

The impact of water stress on stomatal patterns in wheat leaves

OWDAH A. AL-SOBHI

Biology Department, Faculty of Science, Taibah University, P.O. Box 6780,
Al-Madinah Al-Munawwarah, (Kingdom of Saudi Arabia).

(Received: September 26, 2007; Accepted: October 19, 2007)

ABSTRACT

The present investigation focuses on the effect of water stress on the stomatal pattern in wheat leaves and the role of herbicide to protect plants from such stress. The mean plant height and number of leaves per plant were reduced as the water stress increased, either when the osmotic potential of the polyethylene glycol (PEG) solutions were increased or when water stress withheld from plants grown in compost. The seedlings grown in PEG had smaller roots compared to the control and showed a bright yellow in color. As the osmotic potential of the solution was increased, the Stomatal Index of the leaf was reduced.

Key words: wheat leaves, water stress, stomata patterns, PEG

INTRODUCTION

Many plant species are able to adapt to conditions of stress and undergo changes in their morphology, anatomy and physiology as a survival strategy. An example of this is seen in the work of Clausen *et al.* (1940) with *Potentilla glandulosa*, where plants from different habitats showed variable growth forms as a response to different environmental factors. Climatic changes have the potential to alter patterns of specific differentiation of stomata in plants (Woodward, 1987). It is well known that stomata density extremely varies between species, habitats, height of insertion and leaf area (Ticha, 1982; Smith *et al.*, 1989). Stomata density can also be affected by a number of environmental factors including altitude, light, shade, temperature and water availability (Schoch, Zinsou & Sibi, 1980; Yegappan *et al.*, 1982; Rahin & Fordhan, 1991). Many previous studies showed that the effects of elevated CO₂ were confounded by other environmental factors affecting the expansion of leaf area (Beerling & Chalone, 1993). Drought

can cause stomata density to increase and low irradiance can have the opposite effect, both owing to changes in the degree of leaf cell expansion (Ticha, 1982).

MATERIAL AND METHODS

Growth conditions and water treatments

Wheat seeds were soaked for 24 hours in water prior to planting into either soil or vermiculate. The trays were placed in a dark room for five days and seedlings were allowed to grow at 25°C with 100% humidity. They were then transferred to an illuminated growth room for the required length of time at 24°C ± 2°C with a photoperiod of 16 hours and at a light intensity of 160mmol m⁻¹ S⁻¹ at plant level. The seedlings were removed from the trays and the roots were soaked with water to remove any vermiculate. Small pots were filled with compost and two seedlings were placed in each pot. They were then left to grow for 7 days before treatment begun. They were then treated as follows: 100 ml of water every day, every 4 days or every 8 days.

The pots were placed in the illuminated growth room for a period of 16 days. The third and youngest leaves of each plant were removed in order that stomata peels can be taken.

PEG treatments

Seedlings of approximately equal height were removed from the trays and the roots washed with water. They were then inserted into the jars and secured with cotton wool with the coleoptiles just below the lids. The following solutions were placed in separate culture jars: -5 bar PEG, -10 bar PEG, 2, 4-D, -5 bar PEG+ 2, 4-D and water as a control. The seedlings were inserted into the jars and transferred to the illuminated growth rooms for 7 days, 14 days or 28 days; after these periods stomata peels were taken from the third leaf and the youngest leaf possible.

Measurements of Stomatal Index

Five plants of each treatment were sampled at each period of growth (see section above). Leaves were gently washed in a 1% solution of tee pal when dry; a thin layer of clear nail varnish was applied on to the upper side of each leaf. The part of the leaf used in this study was 1 cm from the tip and 1 cm from the base of the leaf. The varnish was allowed to dry for 20-30 min before being gently peeled off with forceps, placed on a microscope slide and covered with a cover slip. The leaf replicas were examined under the light microscope to obtain the stomata density and the Stomatal Index (SI) for each leaf. The number of stomata and the number of other epidermal cells were counted from ten half fields of view; Stomatal Index (SI) that relates the number of stomata per unit area (S) to the number of epidermal cells per unit area (E), was calculated according to Salisbury (1927) as follows: $SI = [S / (E+S)] \times 100$.

RESULTS AND DISCUSSION

The results obtained in this investigation clearly showed that the fresh and dry weight were reduced as the water stress increased, either when the osmotic potential of the PEG salutations were increased or when water stress withheld from plants grown in compost (Table. 1). Many of the leaves were dry and yellow in color, particularly when treated with -10 bar PEG Solution. It was

Table 1: Mean fresh and dry weight of root and shoot per wheat plant (g).

PEG osmotic potential (bars)	Mean weight per plant (g)			
	Shoot		Root	
	Fresh	Dry	Fresh	Dry
-10	0.09	0.018	0.057	0.010
-8	0.10	0.020	0.058	0.010
-6	0.12	0.022	0.059	0.011
-4	0.14	0.025	0.100	0.013
-2	0.16	0.030	0.180	0.014
0	0.19	0.032	0.190	0.015

visible, when compared with the control, that the PEG solution greatly reduced the number of roots produced and also limited their branching. The seedlings grown in PEG had very slimy roots which were bright yellow in color compared with the controls. The fresh weight of the roots was shown to decrease as the osmotic potential increased. A similar decrease was seen in the dry weight except for plants grown in -10 bar solution which showed a slight increase (Table. 1). Under conditions of water stress wheat seedlings undergo changes in their morphology, anatomy and physiology in order to reduce water loss (Al-Sobhi, 2002). The imposition of the water stress resulted in a reduction in growth, both in the roots and shoot. Since water is essential for photosynthesis and hence growth, as less became available the growth was reduced in order to conserve water (Al-Sobhi, 2002). Also, the surface area of the leaf is proportional to the amount of water lost, therefore the reduction in leaf growth reduces the surfaces area and hence the amount of water lost from the leaf.

The results showed without exception that as the amount of water was limited, the Stomatal Index from the tip and base of the leaf were also reduced. Similar trends were seen with the youngest leaf, with one exception. The results from the leaf base of the seedlings watered every 4 days, showed a slight increase in Stomatal Index. The results showed conclusively that, as the osmotic potential of the solution was increased, the Stomatal Index of the leaves was reduced (Fig. 1). In order to assess the validity of the stomatal counting method, peels were taken from the third leaf of four individual wheat

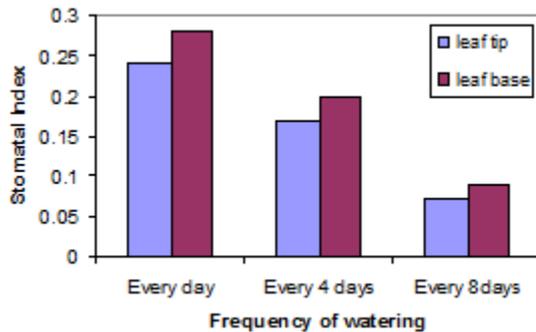


Fig. 1: Effect of frequency of watering on the stomatal index.

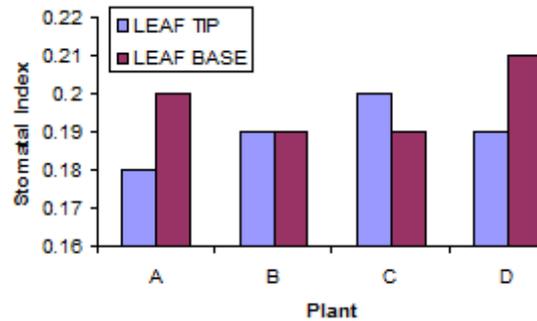


Fig. 2: Confirmation of the effect of the osmotic potential on the stomatal index.

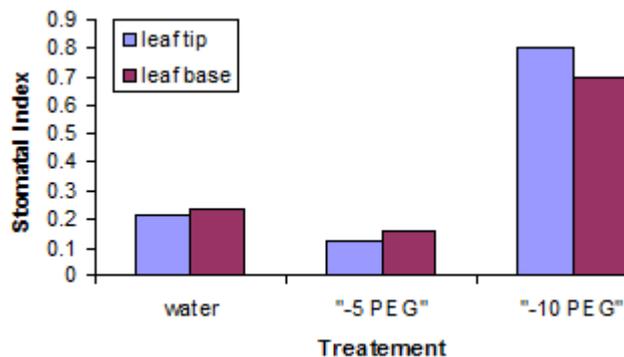


Fig. 3: Effect of the PEG osmotic potential on the stomatal index.

seedlings. The variation in the stomatal index was not significant as shown in Fig. 2. The results also showed that as the osmotic potential of the solution was increased, the stomatal index of the leaf was reduced (Fig. 3). Stomata control the amount of water lost from the leaf by transpiration, therefore a reduction in Stomatal Index reduces water loss. Water stress influences the amount of water that is available to the plant and therefore it must adapt to

conserve water and allow the plant to survive the stressful conditions (Al-Sobhi, 2002). The long term investigation however, revealed no reduction in the number of stomata and instead an increase was seen. This indicates that over longer periods of time the seedlings can overcome the imposed drought stress and produce stomata as reported by Hay (1976) and Zemskaya (1984) by reducing the levels of proline accumulation under water stress.

REFERENCES

1. Al-Sobhi, O. A., Effect of NaCl salinity on stomatal abundance and stomatal index of *Calotropis procera* (AIT). *Bulletin of pure and applied Sciences.*, 21B: 123-130 (2002).
2. Beerling, D. J. and Chaloner, W. G., The impact of atmospheric CO₂ and temperature change on stomatal density: observations from *Quercus robur lammas* leaves. *Annals of Botany.* 71: 231-235 (1993).
3. Hay, E. W., Herbicide transport in plants. In *Herbicides: Physiology, Biochemistry, Ecology*, 2nd ed. Vol.1(ed. I. J. Audus) pp. 363-396, Academic Press London New York and San Francisco (1976).

4. Rabin, M.A., and Fordham, R., Effect of shade on leaf and cell size and number of epidermal cells in garlic (*Allium sativum* L.) *Annals of Botany*. **67**: 167- 171 (1991).
5. Salisbury, E. J. Ecological significance of stomatal frequency with special reference to the woodland flora. *Philosophical Transaction of the Royal Society*. **216B**: 1-65 (1927).
6. Schoch, P. G., Zinsou, C. and Sibi, M., Dependence of the stomatal index on environmental factors during stomatal differentiation in leaves of *Vigna sinensis* L. *J. of Experimental Botany*. **31**: 1211-1216 (1980).
7. Smith S., Weyers J . D. B. and Berry, W.G., Variation in stomatal characteristics over the lower surface of *Commelina communis* leaves. *Cell and Environment*. **12**: 653-659 (1989).
8. Ticha, I., Photosynthetic characteristics during ontogenesis of leaves, stomata densities and sizes. *Photosynthetica*. **11**(2): 365-471 (1982).
9. Woodward, F. I., Stomatal number is sensitive to increases in CO₂ from pre-industrial levels. *Nature.*, **327**: 617-618 (1987).
10. Yegappan, T. M., Patton, D. M., Gates, C. T. and Maller, W.J., Water stress in Sunflower (*Helianthus annuus* L.) II: Effects on leaf cells and leaf area *Annals of Botany*, **49**: 63-68 (1982).
11. Zenskaya, V. A., Khilik, L. A., Karpova, G. YA., Kalibernaya, Z. V. and Chernikova, L. M., Penetration, transport and conversion of 2, 4-D in the lavender plant. *Soviet Plant Physiology English Translation.*, **30**(52): 549-556 (1984).