal aperture in all species. Like most other species, these four species studied seemed to respond to water stress by stomatal closure. Thus, a reduction in the rate of water loss due to higher transpiration occured (e.g. Biehler *et al.*, 1997).

3.3.8 Recovery from Water Stress

Generally, the results show that the parameters of plant water relations of some species had been improved after a few cycles of stress. Photosynthesis of *T.erecta* recovered to the control value of watered plants whereas *J.betonica* and *L.camara* recovered almost to the control value (Fig. 3.5). Unlike other species, water stress had a greater effect on photosynthesis of *H.mutabilis*. This species showed non-consistent

Fig. 3.3 : Relationship between photosynthesis rate and stomatal conductance. Each point represents the mean of 7-10 determinations. The line was not forced through the (0, 0) origin.

Fig.3.3: Relationship between photosynthesis rate and stomatal conductance

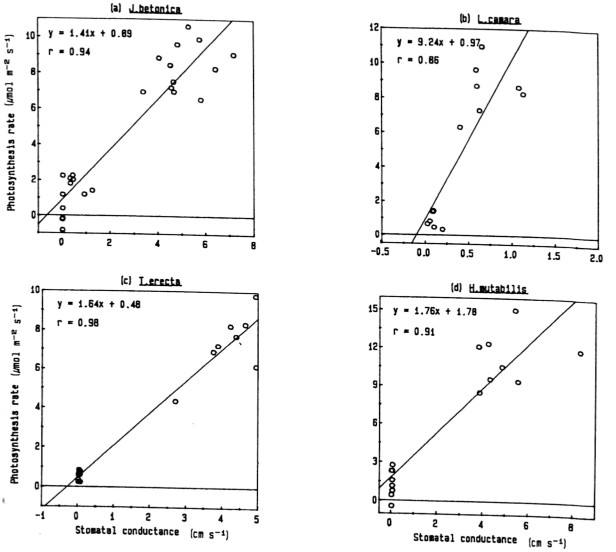


Fig. 3.4 : Relationship between transpiration rate and stomatal conductance. Each point represents the mean of 7-10 determinations. The line was forced through the (0, 0) origin, assuming the cuticular transpiration is not significant.

Fig.3.4: Relationship between transpiration rate and stomatal conductance

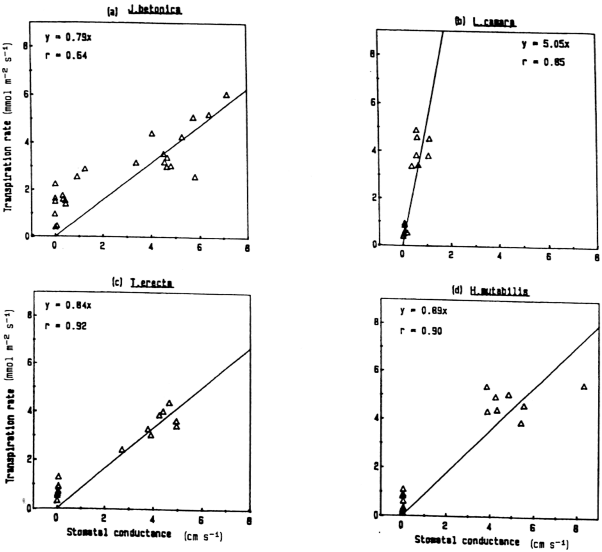
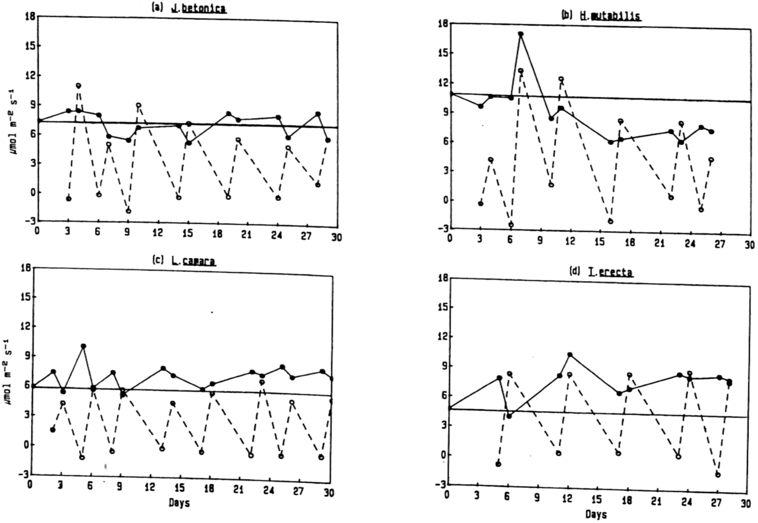


Fig. 3.5 : Photosynthesis rates in (●) well watered and (○) water-stressed shrubs. Each point represents the mean of 7-10 determinations. Each point where photosynthesis value goes up indicate also watering times.

Fig.3.5: Photosynthesis rates in well-watered (•) and water-stressed (○) shrubs



with abrupt changes of the photosynthesis rates under water-stress condition. Transpiration rate of all species did not seem to recover and the plant maintained lower transpiration rate throughout the experiment (Fig. 3.6). This is perhaps due to protective mechanism by stomatal closure mentioned earlier (3.3.6.) Interestingly, WUE of all treated shrubs, except *H.mutabilis*, reached values above the control throughout the experiment (Fig. 3.7). High photosynthesis and low transpiration rate is likely the cause of this.

The RWC value of treated *T.erecta* returned to above the control value after day 18 while *J.betonica* and *L.camara* returned to almost the same level of well watered plants throughout the experiment (Fig. 3.8). However, RWC of treated *H.mutabilis* did not reach the control values of well watered plants.

Thus, overall results imply that *J.betonica, L.camara* and *T.erecta* have the ability to maintain higher photosynthesis rate and RWC, and improved WUE when water was limited. These criteria could be considered as possible mechanism of drought resistance in these species.

3.3.9 The Effects of Water Stress on Leaf Area

Leaf area of treated *H.mutabilis* and *T.erecta* were very much reduced by water stress (Fig. 3.9). The results were consistent with the evidence that photosynthetic activity, and thus CO_2 assimilation, tended to be depressed with stomatal closure during an episode of water stress (Chaves and Pereira, 1992; Quick *et al.*, 1992). Upon stress recovery, these species recovered for most other physiological parameters except leaf area.

Water deficits did not give much impact on the leaf area of *J.betonica* and *L.camara*. Somehow, there was no definite explanation to describe these observations. It may be speculated that maintenance of high RWC exhibited by these species during the episode

Fig. 3.6 : Transpiration rates in (■) well watered and (□) water-stressed shrubs. Each point represents the mean of 7-10 determinations. Each point where transpiration value goes up indicate also watering times.

Fig.3.6: Transpiration rate in well-watered (■) and water stressed shrubs (□)

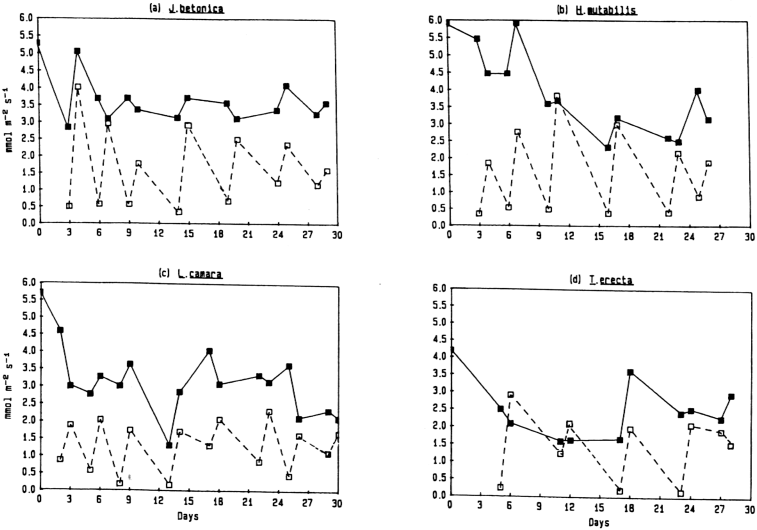
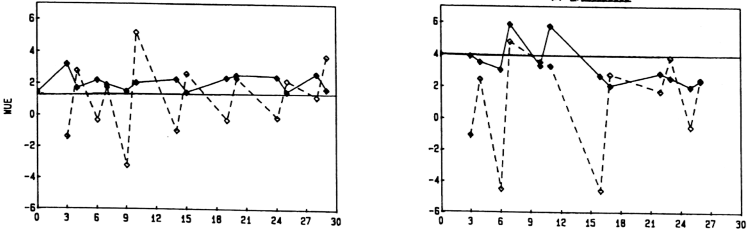


Fig. 3.7 : WUE in (◆) well-watered and (◊) water-stressed shrubs. Each point represents the mean of 7-10 determinations. Each point where WUE value goes up indicate also watering times.

Fig.3.7 : WUE in well-watered (◆) and water stressed shrubs (◇)

(a) J.betonica

(b) H.mutabilis



(c) L.camara

(d) T.erecta

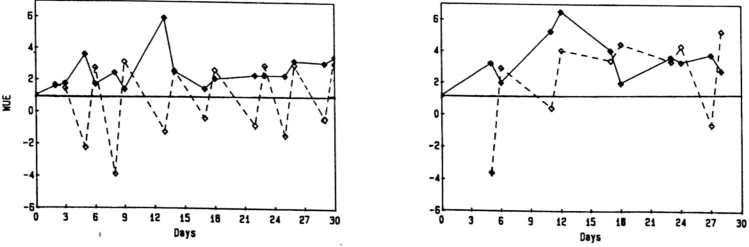
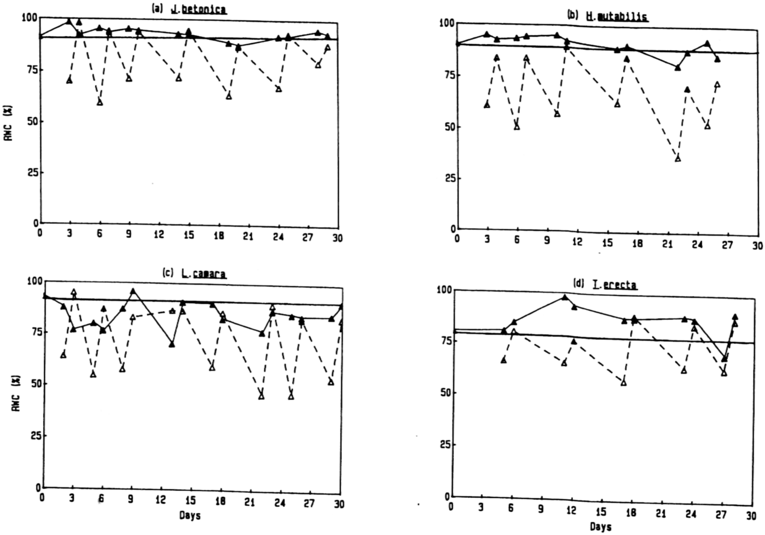


Fig. 3.8 : RWC in (▲) well-watered and (Δ) water-stressed shrubs. Each point represents the mean of 7-10 determinations. Each point where RWC value goes up indicate also watering times.

Fig.3.8 : RWC in well-watered (▲) and water stressed shrubs (△)



(a) J.betonica

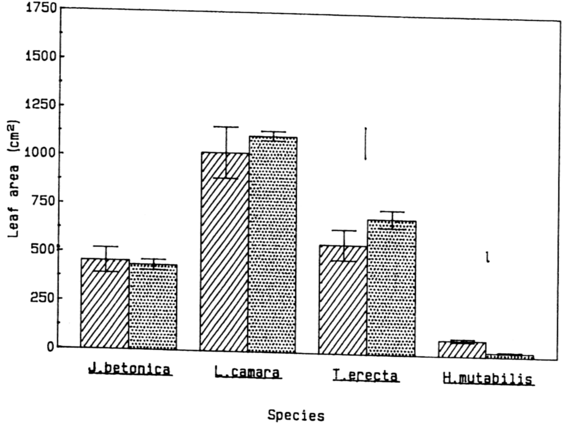
(b) H.mutabilis

(c) L.camara

(d) I.erecta

Fig. 3.9 : Leaf area of (▨) well-watered and (⊞) water-stressed of the plant studied. $LSD_{p<0.05}$=131.0 and 13.4 for *T. erecta* and *H. mutabilis*, respectively.

Fig.3.9: Leaf area of well-watered and water-stressed of the plant studied



of water-stressed conditions maintained physiological processes affecting production of leaf area (Schopfer, 1995).

3.4 General Discussion

The stomatal conductance of all species studied were conceivably sensitive to water stress condition (Fig. 3.1). This implies a conservative behaviour which was pronounced in all species. In addition, all species, except *H.mutabilis,* maintained high WUE throughout the experiment (Fig. 3.7). Apart from WUE, other physiological criteria showed different responses among the four species to water stress. The drought resistant character had been observed in these species in terms of photosynthesis, WUE and RWC. In view of this, *T.erecta*, *L.camara* and *J.betonica* are considered to be suitable species as slope plant (*H.mutabilis* did not pass this screening procedure and it is not included in the list of suitable species as slope plant). In the next chapter, water relations of these three species are examined further, in particular respect to the significance of pre-treatment.