Nitric oxide accumulation and glycinebetaine metabolism in two osmotically stressed maize cultivars supplied with different nitrogen forms

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Abstract

Hydroponic experiments were conducted to investigate the effects of nitrogen forms on nitric oxide accumulation and glycinebetaine (GB) metabolism under osmotic stress (OS) simulated by the addition of polyethylene glycol (PEG-6000) to two maize (Zea mays L.) cultivars Zhengdan 958 (ZD958; drought-tolerant) and Jundan 20 (JD20; drought-sensitive). OS led to the accumulation of NO, GB, and choline, as well as to an increase of betaine aldehyde dehydrogenase (BADH) activity in both the cultivars. The responses of both the maize cultivars to OS were significantly higher under nitrate nutrition than under the mixed supply of ammonium and NO₃⁻ or sole NH₄⁺. Cultivar ZD958 was superior to JD20 under the osmotic stress. Furthermore, compared with sole NH₄⁺, sole NO₃⁻ and the mixed supply of NH₄⁺ and NO₃⁻ increased all the parameters in cv. ZD958 but only the GB and choline content in JD20. Therefore, it is concluded that ZD958 showed a higher accumulation of NO and enhanced GB metabolism under OS than JD20 regardless of N form.

Additional key words: betaine aldehyde dehydrogenase, choline, drought stress, Zea mays.

Drought stress is one of the major environmental limitations which adversely affect plant growth and yield of crops worldwide (Ashraf 2010). Plants have evolved various strategies including an enhanced accumulation of some organic osmolytes including glycinebetaine (GB) to alleviate detrimental effects of drought stress on plant metabolism and growth (Anjum et al. 2011). Nitric oxide (NO) is one of the stress signaling molecules (Misra et al. 2011). Zhang et al. (2013) provided a sound evidence of regulation of GB metabolism in drought stressed maize plants by exogenous application of NO. Foliar-applied urea causes a marked accumulation of NO especially in a drought sensitive maize cultivar (Zhang et al. 2012b). It is also very effective in enhancing a GB accumulation by modulating GB metabolism (Zhang et al. 2012a). Betaine aldehyde dehydrogenase (BADH, EC 1.2.1.81) is the key enzyme involved in the GB biosynthetic pathway (Rhodes and Hanson 1993). Nitrogen forms (NO₃⁻ versus NH₄⁺) influence plant responses to stress, including the pattern of accumulation of osmolytes proline and GB (Walch-Liu et al. 2000). Therefore, the aim of this study was to investigate the interactive effects of N forms and osmotic stress (OS) on NO accumulation and GB metabolism in two maize cultivars with contrasting drought tolerance.

Seeds of maize (Zea mays L.) cvs. Jundan 20 (JD20; drought sensitive) and Zhengdan 958 (ZD958; drought tolerant) were surface-sterilized in a 1 % (m/v) sodium hypochlorite solution on a magnetic stirrer for 20 min and thoroughly rinsed with sterile deionized water. Then the seeds were germinated between two layers of blotter paper moistened with sterile water at a temperature of 28 ± 1 °C in the dark for 72 h. The seedlings were cultured in a hydroponic culture in a growth chamber with day/night temperatures of 25/18 °C, a relative humidity of 60 - 70 %, an irradiance of 350 μmol m⁻² s⁻¹, and a 16-h photoperiod.

In the first experiment, OS was implemented by adding 30, 90, and 120 g(PEG-6000) kg⁻¹ (distilled water) in a full strength nutrient solution (Hoagland and Arnon 1950) to gradually achieve osmotic potentials of -0.08, -0.13, and -0.23 MPa, respectively (Wang and Li 2002). In the second experiment, the seedlings were grown under

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Abbreviations: BADH - betaine aldehyde dehydrogenase; GB - glycine betaine; OS - osmotic stress; PEG - polyethylene glycol.

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control conditions or PEG (osmotic potential of -0.23 MPa) and subjected to the following N forms: \( \text{NH}_4^+ - \text{N} \) supplied as \((\text{NH}_4)^2\text{SO}_4\), \( \text{NO}_3^- - \text{N} \) supplied as \( \text{Ca(NO}_3\text{)}_2 \), and the mixture of \( \text{NH}_4^+ + \text{NO}_3^- \) in the ratio of 50:50. The maize plants were harvested after 0, 1, 2, 3, 5, 7, and 10 d after the onset of the osmotic stress in the first experiment, and after 10 d of the onset of the drought or N form treatments in the second experiment. The osmotic potential of the PEG solution was measured by a vapor pressure osmometer (Wescor model 5500, Logan, UT, USA).

The GB content in leaf was determined by the method of Grieve and Grattan (1983). The choline content was determined following Feng and Ren (2004) and Winzler and Meserv (1945). The NO content was assayed as described by Griess (1879) following the guidelines on the Griess reagent colorimetric kit (Beyotime Institute of Biotechnology, Shanghai, China). The NO content was determined through a standard curve using NaNO2 and expressed as nmol g\(^{-1}\) (d.m.). For determination of BADH activity, plant material was homogenized in 250 mm\(^3\) of a buffer containing 50 mM HEPES-KOH (pH 8.0), 1 mM Na\(_2\)EDTA, 20 mM sodium disulfite, 10 mM sodium borate, 5 mM ascorbic acid, 5 mM dithiothreitol, and 2 % (m/v) polyvinylpyrrolidone. Homogenates were then centrifuged at 10 000 \( g \) and 4 °C for 15 min and the supernatants were used for determination of BADH activity according to Daniell et al. (2001) by monitoring the absorbance at 340 nm with 0.05 mM betaine aldehyde chloride as substrate. The activity was calculated using the coefficient of absorbance of 6 220 M\(^{-1}\) cm\(^{-1}\) for NADH. Protein content of the crude extract was measured by Coomassie Brilliant Blue G-250 method (Bradford 1976).

All data were subjected to the analysis of variance (ANOVA) using the SAS v. 9 software (SAS Institute, Chicago, USA). The significance of the treatment effect was determined using the \( F \)-test, and the significance of difference between means was determined by the LSD test at the 0.05 probability level.

Osmotic stress can induce some physiological mechanisms to adapt to it, including an accumulation of NO and organic osmoytes, such as GB (Bartels and Sunkar 2005, Ashraf et al. 2011, Zhang et al. 2013). In our experiments, OS caused a marked accumulation of NO and GB in two maize cultivars, and the accumulation of NO reached the maximum earlier than that of GB, which suggests that NO possibly acted as signal molecule to regulate other processes involved in drought tolerance in correspondence with our previous results (Zhang et al. 2013).

![Fig. 1](image.png)

Fig. 1. Interactive effects of nitrogen forms and osmotic stress (OS) on the content of glycinebetaine (GB; A), NO (B), choline (C), and betaine aldehyde dehydrogenase (BADH) activity (D) in seedlings of two maize cultivars after 10 d of PEG treatment. JD20 and ZD958 represent cultivars Zhengdan 958 and Jundan 20, respectively. N-N, NA-N, and A-N represent plants supplied with \( \text{NO}_3^- \), \( \text{NH}_4^+ - \text{NO}_3^- \), and \( \text{NH}_4^+ \), respectively. Means ± SE, \( n = 8 \), different letters indicate significant differences among treatments at \( P \leq 0.05 \).
Correlations between the NO content and GB content in both the cultivars increased under OS regardless of OS (Table 1). Moreover, the BADH activity and choline content under OS were greater in cv. ZD958 than in cv. JD20. A positive correlation of the above responses was significantly greater in cv. ZD958 than in cv. JD20 during 0 - 3 d of OS. Afterwards, the NO accumulation began to decrease earlier than that of the GB accumulation. The changes in all the measured parameters in response to OS were greater under the NO3- nutrition than under sole NH4+ nutrition. The cultivar ZD958 was more responsive than JD20 to the OS and N-nutrition. Furthermore, OS obviously increased the accumulations of NO, GB, and choline, and enhanced the BADH activity more under both sole NO3- and the mixed supply of NH4+ and NO3- than under sole NH4+, especially in cv. ZD958. Thus, the content of NO, GB, and choline, as well as the BADH activity could be used as potential selection criteria for developing stress-tolerant cultivars.

Table 2. Correlation coefficients between the glycinebetaine (GB) content [nmol g\(^{-1}\)(d.m.)], betaine aldehyde dehydrogenase (BADH) activity [nmol mg\(^{-1}\)(protein) min\(^{-1}\)], choline content [nmol g\(^{-1}\)(d.m.)], or NO content [nmol g\(^{-1}\)(d.m.)] in two maize cultivars under osmotic stress. Significant differences at * - \(P < 0.05\), ** - \(P < 0.01\), *** - \(P < 0.001\).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>GB</th>
<th>BADH</th>
<th>Choline</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>GB</td>
<td>0.683***</td>
<td>0.889***</td>
<td>0.024</td>
<td></td>
</tr>
<tr>
<td>BADH</td>
<td>0.853***</td>
<td>0.391</td>
<td>-0.258</td>
<td></td>
</tr>
<tr>
<td>Choline</td>
<td>0.966***</td>
<td>0.902***</td>
<td>0.303</td>
<td></td>
</tr>
<tr>
<td>NO</td>
<td>0.983***</td>
<td>0.897***</td>
<td>0.983***</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. \(F\)-values of leaf glycinebetaine (GB) content [nmol g\(^{-1}\)(d.m.)], betaine aldehyde dehydrogenase (BADH) activity [nmol mg\(^{-1}\)(protein) min\(^{-1}\)], choline content [nmol g\(^{-1}\)(d.m.)], and NO content [nmol g\(^{-1}\)(d.m.)] in osmotic stressed maize cultivars supplied with different N forms. Significant differences at * - \(P < 0.05\), ** - \(P < 0.01\), *** - \(P < 0.001\).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>GB content</th>
<th>BADH activity</th>
<th>Choline content</th>
<th>NO content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water regime (W)</td>
<td>1</td>
<td>11968.50***</td>
<td>13234.90***</td>
<td>1835.59***</td>
<td>6396.68***</td>
</tr>
<tr>
<td>Cultivar (Cv)</td>
<td>1</td>
<td>323.44***</td>
<td>446.00***</td>
<td>17.69***</td>
<td>140.54***</td>
</tr>
<tr>
<td>N form (NF)</td>
<td>2</td>
<td>103.53***</td>
<td>121.85***</td>
<td>139.24***</td>
<td>59.36***</td>
</tr>
<tr>
<td>W+Cv</td>
<td>1</td>
<td>558.71***</td>
<td>334.31***</td>
<td>77.54***</td>
<td>179.85***</td>
</tr>
<tr>
<td>W+NF</td>
<td>2</td>
<td>83.47***</td>
<td>149.75***</td>
<td>50.48***</td>
<td>60.43***</td>
</tr>
<tr>
<td>Cv+NF</td>
<td>2</td>
<td>14.04***</td>
<td>8.36***</td>
<td>3.19</td>
<td>8.01***</td>
</tr>
<tr>
<td>Cv=W+NF</td>
<td>2</td>
<td>12.38***</td>
<td>27.53***</td>
<td>0.69</td>
<td>6.67***</td>
</tr>
</tbody>
</table>
criteria for drought tolerance in maize because an effectiveness of N form on all the measured parameters under OS was more pronounced in tolerant ZD958 than that in sensitive JD20. In the control plants of the two cultivars, the less and non-significant effects of different N forms on the NO accumulation and the other attributes measured were found (Fig. 1, Table 1).

The interactions among the OS, cultivars, and N forms on the above parameters were significant (Table 3). It is, therefore, suggested that the optimal application of N forms can promote NO accumulation and up-regulate GB metabolism under OS. Consequently, the optimal N forms could promote accumulation of NO thereby causing enhanced accumulation of GB by up-regulating BADH activity and enhancing choline content.

In conclusion, an increased ratio of NO\textsubscript{3}\textsuperscript{−} to NH\textsubscript{4}\textsuperscript{+} in nutrient solution (i.e. 0:100, 50:50, 100:0) under OS led to an enhanced NO accumulation and an up-regulation of GB metabolism in the two maize cultivars, thereby resulting in a greater GB accumulation, which alleviated drought-induced damage to the maize plants. The GB and NO accumulations increased consistently with the duration and intensity of OS. These efforts were more pronounced in the drought tolerant cultivar (ZD958) than in the sensitive one (JD20). The results suggest a beneficial role of NO\textsubscript{3}\textsuperscript{−} as compared to that of NH\textsubscript{4}\textsuperscript{+} in improving drought tolerance in the maize plants.

References


