EFFECT OF OSMOTIC STRESS ON PROLINE ACCUMULATION, PHOTOSYNTHETIC ABILITIES AND GROWTH OF SUGARCANE PLANTLETS (SACCHARUM OFFICINARUM L.)

SURIYAN CHA-UM* AND CHALERMPOL KIRDMANEE

National Center for Genetic Engineering and Biotechnology (BIOTEC)
National Science and Technology Development Agency (NSTDA) 113 Thailand Science Park,
Paholyothin Road, Klong I, Klong Luang, Pathumthani 12120, Thailand

Abstract

Disease-free sugarcane plantlets derived from meristem cutting were photoautotrophically grown on the MS medium and subsequently exposed to 0, 100, 200, 300 or 400 mM mannitol for 7 days. Osmotic pressure in the culture medium was increased with increase in mannitol concentration, causing low water use efficiency (WUE) \( (r^2 = 0.88) \) and chlorophyll degradation \( (r^2 = 0.92) \). Chlorophyll a (Chl a), chlorophyll b (Chl b) and total carotenoids (C x+c), concentrations in the osmotic stressed leaves decreased, especially in 400 mM mannitol treatment, degrading 44, 81 and 72%, respectively when compared to control. In contrast, proline content in osmotic stressed plantlets was accumulated and peaked at 2,236.75 \( \mu \text{mol g}^{-1} \text{FW} \) in 300 mM mannitol treatment. The WUE and chlorophyll degradation were correlated with maximum quantum yield of PSII \( (F_{v}/F_{m}) (r^2 = 0.75) \) and photon yield of PSII \( (\Phi_{\text{PSII}}) (r^2 = 0.83) \), respectively. The \( F_{v}/F_{m} \) and \( \Phi_{\text{PSII}} \) in drought acclimatized plantlets decreased, when non-photochemical quenching (NPQ) reached. The reduction of \( \Phi_{\text{PSII}} \) was positively related to net-photosynthetic rate (NPR) \( (r^2 = 0.85) \) as well as the proline content and NPQ \( (r^2 = 0.81) \). The NPR, stomatal conductance \( (G_s) \) and transpiration rate \( (E) \) in osmotic stressed plantlets were significantly dropped, leading to growth reduction \( (r^2 = 0.95) \). The basic knowledge of osmotic stressed responses may further be applied as effective indices for drought tolerance in sugarcane breeding program.

Introduction

Water limitation is one of the most important factors to reduce agricultural crop production, which is related to global climate changes, especially drought and heat stress (Ciais et al., 2005). Drought stress (water deficit or low water availability) is a major abiotic problem, widely distributed worldwide over 1.2 billion ha in rainfed agricultural land (Chaves & Oliveira, 2004; Kijne, 2006; Passioura, 2007). The drought environment has been reported as key factor to limit plant growth and development prior to the loss of productivity, especially of crop species (Bray, 1997; Chartzoulakis et al., 2002; Yordanov et al., 2003; Reddy et al., 2004; Blum, 2005; Neumann, 2008; Shao et al., 2008). There are many plant defense responses to water deficit such as transcription factors, water channels/transporters, hormonal regulation, osmoregulation and detoxification systems (Valliyodan & Nguyen, 2006; Seki et al., 2007; Shinozaki & Yamaguchi-Shinozaki, 2007; Cattivelli et al., 2008). Proline accumulation in drought stressed plants is one of the vital compatible solutes to function in cellular osmotic adjustment and scavenge detoxify oxidants (Delauney & Verma, 1993; Yamada et al., 2005; Valliyodan & Nguyen, 2006; Seki et al., 2007). There are many ways to enhance on proline accumulation such as \( \Delta^1 \)-pyrroline-5-carboxylate synthetase (P5CS) overexpression, proline dehydrogenase (ProDH) antisense suppression and exogenous proline application for drought tolerant propose (Kavi Kishor et al., 1995; de Ronde et al., 2000; Simon-Sarkadi et al., 2000; Kavi Kishor et al., 2005; Yamada et al., 2005; Ashraf & Foolad, 2007; Ali et al., 2008).

*Correspondence author: Tel.: 662-5646700; Fax.: 662-5646707; E-mail address: suriyanc@biotec.or.th
In drought conditions, water availability in supporting materials such as soil, vermiculite, perlite and peat-moss, is restricted, thereby causing low water use efficiency (WUE) in plant cells (Blum, 2005; Bloch et al., 2006; Costa et al., 2007; Shao et al., 2008). Low WUE is a primary effect on plant responses to water deficit conditions, leading to biochemical changes, including decreased Rubisco (ribulose-1,5-bisphosphatase carboxyase/oxygenase) activity and photochemical efficiency, enhanced accumulation of stress metabolites (proline, glycinebetaine, polyamine, glutathione, polyamines, sugars, sugar alcohols and α-tocopherol), increased antioxidant enzymes (superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase); reactive oxygen species (ROS) reduction and physiological changes i.e. loss of membrane stability, reduced leaf water potential, pigment degradation, decreased stomatal conductance, reduced internal CO₂ concentration, NPR reduction and growth inhibition prior to plant death (Yordanov et al., 2003; Chaves & Oliveira, 2004; Reddy et al., 2004; Cattivelli et al., 2008; Shao et al., 2008). Sugarcane (Saccharum officinarum L.) is a member of Poaceae family which produces and accumulates sugar in the stem for sugar production in tropical and subtropical regions (Cordeiro et al., 2007). Sugarcane is a high biomass producer and it consumes a large amount of water and takes a long time (6-8 months) for plant growth and development prior to harvesting (Allison et al., 2007). Water management is an important factor for sugarcane plantation to achieve maximum yield, especially in arid and semiarid zones (Robertson et al., 1999; Wiedenfeld, 2000; Inman-Bamber & Smith, 2005; Singh et al., 2007). The aim of this study was to investigate the biochemical and physiological responses of sugarcane plantlets to water deficit using mannitol under In-vitro photoautotrophic conditions.

Materials and Methods

Plant materials: Disease-free sugarcane shoots (Saccharum officinarum L. cv. K84-200) derived from meristem cutting (Cha-um et al., 2006a) were propagated on the MS medium (Murashige & Skoog, 1962) containing 8.88 μM benzyl adenine (BA), 3% sucrose and 0.25% Phytagel® for 6 weeks. The multiple shoots were elongated on the MS medium without plant growth regulators for 4 weeks, then the single shoots were excised and the roots were induced on MS medium supplemented with 2.46 μM indole butyric acid (IBA) for 2 weeks. Plantlets were cultured In vitro under conditions of 25±2°C ambient temperature, 60±5% relative humidity (RH) and 60±5 μmol m⁻² s⁻¹ photosynthetic proton flux density (PPFD) provided by fluorescent lamps (TDL 36 W/84 Cool White 3350 Im, Philips, Thailand) with a 16 h d⁻¹ photoperiod. Then, the sugarcane plantlets were transferred to MS sugar-free liquid medium (photoautotrophic condition) using vermiculite as supporting material for 7 days. The number of air-exchanges in the glass vessels was adjusted to 2.32 h⁻¹ by punching a hole on plastic cap (Ø 1 cm) and covering the hole with a microporous filter (0.20 μm of pore size; Nihon Millipore Ltd., Tokyo, Japan). The plantlets were subsequently cultured in a plant growth incubator with the same conditions as previously mentioned and CO₂ enrichment at 1,000±100 μmol mol⁻¹. Mannitol (osmotic stress) concentrations in the culture medium were adjusted to 0, 100, 200, 300 or 400 mM for 7 days. Photosynthetic pigments, proline contents, chlorophyll a fluorescence, net-photosynthetic rate (NPR) and growth characters were measured.
Data measurements: Osmolarities of culture medium containing varying concentrations of mannitol were measured, according to Lanfermeijer et al., (1991) using an osmometer. Chlorophyll a (Chlₐ), chlorophyll b (Chlₜ), total chlorophyll and total carotenoids (Cᵥ+c) concentrations were determined following the methods of Shabala et al., (1998) and Lichtenthaler (1987), respectively. One hundred milligrams of leaf material were collected, placed in a 25 mL glass vial, added with 10 mL of 95.5% acetone and blended with a homogenizer. The Chlₐ, Chlₜ, and Cᵥ+c concentrations were measured using an UV-visible spectrophotometer. A solution of 95.5% acetone was used as a blank. Pigment degradation percentage was calculated as:

\[
\text{Pigment degradation (\%) = } \frac{1 - \frac{\text{Salt treatment}}{\text{Control}}} {\times 100}
\]

Proline content from leaves was extracted according to the method of Bates et al., (1973). One hundred milligrams of leaf tissues were ground in liquid nitrogen. The homogenate powder was mixed with 1 mL aqueous sulfosalicylic acid (3% w/v) and filtered through filter paper (Whatman #1). Extracted solution was reacted with an equal volume of glacial acetic acid and ninhydrin reagent (1.25 mg ninhydrin in 30 mL of glacial acetic acid and 20 mL of 6 M H₃PO₄) and incubated at 95°C for 1 h. The reaction was terminated placing on an ice bath. The reaction mixture was vigorously mixed with 2 mL toluene. After warming at 25°C, the chromophore was measured on spectrophotometer (DR/4000, HACH, Loveland, Colorado, USA) at 520 nm. L-proline (Fluka, Switzerland) was used as a standard.

Chlorophyll a fluorescence emission from the adaxial surface of leaf was monitored with a Fluorescence Monitoring System (FMS 2; Hansatech Instruments Ltd., Norfolk, UK) in the pulse amplitude modulation mode, as previously described by Loggini et al., (1999) & Maxwell and Johnson (2000).

The net-photosynthetic rate (NPR), transpiration rate \((E; \text{mmol} m^{-2} s^{-1})\) and stomata conductance \((G_s; \mu\text{mol} H_2O m^{-2} s^{-1})\) were measured using Infra-red Gas Analyser (IRGA; Model Portable Photosynthesis System LI 6400, LI-COR® Inc, Lincoln, Nebraska, USA). The \(E\) and \(G_s\) were measured continuously monitoring H₂O of the air entering and existing in the IRGA headspace chamber. Water use efficiency (WUE) of acclimatized plantlets was calculated by the ratio of NPR to \(E\) (Cha-um et al., 2007).

Fresh and dry-weights, shoot height, root length and leaf area of sugarcane plantlets were measured as described by Cha-um et al., (2006b). Sugarcane plantlets were dried at 110 °C in a hot-air oven (Model 500, Memmert, Buchenbach, Germany) for 2 days, and then incubated in desiccators before measurement of the dry weight. Leaf area of plantlets was measured using a Leaf Area Meter DT-scan (Delta-Scan Version 2.03, Delta-T Devices, Ltd., Burwell, Cambridge, UK).

\[
\text{Water used efficiency (WUE) = } \frac{\text{Net photosynthetic rate (NPR)}} {E}
\]

Experimental designs: The experiment was setup in a Completely Randomized Design (CRD) with six replicates and four plantlets per replicate. The mean values were compared by Duncan’s New Multiple Range Test (DMRT) and analyzed by SPSS software (SPSS for Windows, SPSS Inc., Chicago, USA). The correlations between physiological and biochemical parameters were evaluated by Pearson’s correlation coefficients.
Results

Water deficit of sugarcane *in-vitro* plantlets was established using mannitol in the medium to control the osmotic potential (Ψₛ) or water available in the root zone. The osmotic pressure in the culture medium containing mannitol was reduced, leading to low water use efficiency (WUE) (ᵣ² = 0.88) and pigment degradation in the osmotic stressed plantlets (ᵣ² = 0.92) (Fig. 1). Chlorophyll a (Chlₐ), chlorophyll b (Chlb), total chlorophyll (TC) and total carotenoids (Cᵥcᵥ) in osmotic stressed plantlets were significantly dropped, especially in the extreme water deficit treatments (300-400 mM mannitol). In mild drought conditions (100-200 mM mannitol), the Chlₐ and Chlb contents in the leaf tissues were maintained, while the TC and Cᵥcᵥ contents were significantly reduced (Table 1). The Chlₐ, Chlb, TC and Cᵥcᵥ contents in the sugarcane plantlets grown under 400 mM mannitol were more degraded 44, 81, 60 and 72% when compared to those control plantlets. In contrast, proline content in osmotic stressed plantlets increased, relating to mannitol concentrations in the culture medium and it was peaked at 2,236.75 μmol g⁻¹ FW in the plantlets treated...
with 300 mM mannitol. The WUE reduction and pigment degradation in drought acclimatized plantlets were strongly related to maximum quantum yield of PSII ($F_v/F_m$) ($r^2 = 0.75$) and photon yield of PSII ($\Phi_{\text{PSII}}$) ($r^2 = 0.83$), respectively (Fig. 2). The $F_v/F_m$ and $\Phi_{\text{PSII}}$ in osmotic stressed plantlets significantly decreased depending on mannitol concentrations in the culture medium, while non-photochemical quenching (NPQ) was increased (Table 2). The results showed that the $\Phi_{\text{PSII}}$ was positively correlated with net-photosynthetic rate (NPR) ($r^2 = 0.85$), as well as the proline content was positively related to NPQ ($r^2 = 0.81$) (Fig. 3). The NPR, stomatal conductance ($G_s$) and transpiration rate (E) in osmotic stressed plantlets at 300 mM mannitol drastically declined when compared to those of control plantlets (Table 2). In addition, there were strong relationships between
biochemical and physiological parameters in the osmotic stressed plantlets (Table 3). The Chla, Chlb, Cx+c, Fv/Fm, NPR, Gs and E parameters showed the positive correlation, whereas NPQ and proline demonstrated negative relationships (Table 3). The NPR reduction in osmotic stressed plantlets was positively related to growth inhibition ($r^2 = 0.95$) (Fig. 4). Growth performances, fresh and dry weights and leaf area, in drought acclimatized plantlets were expressed in the similar pattern as the pigment degradation and photosynthetic reduction (Table 4). The leaf area was a sensitive parameter in drought stressed sugarcane, which was significantly reduced when exposed to water deficit. In addition, fresh and dry weights in mild-drought acclimatized plantlets (100-200 mM mannitol) were maintained better than those in extreme drought conditions (300-400 mM mannitol).

Fig. 3. Relationship between photon yield of PSII ($\Phi_{\text{PSII}}$) and net-photosynthetic rate (NPR) (A), proline and non-photochemical quenching (NPQ) (B) of sugarcane plantlets acclimatized under drought condition (mannitol) for 7 days. Error bars represent ±SE.
Fig. 4. Relationship between net-photosynthetic rate (NPR) and dry weight of sugarcane plantlets acclimatized under drought condition (mannitol) for 7 days. Error bars represent ± SE.

Table 1. Chlorophyll a (Chla), chlorophyll b (Chlb), total chlorophyll (TC), total carotenoids (Cx+c) and proline contents of sugarcane plantlets acclimatized under drought condition (mannitol) for 7 days. Different letters in each column show significant difference at \( p \leq 0.01 \) (***) by Duncan’s New Multiple Range Test (DMRT).

<table>
<thead>
<tr>
<th>Mannitol (mM)</th>
<th>Chla (μg g(^{-1})FW)</th>
<th>Chlb (μg g(^{-1})FW)</th>
<th>TC (μg g(^{-1})FW)</th>
<th>Cx+c (μg g(^{-1})FW)</th>
<th>Proline (μmol g(^{-1})FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Control)</td>
<td>231.08a</td>
<td>170.36a</td>
<td>401.44a</td>
<td>79.28a</td>
<td>318.34c</td>
</tr>
<tr>
<td>100</td>
<td>213.42a</td>
<td>117.50ab</td>
<td>330.92b</td>
<td>58.73b</td>
<td>517.34c</td>
</tr>
<tr>
<td>200</td>
<td>163.73b</td>
<td>64.00bc</td>
<td>227.73c</td>
<td>37.92c</td>
<td>1027.16b</td>
</tr>
<tr>
<td>300</td>
<td>159.53b</td>
<td>52.96c</td>
<td>212.49c</td>
<td>33.13c</td>
<td>2236.75a</td>
</tr>
<tr>
<td>400</td>
<td>129.68b</td>
<td>32.73c</td>
<td>162.41c</td>
<td>21.94d</td>
<td>1235.02b</td>
</tr>
</tbody>
</table>

ANOVA ** ** ** ** **

Table 2. Maximum quantum yield of PSII (F\(_{v}/F_{m}\)), photon yield of PSII (\( \Phi_{PSII} \)), non-photochemical quenching (NPQ), net-photosynthetic rate (NPR), stomatal conductance (G\(_{s}\)) and transpiration rate (E) of sugarcane plantlets acclimatized under drought condition (mannitol) for 7 days. Different letters in each column show significant difference at \( p \leq 0.01 \) (***) by Duncan’s New Multiple Range Test (DMRT).

<table>
<thead>
<tr>
<th>Mannitol (mM)</th>
<th>F(<em>{v}/F</em>{m})</th>
<th>( \Phi_{PSII} )</th>
<th>NPQ (μmol CO(_{2}) m(^{-2}) s(^{-1}))</th>
<th>G(_{s}) (mol m(^{-2}) s(^{-1}))</th>
<th>E (mmol H(_{2}O) m(^{-2}) s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Control)</td>
<td>0.889a</td>
<td>0.705a</td>
<td>0.095d</td>
<td>5.60a</td>
<td>0.79a</td>
</tr>
<tr>
<td>100</td>
<td>0.867b</td>
<td>0.677b</td>
<td>0.119d</td>
<td>3.54b</td>
<td>0.64b</td>
</tr>
<tr>
<td>200</td>
<td>0.841c</td>
<td>0.622c</td>
<td>0.162c</td>
<td>2.33c</td>
<td>0.42c</td>
</tr>
<tr>
<td>300</td>
<td>0.807d</td>
<td>0.573d</td>
<td>0.224b</td>
<td>1.16d</td>
<td>0.12d</td>
</tr>
<tr>
<td>400</td>
<td>0.765e</td>
<td>0.456e</td>
<td>0.270a</td>
<td>0.23d</td>
<td>0.08d</td>
</tr>
</tbody>
</table>

ANOVA ** ** ** ** ** **
Table 3. Relationship between physiological and biochemical parameters of sugarcane plantlets acclimatized under drought condition (mannitol) for 7 days. Significant levels at $p \leq 0.01$ are represented by ** using Pearson’s correlation coefficients.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Chl_a</th>
<th>Chl_b</th>
<th>C_{svc}</th>
<th>PRO</th>
<th>F_{v/Fm}</th>
<th>NPQ</th>
<th>NPR</th>
<th>G_s</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chl_a</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chl_b</td>
<td>0.931**</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C_{svc}</td>
<td>0.893**</td>
<td>0.896**</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PRO</td>
<td>-0.664**</td>
<td>-0.680**</td>
<td>-0.719**</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>F_{v/Fm}</td>
<td>0.861**</td>
<td>0.802**</td>
<td>0.915**</td>
<td>-0.679**</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NPQ</td>
<td>-0.849**</td>
<td>-0.773**</td>
<td>-0.903**</td>
<td>0.717**</td>
<td>-0.958**</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NPR</td>
<td>0.895**</td>
<td>0.871**</td>
<td>0.957**</td>
<td>-0.741**</td>
<td>0.940**</td>
<td>-0.901**</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>G_s</td>
<td>0.878**</td>
<td>0.865**</td>
<td>0.935**</td>
<td>-0.841**</td>
<td>0.937**</td>
<td>-0.941**</td>
<td>0.930**</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E</td>
<td>0.892**</td>
<td>0.881**</td>
<td>0.973**</td>
<td>-0.771**</td>
<td>0.943**</td>
<td>-0.916**</td>
<td>0.962**</td>
<td>0.975**</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 4. Growth characters, fresh weight (FW), dry weight (DW) and leaf area (LA) of sugarcane plantlets acclimatized under drought condition (mannitol) for 7 days. Different letters in each column show significant difference at $p \leq 0.01$ (**), by Duncan’s New Multiple Range Test (DMRT).

<table>
<thead>
<tr>
<th>Mannitol (mM)</th>
<th>FW (mg)</th>
<th>DW (mg)</th>
<th>LA (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Control)</td>
<td>147.6a</td>
<td>23.5a</td>
<td>1058a</td>
</tr>
<tr>
<td>100</td>
<td>134.8a</td>
<td>18.3b</td>
<td>935b</td>
</tr>
<tr>
<td>200</td>
<td>95.2b</td>
<td>14.8bc</td>
<td>782c</td>
</tr>
<tr>
<td>300</td>
<td>67.6c</td>
<td>14.8bc</td>
<td>485d</td>
</tr>
<tr>
<td>400</td>
<td>56.0c</td>
<td>12.4c</td>
<td>346e</td>
</tr>
</tbody>
</table>

ANOVA ** ** **

Discussion

The osmotic control in the root zone of plant cultivation using mannitol and polyethylene glycol (PEG) solution has been well established in crop species i.e., sugarcane (Errabi et al., 2006; Errabi et al., 2007), rice (Ahmad et al., 2007; Liu et al., 2007; Lefèvre et al., 2001), cowpea (Costa et al., 2007), alfalfa (Safarnejad, 2008), lentil (Yupsanis et al., 2001), three grass species (van den Berg & Zeng, 2006), maize (Ashraf et al., 2007) and halophyte species i.e. Sevium portulacastrum (Slama et al., 2007), Cantaurea ragusina (Radić et al., 2005; Radić et al., 2006), Suaeda salsa and Kalanchoe claigremontiana (Kefu et al., 2003). In the present study, the osmotic pressures of the culture medium declined consistently with increase in mannitol concentration, leading to low WUE in sugarcane plantlets grown under water deficit conditions. Similar results were demonstrated where the relative water content in the callus tissues was positively decreased with 0 (-0.4 MPa), 100 (-0.62 MPa), 200 (-0.84 MPa) and 300 mM mannitol (-1.08 MPa) contained in MS medium (Errabi et al., 2006; Errabi et al., 2007). The total chlorophyll and total carotenoid pigments in the leaf tissues of extreme water deficit were
degraded by 60 and 72%, respectively. Reduction in WUE in water-deficit sugarcane directly affects on photosynthetic pigment degradation, leading to reduce water oxidation in photosystem II defined by $F_v/F_m$ and $\Phi_{PSII}$, especially under extreme drought conditions (Nable et al., 1999; Robertson et al., 1999; de Silva & de Costa, 2004; Inman-Bamber & Smith, 2005; Smit & Singels, 2006; Silva et al., 2007; Shao et al., 2008). The chlorophyll degradation and chlorophyll fluorescence diminution in sugarcane varieties CP72-1210, CP92-675, H99-295 and TCP02-4624 cultivated under drought condition reduced by 19.4 and 7.6%, respectively (Silva et al., 2007). In addition, the primary response of drought stressed sugarcane plantlets was osmotic adjustment through proline accumulation, which is well established in many plant species (Raymond & Smirnoff, 2002; Errabi et al., 2006; Ahmad et al., 2007; Errabi et al., 2007). In this study, proline content reached to maximum in the drought acclimatized plantlets under 300 mM mannitol (-0.94 MPa) and then dropped. In sugarcane varieties, R570 and CP59-73, the water content in calli treated with mannitol induced osmotic stress was significantly dropped with increase in mannitol concentration, whereas proline content was accumulated (Errabi et al., 2006). The proline accumulation in drought-stressed plants may play a role as osmolyte to maintain the organelles, resulting in the greenish leaf when exposed to water deficit condition (Yamada et al., 2005; Sankar et al., 2007; Safarinejad, 2008). Moreover, the sugarcane plantlets grown under extreme drought environments showed pigment damages, low $F_v/F_m$ and NPR reduction, causing growth inhibition of sugarcane plantlets. There are many reports which show physiological and morphological changes such as leaf water potential, stomatal conductance, leaf area and productivity in sugarcane in response to drought stress, which are used as potential and rapid tool for screening for drought tolerance (Nable et al., 1999; Robertson et al., 1999; de Silva & de Costa, 2004; Inman-Bamber & Smith, 2005; Smit & Singels, 2006; Silva et al., 2007), especially under in vitro environments.

In conclusion, sugarcane variety K84-200 was very sensitive to water deficit ($\Psi_s < -0.67$ MPa), as it had a maximum pigment degradation, low photosynthetic abilities and maximum growth reduction. The relationship between biochemical and physiological characters and growth of osmotic stressed plantlets was found to be positive in this investigation. They could be applied as effective indices for screening elite sugarcane varieties for drought tolerance.

Acknowledgements

The authors are grateful to the Mitr Phol Sugarcane Research Center, Mitr Phol Group Co. Ltd. for sugarcane seed stock. This experiment was funded by Mitr Phol Sugarcane Research Center and partially supported by the National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA) Thailand.

References


(Received for publication 13 September 2008)