Leaf turgor pressure in maize plants under water stress

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Abstract

Maize grown is affected by water stress reducing photosynthetic rate and availability of water in its tissues, decreasing plant yield. Monitoring plant water potential is an important indicator of the degree of water stress. With the new magnetic probe for determining leaf turgidity, it is possible to evaluate the water status of the plant and, in some cases, to indicate the optimal relative tolerance to water stress. The union of new and old approaches gives us a better knowledge of water relations in plants. Therefore, the aim of this study was to understand the behavior of maize plants subjected to water stress, using novel and conventional approaches. Maize plants were grown in pots in a greenhouse for 45 days. After this period, plants were subjected to water stress, where turgor measurements expressed by the variable Pp (patch pressure) were monitored. In addition, the leaf water potential, stomatal conductance, CO2 assimilation, transpiration rate, and variable growth (height, leaf area and dry weight) had been monitored for 30 days. Two treatments were conducted, one in which the plant was irrigated and the other one in which irrigation was fully suspended for a period of time and monitored the water status. As the days passed, the plants showed the first visual signs of stress like leaf rolling. During this period, we observed fluctuating Pp values throughout the day, but with a recovery of turgor at night. There were significant differences between treatments for stomatal conductance, water potential, photosynthesis, and Pp, mainly before irrigation. After each irrigation, there has been a rapid recovery in all parameters. There was five periods of stress and it is possible to see a pattern of decreasing and increasing the Pp as the advance of stress, mainly in the last two. Maize plants had a big resilience in water stress conditions, due to mechanisms of water loss mitigation, like leaf rolling and possibly osmotic adjustment. Thus, it was concluded that Pp introduced a new approach to study plants subjected to water stress and it is a complement to other variables as CO2 assimilation rate, stomatal conductance, transpiration rate, and leaf water potential.

Keywords: new methodology, turgor probes, monitoring.

Introduction

When a plant is subjected to drought, many of its physiological processes are affected, such as stomatal opening, photosynthesis, protein synthesis, enzymatic and hormonal activity, among others (Villega and Bull, 1999). Abscisic acid is the head hormone accumulated in the plants, due to this effect in stomatal conductance (Ache et al., 2010). Photosynthesis in plants is adversely affected by water stress determined by changes in the leaf water potential (Ghannoum, 2009). Maize plants subjected to drought conditions exhibit a decreased water potential, causing stomatal closure and, therefore, a decrease in photosynthesis and carbon assimilation (Otegui et al., 1995). The monitoring of water conditions in plants is important for determining the optimal timing of irrigation, thus avoiding losses arising from lack of water. To this end, indicators of plant water status through measurement of water potential of their organs (mainly leaves) have been frequently used by means of either a method based on equilibrium vapor pressure (psychometric/thermocouple techniques) (Martinez et al., 2011) or on pressure balance (pressure chamber) (Boyer, 1967); linear transducers to determine inference from variation in leaf thickness (Seelig et al. 2012) and/or microedendrometry technique to assess variation in trunk/stem diameter (Simmonceau et al., 1995; Junjittakarn et al., 2011) are also used. Despite all these alternatives for evaluating plant water status, studies in this research field present problems involving the representativeness of these stress indicators based on the plant itself (Zimmermann et al., 2013), especially those that use inferences from physiological responses. Furthermore, for practical implementation issues, the difficulty of automating steps and the time required for each measurement, which occurs in determining the water potential and leaf diffusive conductance to vapor, are a complementary problem. Moreover, the results are not always consistent with the actual condition of the plant (Zimmermann et al., 2004). In addition to the fact that the use of a magnetic probe can reveal water dynamics in a plant, it has been assumed that it helps to evaluate possible methodological errors performed in the past (Zimmermann et al., 2008). Thus, the employment of a probe constitutes a useful technique for assessing plant water condition (Bramley et al., 2013; Kant et al.; 2014; Fernandez, 2014). So, in our research, was applied the LCPC (leaf patch clamp pressure) technique, introduced by Zimmermann et al. (2008). The LPCP probe allows non-invasive online monitoring of cell turgor pressure changes, with high precision. The probe consists of a miniaturised pressure sensor integrated into a magnetic clamp that is clipped to a patch of an intact plant.
leaf (Zimmermann et al., 2010). The probe measures the pressure transfer function of the leaf patch, i.e. the attenuated output pressure, Pp, in response to the clamp pressure, Pelamp. The magnitude of the leaf pressure transfer function and thus the attenuation of the constant external pressure, is dictated by a plant-specific, turgor pressure-independent term and a turgor pressure-dependent term (Westhoff et al., 2009). The behavior of many plants, like banana (Zimmermann et al., 2010), olives (Fernandez et al., 2011; Ben-Gal et al., 2010), wheat (Bramley et al., 2013), grapevines (Rüger et al., 2010), canola (Kant et al., 2014) and oak (Ehrenberger et al., 2012) were tested and concluded that this technique have highly precision to measure water status in intact plants. However, there is no observation for maize yet.

Thus, the aim of this work was to determine if leaf turgor measured by a magnetic probe serves as a basis for assessing the degree of water stress in maize plants and how these plants respond to water stress, subsidized by measurements of leaf water potential, CO₂ assimilation, stomatal conductance, and transpiration rate.

**Results**

**Turgor analysis**

Analysis of variance showed that there were significant differences between treatments for the variables Pp (patch pressure), water potential (Ψ), net photosynthetic rate (A), stomatal conductance (gₛ), and transpiration rate (E) (Table 1). The amplitudes of the curves were approximately constant over time in plants without water stress in the first period (Fig 1). In the second period, on days 24, 29 and 32 (Figure 2), the amplitude of the curves decreased due to variations in temperature and in relative humidity, as the temperature was much lower than on other days (average 20°C) and the relative humidity was slightly higher (average 70%), with a decrease in maximal Pp. In well-watered plants there was no significant increase in the maximum values of Pp, which usually occurred near noon; however, for minimum values that occurred during the night, there was a gradual decrease. This increased turgor was more pronounced at night, (Figures 1 and 2). There were small specific variations at most Pp in both periods, but it can be clearly seen that this was due to environmental effects on those days. In water-stressed plants there was a daily gradual increase of Pp after each irrigation (Fig 3 and 4). When the maximum Pp was observed, the plants showed very negative water potential as well as null photosynthesis (Fig 4 and 6). Over the days, there was a gradual increase of maximum Pp after the first irrigation (day 0) and a decrease of minimum Pp, but after the second irrigation (day 5) the maximum values of Pp showed a downward trend until day 12.

After day 12, the sensor was placed on another leaf, due to early senescence of the former leaf. This change resulted in a change in Pp, due to natural variation of each leaf. Thus, it is noteworthy that measurements with turgor probes should take into account the temporal variation of each leaf and not the absolute values found (Zimmermann et al. 2010).

In the last 3 cycles of stress between days 14-34 a greater variation of Pp was observed (Figure 4). In the period between days 16 and 19, there was an increase of Pp according to the intensity of the stress and a decrease of Pp after irrigation performed on this last mentioned day. The lines presented in Figure 4 correspond exactly to the increase in stress and its decrease after irrigation. On day 19, the Pp reached the maximum value of 45 kPa, but after day 20 an irrigation was performed to which the plant responded quickly, with a decrease of Pp 30 kPa the following day.

**Water potential and gas exchange**

During the fourth stress cycle (between day 20 and 27 (Figure 5), the minimum water potential reached was near -2.5 MPa, but soon after irrigation, the plant quickly recovered, with values near or above the control. Water potential in plants which the water stress was not applied varied from -1.0 to -1.5 MPa, only due to the high evaporative demand of the atmosphere, especially if we look at temperature and humidity every day (Figures 1-4), as previously described by Kramer and Boyer (1995).

During the experimental period, there was a variation in photosynthesis (Figure 6). The most common response to water stress is stomatal closure, causing reductions in the photosynthesis rate, as it reduces the availability of CO₂ on the sites of carboxylation (Santos et al., 2009). On the days when the plant was maintained under severe water stress, there was a clear decrease in assimilation of CO₂. On the 3 and 4 day of major water stress, it can be seen that there was no CO₂ assimilation.

Similarly, stomatal conductance showed a variation along the days (Figure 7), close to zero on the maximum stress days. In this case, in addition to decreasing the conductance, plant leaf rolling was a further strategy for maintaining water in their tissues. On the first 2 days of maximum stress, the stomatal conductance of stressed plants was higher. We can assume that these plants were not yet adapted to the lack of water. On the other days, we can see a change in this pattern; stomatal conductance of stressed plants was lower than control.

On the other hand, transpiration rate was different on those 4 days; water-stressed plants had lower transpiration rates than control plants (Figure 8). We can assume that the availability of water in the tissues allowed this behavior.

**Growth analysis**

We can observe that growth of plants under water stress was lower than growth of plants under irrigation. For the leaf area, the same behavior was observed. Plants under stress conditions had a smaller leaf area, due to the lack of water. A smaller leaf area for plants under stress may have favored a reduced water loss through the stomatal on days when there was no maximum stress; thus, maintenance of water potential and turgor may be related to a small evaporative area, in addition to an aspect previously discussed, such as leaf rolling. This behavior was the same as found in the transpiration of plants.

The plant had its growth in dry mass decreased in the treatment of water stress (Figure 10). Basically, until day 5, there was no significant change in dry mass growth among the plants. From the tenth day of analysis, there was a greater dry weight increase.

**Discussion**

As observed in other studies (Zimmermann et al., 2008, Zimmermann et al., 2010; Ehrenberger et al., 2012), there are some indications to be used for the evaluation of drought using probes such as the following: enhancing Pp peaks at noon (when this increase becomes maximum, it is also called Pp peak or Pp max), increasing Pp night values (increase of minimum values), and Pp recovery between maximum and minimum values.
Table 1. Test comparing averages between water stress and control treatments, involving the variables Patch pressure - Pp (kPa), Ψ (MPa), net assimilation rate - A (µmol CO$_2$ m$^{-2}$ s$^{-1}$), stomatal conductance - g$_s$ (mol H$_2$O m$^{-2}$ s$^{-1}$), and transpiration rate - E (mmol H$_2$O m$^{-2}$s$^{-1}$).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Pp  (kPa)</th>
<th>Ψ    (MPa)</th>
<th>A   (µmol CO$_2$ m$^{-2}$ s$^{-1}$)</th>
<th>g$_s$ (mol H$_2$O m$^{-2}$ s$^{-1}$)</th>
<th>E   (mmol H$_2$O m$^{-2}$s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water stress</td>
<td>31.72 a</td>
<td>-1.39 a</td>
<td>22.45 a</td>
<td>0.14 a</td>
<td>2.42 b</td>
</tr>
<tr>
<td>Control</td>
<td>19.70 b</td>
<td>-1.08 b</td>
<td>18.04 b</td>
<td>0.10 b</td>
<td>3.72 a</td>
</tr>
</tbody>
</table>

* Means followed by different letters in the column differ statistically at a 5% probability by Tukey test. The test was performed using the whole analysis period: 31 days for the variable Pp and 17 days for Ψ, A, g$_s$, and E. Pp was used for the daily mean values.

Fig 1. Top - Temporal variation of Pp; Middle - Ppmax and Ppmin; and Bottom - temperature (T) and relative humidity (RH) during the first period of analysis in maize plant without water stress (control). White bars refer to daytime, dark bars refer to nighttime.

Fig 2. Top - Temporal variation of Pp; Middle - Ppmax and Ppmin; and Bottom - temperature (T) and relative humidity (RH) during the second period of analysis in maize plant without water stress (control). White bars refer to daytime, dark bars refer to nighttime.

Fig 3. Top - Temporal variation of Pp; Middle - Ppmax and Ppmin; and Bottom - temperature (T) and relative humidity (RH) during the first period of analysis in maize plant under water stress. White bars refer to daytime, dark bars refer to nighttime.
Fig 4. Top - Temporal variation of Pp; Middle - Ppmax and Ppmin; and Bottom - temperature (T) and relative humidity (RH) during the second period of analysis in maize plant under water stress. White bars refer to daytime, dark bars refer to nighttime.

Fig 5. Average daily variation of leaf water potential on days that had major effects of water stress. These days comprise 5, 12, 19, 29.

Fig 6. Variation of CO₂ assimilation on days that had major effects of water stress. These days comprise 5, 12, 19, 29.
**Fig 7.** Stomatal conductance variation on days that had major effects of water stress. These days comprise 5, 12, 19, 29.

**Fig 8.** Transpiration rate (E) on days that had major effects of water stress. These days comprise 5, 12, 19, 29.

**Fig 9.** Height growth (cm) in maize plants during 30 days of treatment, representing the whole cycle of analysis. Parallelism tests were significant for the “days” factor; “17 days” refers to the days when samples were collected.
Fig 10. Maize leaf area (cm²) under and without drought conditions during 30 days of treatment, which represents the whole cycle of analysis. Parallelism tests were significant for the “days” factor; “17 days” refers to the days when samples were collected.

Fig 11. Variation of dry weight (g) in maize plant under and without drought conditions during 30 days of treatment, which represents the whole cycle of analysis. Parallelism tests were significant for the “days” factor; “17 days” refers to the days when samples were collected.

Thus, it can be assumed that the gradual and daily high peak is a condition in which the degree of stress is increasing, reaching extreme values; hereafter, the plant will lose both night and daytime resilience. However, the decrease in maximal Pp after the irrigation of day 5 only tended to increase from day 18 on (Figures 3 and 4), i.e., the stress period was longer than the previous period (between the first and second irrigation). In this study, however, it was only possible to observe an increase of the maximum values of Pp (Pp max), bearing in mind that the higher the Pp, the lower the turgor. Hence, there was a loss of turgor maintenance ability with increasing stress, easily detected by the presence of leaf wilting and leaf rolling, at the hottest times of the day.

Some authors observed that stressed plants tend to lose turgor recovery capacity, especially after sunset, with the addition of water stress. These values are re-established after irrigation (Ben-gal et al., 2010; Bramley et al., 2013). To explain the gradual decrease of Pp minutes after each irrigation (Figures 3 and 4) some possibilities are considered, such as a limited recovery in the turgidity of the plant, improvement of plant tolerance to drought in every cycle - recovering and maintaining it more hydrated - and the presence of bulliform cells. Poaceae family plants, of which maize is part, have some strategies to resist drought. One of them is the presence of a group of cells called motor bulliform cells, which are present in the upper side of the leaves, arranged in longitudinal rows parallel to the ribs, and may be grouped in
a cross section of 2 to 8 cells (Alvarez et al., 2008). These cells promote curling of leaves under conditions of excessive loss of water or even flooding, reducing its specific area and forming a spiral (Souza et al., 2010). It was observed that most plants under a drought treatment had this leaf rolling condition after two days of irrigation, especially during the hottest times of the day. Thus, neither the water potential nor Pp highly decreased during the period when the leaves had curled. During leaf rolling, with decreased stomatal conductance, there may be a change in the water status of the plants, causing the false impression that they are better adapted to this stress condition by increasing the turgor. These values remained relatively constant over the entire experimental period, being less negative in the subsequent measurements conducted at dawn. The average daily leaf water potential in the experiment underwent fluctuations over the day, but there was more marked difference between treatments from day 20 (Figure 5). However, in periods in which the water stress was more pronounced, a marked variation in the treatments without stress can be observed (Figure 5). In the treatments with stress, variation throughout the day intensified from day 24 on. This behavior observed in some plants such as C4 is called isohydric (Tardieu and Simonneau, 1998), where the daily variation of water potential is small, assisted by an efficient stomatal closure, which prevents excessive water loss. On day 28, shortly after irrigation, there was a quick increase in the water potential, which remained constant during the rest of the day. The reduction of water potential as a plant response demonstrates mainly the decrease of the cell pressure. Thus, as seen in Bianchi et al. (2005), this potential reduction may not affect the plant growth depending on the intensity of drought. Furthermore, the plant can develop an osmotic-adjusting mechanism, which permits a stomatal cell growth and activity even at negative leaf water potentials (Babu et al., 1999). This would also be a plausible explanation for the increase of leaf turgor, measured by the probes in plants under water stress. Nevertheless, more analyses are needed to confirm this hypothesis of osmotic adjustment. Maintenance of a relatively constant water potential throughout the day could be observed when the plant is subjected to severe stress conditions, but lower potential values generally occur between 13 and 16 hours, as observed by Vieira Junior et al. (2007) and Martins et al. (2010), as well as in the present study. This was due to the increasing vapor pressure of the air, caused mainly by the higher air temperature (Figures 1 to 4) during this period of the day.

In days when stress was high, there was also a dramatic decrease in photosynthesis. As the plant was subjected to water stress and posterior irrigation, it appeared to be more resistant, since after the irrigation it had a higher photosynthetic rate than in the previous period (Figure 6). This time for maximum photosynthesis recovery was generally one day. Somehow, these plants have improved their performance, which shows its resilience, as occurred in the previous steps, its measurements signaled a possible maintenance of turgor, were not consistent with the actual condition of the plant. The reduced plant growth can lead us to the conclusion that there was no maintenance of turgor as previously speculated, but there is the possibility of improvement of the measurements with the probe, influenced by occasional leaf rolling. It was confirmed in this study that turgor probe (ZIM-Probe) is a versatile device with advantages over others used in monitoring plant water relations, allowing for an instantaneous and continuous determination of the relative turgor pressure (Pp); it was possible to monitor the water status of maize in both treatments. However, on the final 10 days of drought stress, unlike what was observed in previous steps, its measurements signaled a possible maintenance of turgor not consistent with the display of leaf rolling and the low values of leaf water potential, stomatal conductance, and CO₂ assimilation. Therefore, further studies are needed to understand the causes of this behavior of the probe under such circumstances.

Materials and Methods

**Plant material**

Maize seeds (cultivar AG5055) were sown and grown in 5-liter pots filled with loamy soil (clayey ultisol; pH 5.6) and organic substrate in a proportion of sieved mixture (60:40 v/v). They were fertilized weekly with Hoagland solution with addition of micronutrients. The plants were grown in a greenhouse, temperatures between 32 (max) and 14 (min) °C (Figures 1-4), humidity between 90 (max) and 30 (min) % (Figures 1-4), with 1000 µmol m⁻² s⁻¹ of light intensity and 12/12 h of photoperiod. After a growth period of about 45 days, plants were subjected to water stress conditions. Magnetic turgor probes (ZIM-Probe™) were installed at the fifth fully expanded leaf. There were two treatments: plants in hydrated condition (control) and plants in water stress.
Coordination for the decreased stomatal conductance, photosynthesis and water situations, expecting to find a pattern) for biometric analysis. Perform (comparison of relations adjusted to different Tukey’s test for comparison of means, both at the 5% level of water-stressed plants). The F test analysis of variance and the in the experiment were the water regimes (hydrated and repetitions were the days of experiment. The factors involved The experimental design was a randomized block where the involved the probe on the leaf and the leaf turgor). Traits measured In one plant of each treatment a probe was installed to monitor the leaf water status for 31 days. Water potential analysis, leaf gas exchange, and growth samplings were done three times per week in the same treatment plant, in order to correlate the measurements, but in different plants each day. After each rehydration of plants under water stress, the same measurements were performed in order to check plant recovery. Each day of experiment consisted in treatment replicates, i.e., blocks of statistical design. Probe evaluations were divided into two distinct periods in both treatments. For the determination of leaf water potential, we used the equilibrium vapor pressure method by means of a hygrometric technique using a microvoltmeter model HR-337 (Wescor, Logan, UT, USA) coupled to Wescor C-52 chambers. Analyses were performed three times a week and on the days when the rehydration was carried out, in order to find a possible correlation with measurements of the probe. On each day of analysis, a plant from each treatment was selected for the determination of leaf water potential, for comparison with the other two in which the probe had been installed. Water potential measurements were performed throughout the day, at dawn, noon, and sunset. Measurements of CO₂ assimilation rates (µmol CO₂ m⁻² s⁻¹) and stomatal conductance (mol H₂O m⁻² s⁻¹) were obtained using an infrared gas analyzer (IRGA, LI-6400XT model, LI-COR, Lincoln, USA). Measurements were performed in a greenhouse, using the external solar radiation as a reference value, which remained around 1000 µmol m⁻² s⁻¹. The measurements of CO₂ assimilation rates, stomatal conductance, and transpiration rate occurred on the same days when there were measurements of water potential. Only four days were selected, when water stress had major influence on the rates. These days correspond to April 29 and May 06, 13, and 21. Biometric analyses – consisting in measuring leaf area, plant height, and dry weight for each treatment – were performed at the end of each day when water potential was analyzed. Leaf area was measured trough leaf area meter (LI-3100C Area Meter, LI-COR, Lincoln, USA). Statistical analysis The experimental design was a randomized block where the repetitions were the days of experiment. The factors involved in the experiment were the water regimes (hydrated and water-stressed plants). The F test analysis of variance and the Tukey’s test for comparison of means, both at the 5% level of significance, were conducted. Parallelism tests were performed (comparison of relations adjusted to different situations, expecting to find a pattern) for biometric analysis. Conclusion Maize plants submitted to drought had a reduced growth and a decreased stomatal conductance, photosynthesis and water potential. The decrease of the last three variables tended to be gradual, performing maximum only on the last day of each stress cycle. There was recovery of photosynthetic activity, as well as other variables measured, usually one day after irrigation, demonstrating high capacity of this plant to recover hydration. 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