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Water Stress Induced Stomatal Closure in Two Maize Cultivars

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Abstract: The objective of this research was to evaluate the effect of water stress on stomatal closure of 2 maize cultivars. Water stress was generated by additions of Polyetylene glycol 6000 to the root medium. Water potentials were: zero (control), - 0.15 (PEG 10%), - 0.49 (PEG 20%), -1.03 (PEG 30%) and -1.76 (PEG 40%) MPa. After 24 h treatment, the leaves stomatal of 2 maize (*Zea mays* L.) cultivars -704 and 301- were photographed in various concentrations of PEG 6000. Stomatal closure was particularly apparent at low water potentials. In low water stress, stomata were open and in moderate water stress, leaves started to close their stomata. Maize leaves closed their stomata after 24 h PEG treatments and stomatal closure in 704 var. was higher than 301 var. Therefore, the stomata of 704 var. were sensitive to water stress than 301 var.

Key words: Maize, polyethylene glycol 6000, stomatal closure, water stress

INTRODUCTION

Selection of plant species/crop cultivars with considerable resistance to drought stress has been considered an economic and efficient means of utilizing drought-prone areas when appropriate management practices to reduce water losses (Turner, 1991). Drought is one of the most limiting environmental stresses for plant production (Kramer and Boyer, 1995). The growth and development of plants on sites experiencing occasional periods of drought stress depends on the ability of stomata to control water loss while maintaining growth. Plants respond to drought by closing their stomata, which reduces leaf transpiration and prevents the development of excessive water deficits in their tissues. The drawback of the stomatal closure for plants is that their carbon gain is lowered and their growth is impaired.

Guard cells are highly specialized epidermal cells that are located in pairs on the aerial organs of plants. Each pair of guard cells forms a pore or "stoma" that closes and opens in response to osmotic shrinking and swelling of the guard cells, respectively. Stomata play a major role in controlling gaseous exchange, especially of photosynthetic carbon dioxide uptake and in water release by transpiration in response to changes in the surrounding environment. The regulation of stomatal closure is thus extremely important for the survival of plants. The rate of transpiration can be maintained until a critical amount of soil moisture is reached (Dunin and Aston, 1984), but some studies suggest a linear decline in transpiration with decreasing soil water (Gollan *et al*., 1985).

Water potential is considered to be a reliable parameter for measuring plant water stress response. It varies greatly, depending on the type of plant and on environmental conditions. When leaf water potential declines, stomatal aperture decreases, reducing transpiration and allowing leaf water potential to recover. Leaf water potential per se may not be the transducer of stomatal response to drought stress (Hinckley *et al*., 1991) but, in general, there is usually a range of leaf water potential over which stomatal conductance remains unaffected. When a certain threshold value of leaf water potential is reached, photosynthesis declines, internal CO_2 concentration increases and the stomata close in a linear or curvilinear way until stomatal conductance approaches zero (De Lucia and Heckathorn, 1989).

Increased field survival in hardened or stress-tolerant transplants may result from their altered stomatal regulation. For example, Spence *et al*. (1986) reported that plant stomata, adapted to drought stress, maintain stomatal opening at lower plant water potentials than no adapted plants. Stomata from drought-stressed plants were smaller, had a different shape and had a mechanical advantage over no stressed plants in opening.

Stomata closure in response to leaf desiccation and/or a transported hormonal signal produced in the root in response to root desiccation (Davies *et al*., 1994). The control of leaf stomatal closure is a crucial mechanism for plants, since it is essential for both CO_2 acquisition and desiccation prevention (Dodd, 2003). The fundamental role played by plant water status (Meidner and Mansfield, 1968) in controlling stomatal aperture

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in most plants is well documented. Stomata (holes) in the leaves call stomata close to reduce water loss. This not only reduces water loss, but also limits carbon dioxide from entering the leaf.

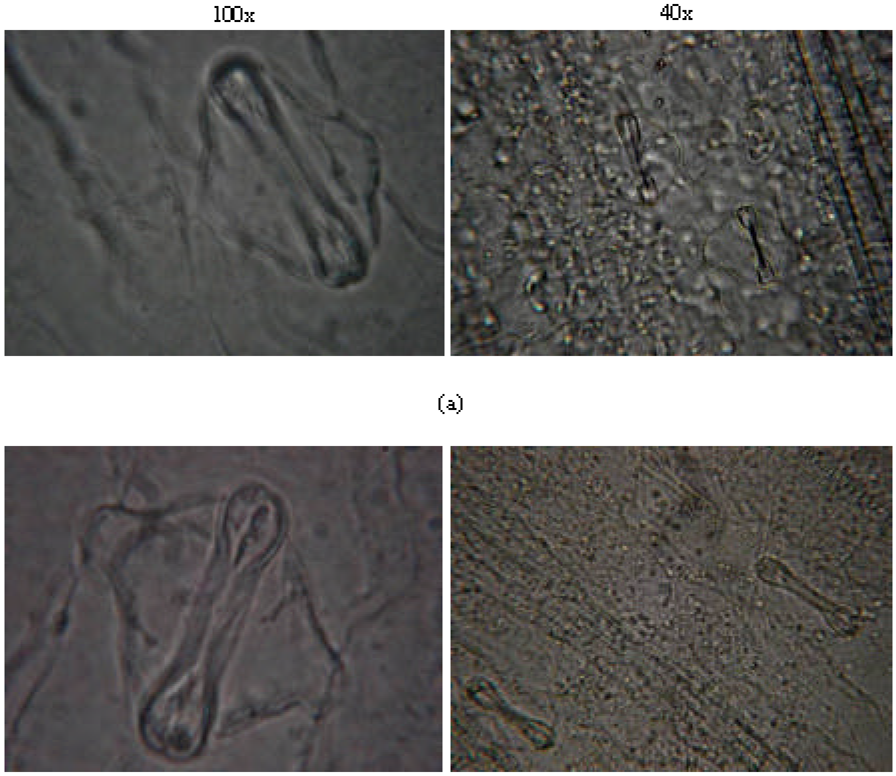
The aim of the present study was to undertake a comparative analysis of the effects of water stress on stomatal closure and assessing stomatal closure as a water-saving mechanism in leaves of 2 maize cultivars.

MATERIALS AND METHODS

Plant materials and growth conditions: This study was conducted at biochemistry laboratory, Department of Biology, Urmia University, Iran, during the spring of 2007. Two genotypes of maize (*Zea mays* L.) -var 0.704 and var.301- were used. The seeds of both cultivars were germinated in Petri dishes on 2 layers of filter paper at 25°C in an incubator. After three days, the seedlings transferred to plastic pots (15 cm diameter, 20 cm depth) filled with sand and irrigated with half strength of Hoagland nutrient solution. Six days seedlings were removed from the sand, washed with tap water, dried and transferred to hydroponics culture of aerated test tubes containing Polyethylene Glycol (PEG) 6000 solutions of 10, 20, 30 and 40% strengths to achieve water deficit levels of - 0.15,-0.49, -1.03 and -1.76 MPa, respectively (Burlyn and Mirrill, 1973; Steuter *et al*., 1981; Nicholas, 1989) as treatments and aerated test tubes containing half strength Hoagland nutrient solution which served as control. Stress was applied for 24 h.

Stomatal photographs: Photographs of stomata were obtained from maize leaves. The abaxial epidermis was peeled from leaves of 7-day-old plants. The epidermis of control and treatment plants 24 h after water stress was cut with blade and then slices were colored and put in glycerol. The slices of both varieties were placed on the upper side on microscope slides and were observed by light microscopy in a light microscope type Zeiss at magnifications of 40x and 100x were photographed using a digital camera. Figure 1 shows open stomata in control plants of 2 varieties. Figure 2 shows closing stomata in 704 var. and Fig.3 shows closing stomata in 301 var. in various concentrations of PEG 6000.

100x 40x



(a)

(b)

Fig. 1: Photographs of maize leaf surfaces showing open stomata in control plants in 704 var. (top) and 301 var. (bottom) of maize at magnifications of 40x (right) and 100x (left)

RESULTS AND DISCUSSION

Stomata are known to close in response to drought to limit water loss by transpiration. During this process, ABA is synthesized and plays a role in closing stomata. The closure of the stomata can be explained by a drop of water potential. Photographs show stomata in magnifications of 40x (right) and 100x (left). Photographs of control leaves (water potential zero) showed open stomata (Fig. 1). In PEG 10% (water potential -0.15) stomata were open in 2 varieties (Fig. 2, 3 a), too. However, plants closed their stomata 24 h after severe water stress (Fig. 2 and 3d). In PEG 20% (water potential -0.49) stomata started to close in 704 var. (Fig. 2b), but they were open in 301 var. (Fig. 3. b). In PEG 30% (water potential -1.03) and PEG 40% (water potential -1.76) stomata completely closed in both varieties (Fig. 2, 3 c, d), but stomata-closing in 704 var. was higher than 301 var. It means that the stomata of 704 var. were sensitive to water stress than 301 var.

Leaf tissues exposed to the water stress partially but not fully close their stomata in moderate water stress and

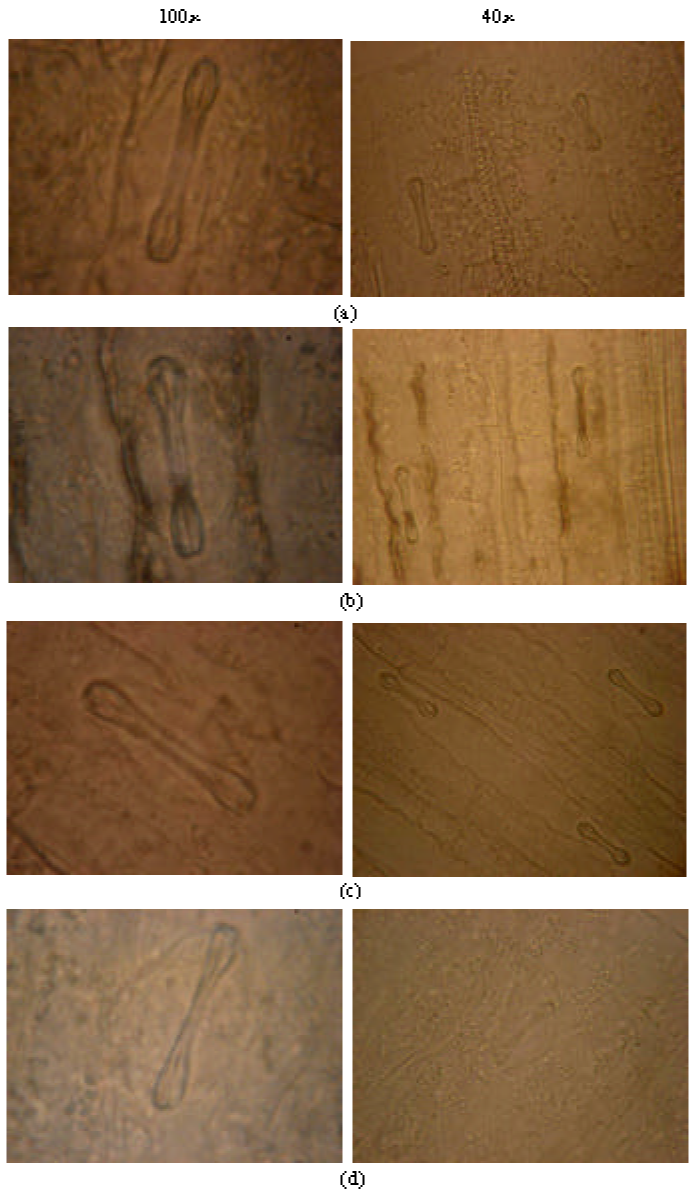


Fig. 2: Photographs of maize leaf surfaces showing closing stomata in water stress in PEG 6000 concentrations 10% (a), 20% (b), 30% (c) and 40% (d), in 704 var. of maize at magnifications of 40x (right) and 100x (left)

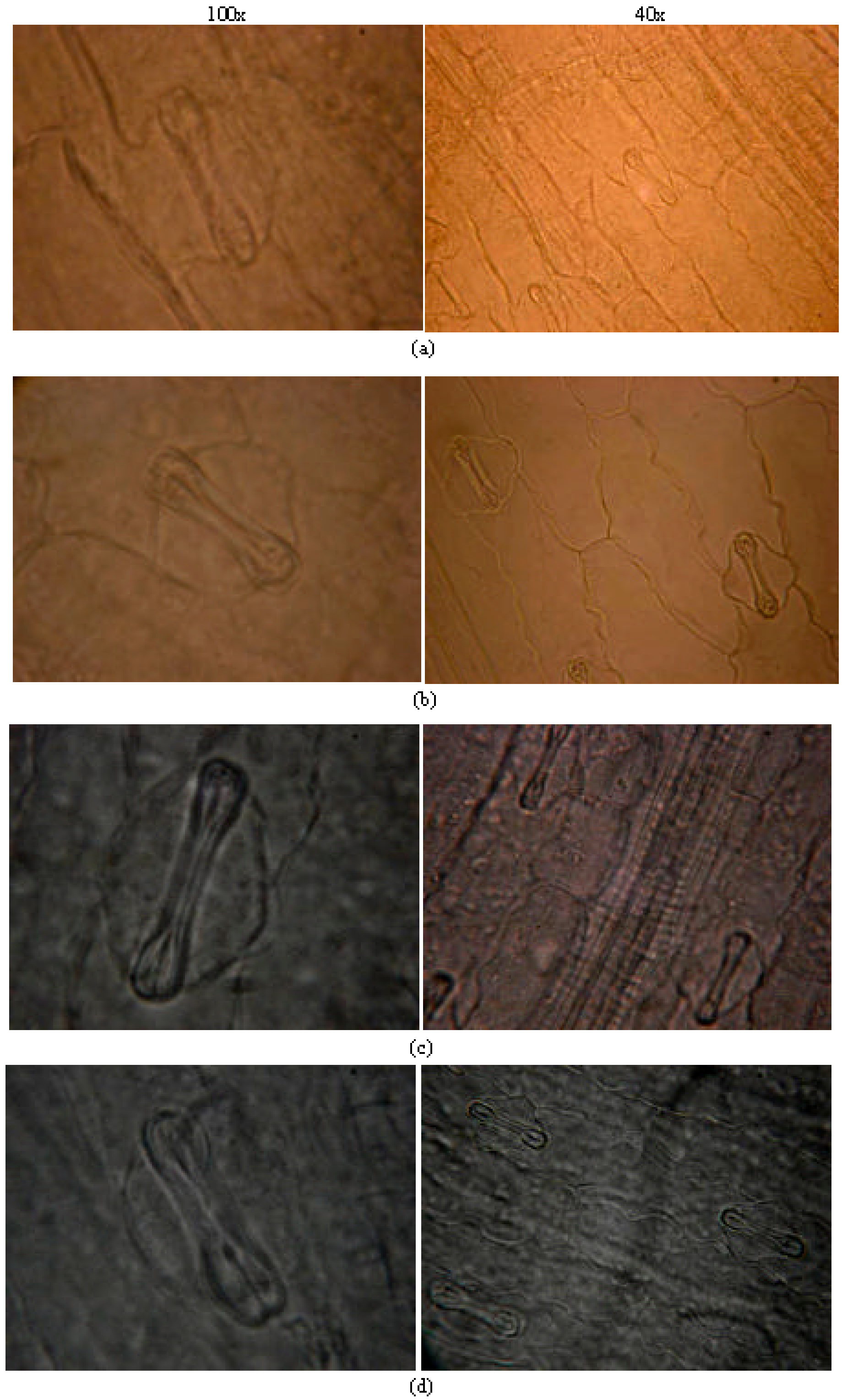


Fig. 3: Photographs of maize leaf surfaces showing closing stomata in water stress in PEG 6000 concentrations 10% (a), 20% (b), 30% (c) and 40% (d), in 301 var. of maize at magnifications of 40× (right) and 100× (left)

fully close their stomata in severe water stress. It has been suggested that at least part of the effect of drought stress on stomatal closing is mediated by ABA levels (Radin and Ackerson, 1982). Stomatal responsiveness to water stress and to apply ABA is increased, perhaps from an alternation of the balance between ABA and endogenous cytokinins. This change is independent of the internal water relations of the leaves, which remain almost unchanged. Hartung *et al.* (1983) demonstrated that osmotic stress alters partitioning of ABA between pools in the mesophyll and thereby increases ABA accumulation in the epidermis.

Stomatal responsiveness to ABA may also be mediated directly at the guard cell plasmalemma, the presumed site of ABA action (Lurie and Hendrix, 1979; Hartung *et al.*, 1983). Jewer and Incoll (1980) reported an effect of cytokinins on stomatal aperture in epidermal peels, implying a direct effect on the guard cells. However, such reports are scarce. Clearly, generalization is not yet possible about mechanisms by which environmental or nutritional stresses affect stomatal behavior.

A stomatal response to changes in leaf water potential has been supported by root pressure chamber experiments on woody plants (Fuchs and Livingston, 1996; Comstock and Mencuccini, 1998). These experiments demonstrate that stomatal closure caused by soil drought or decreased air humidity can be partially or wholly reversed by root pressurization.

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