Alleviation of water stress in beans by exogenous glycine betaine

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Abstract

The effect of exogenously applied glycine betaine on the ability of bean (Phaseolus vulgaris) plants to withstand water stress was investigated. Water stress induced endogenous accumulation of glycine betaine in the leaves, resulting in an increase of ~26% over the level in well-watered plants. Plants treated with exogenous glycine betaine (10 mM) maintained better water status during water stress treatment than the untreated plants. During water stress treatment, glycine betaine-treated plants showed a slower decrease in leaf water potential, thus developing wilting symptoms much later than the untreated plants. In addition, glycine betaine-treated plants showed better ability to recover from wilting than the untreated plants. Water stress adversely affected the photosynthetic activity of plants as indicated by a reduction in the leaf CO₂ absorption rate, the ratio of variable to minimal chlorophyll fluorescence (Fᵥ/Fₒ), and the overall growth. Glycine betaine treatment fully overcame the adverse effects on CO₂ absorption and chlorophyll fluorescence during water stress. However, little or no effect of glycine betaine on shoot biomass and pod yield was observed in water stressed plants. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Glycine betaine; Water stress; Phaseolus vulgaris

1. Introduction

Environmental stresses including water-deficits, salinity and low temperatures can induce a significant accumulation of glycine betaine in a number of diverse groups of plants [1,2]. This accumulation is thought to be a protective mechanism against many environmental stresses [3,4]. Glycine betaine has been shown to be involved in osmoregulation in bacteria [5] and plants [6–8]. In addition, glycine betaine has been shown to protect the structure and the function of proteins and membranes in plants against high temperatures and osmotic stress [7,9–11]. Specifically, under stress conditions, glycine betaine can protect photosynthetic activity including photosynthetic enzymes [12], proteins and lipids of thylakoid membranes [13], and electron flow in the photosystem II complex [14]. Studies have also shown that maize lines that accumulate glycine betaine are photosynthetically more efficient and exhibit better overall growth under salt stress than glycine betaine-deficient lines. This favorable response was attributed to better water status in the glycine betaine-containing line [15]. Freshwater cyanobacteria transformed with Escherichia coli bet genes acquired resistance to salt stress when supplied with choline, the precursor of glycine betaine [16]. Thus, accumulation of glycine betaine has been linked with plant’s ability to better survive osmotic/water stress conditions [17]. However, the impact of exogenous glycine betaine on plant water relations and on plant’s response to water stress is not clear. In this study we determine the effects of exogenously applied glycine betaine on the ability of bean plants to withstand water stress as indicated plant water relations and growth characteristics.

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2. Materials and methods

2.1. Plant material

Bean (Phaseolus vulgaris cv. Tendergreen) seeds were sown in a growing medium consisting of mixture of 2:2:1 (v/v) soil, peat, and perlite in 15-cm pots. Each pot contained 1.1 kg of the growing medium. Seedlings were thinned to one plant per pot 2 weeks after germination. Plants were grown in a greenhouse at 23/18°C day/night temperatures under natural day light conditions from October to December, 1995 and November 1996 to January 1997. Plants were watered to field capacity once every 2 days and fertilized weekly with Peat-Lite Special (Scotts-Sierra Horticultural Products, OH) with N–P–K of 20–10–20 (ammonium nitrate, potassium phosphate and potassium nitrate) at 250 ppm of nitrogen in irrigation water.

The 2-week-old plants were treated with 10 mM anhydrous glycine betaine (Sigma, MO) as a soil drench once every 2 days along with irrigation water containing fertilizers at the same level as before. The growing medium was irrigated to field capacity (~ 60 ml) with the glycine betaine solution, which was freshly prepared before each application.

2.2. Induction of water stress and recovery

Water stress treatments were initiated when plants were 4 weeks old. Irrigation was withheld until the untreated plants (plants not receiving glycine betaine) reached the permanent wilting point. During the water stress treatment, photosynthetic efficiency and endogenous glycine betaine levels in plants were determined. Stressed plants were rewatered and grown for 1–2 weeks to determine the extent of recovery. The control plants (unstressed) were irrigated with water or glycine betaine solution to field capacity once in 2 days. In a separate study, bean plants were grown under conditions as described above and subjected to a long term water stress. Long term water stress consisted of a series of drought cycles over a 4-week period. After the water stress treatment, plants were irrigated with water or glycine betaine solution to field capacity when initial symptoms of wilting were noticed, typically when the leaf water potential was between −1 and −1.2 MPa. Unstressed control plants were irrigated once in 2 days to the field capacity. Each treatment consisted of ten replications. When plants were 8–10 weeks old, data on growth characteristics such as shoot biomass, pod number, and pod dry weight were obtained.

2.3. Determination of endogenous glycine betaine level

Leaves from well-watered (including glycine betaine-treated) plants and water stressed plants (leaf between −1 and −1.8 MPa) were collected and frozen immediately in liquid nitrogen. Leaf extracts were obtained from thawed samples using a Carver Laboratory Press (Fred S. Carver, IN). A total of 10 µl of the extract was dried over nitrogen gas in a desiccator at room temperature. The final volume of the extract was made up to 1 ml with D2O. Glycine betaine was quantified using a Varian Unity Plus 500 NMR spectrometer [18]. One-dimensional proton spectra were measured at 30°C using a 5 mm triple-resonance inversion detection probe. Spectra were acquired with 16 transients, using 19.2 K data points and a spectral width of 8000 Hz centered on water peak. The free induction decays were subjected to Fourier transformation using VNMR 4.3b software. The peak intensities were measured digitally using the spectrometer’s integration software.

2.4. Water relations

During the preliminary experiments, both the hydraulic pressure method and the pressure bomb method were used to estimate leaf water potential. However, water potential values from the hydraulic press were somewhat higher than those determined with a thermocouple psychrometer. Also, preliminary experiments with pressure bomb did not yield consistent results, particularly at low water potential values. Gas pressure could not be used to express leaf sap because of the somewhat hollow nature of the petiole of bean leaves. Therefore, leaf water potentials were measured by a thermocouple psychrometer [19]. Water potential measurements were made using leaf sections of mature leaves selected from just below the fully expanded leaves. The leaf water potential was measured using a pre-calibrated thermocouple psychrometer (Model 75-1 AC, J.R.D. Merrill...
Speciality Equipment, UT) and a dew point microvoltmeter (Model HR-33T, Wescor, UT). A leaf section was excised and sealed immediately inside a small, water-tight, stainless-steel psychrometer chamber. The sample was equilibrated at 25°C for 5 h before the water potential was measured in the dew point mode with a cooling time of 20 s. To determine the leaf osmotic potential, leaf discs were floated on distilled water for 5 h and blotted dried, placed in a microcentrifuge tube, and frozen at −20°C for 24 h. After the leaf discs were thawed for 30 min, sap was expressed using a laboratory press (Fred S. Carver Inc.). A 10 μl aliquot of the expressed sap was transferred onto a filter paper disc which was then placed in the sampling chamber of the osmometer (Model 5500, Wescor) to measure the osmotic potential.

2.5. Fluorescence measurements

The chlorophyll fluorescence was measured on the dark-adapted leaflets of plants growing in a greenhouse using a fluorometer (Model SF-30; Richard Branacker Research, Ottawa, Canada) following the procedure of Smillie and Hetherington [20]. Fully turgid leaves from unstressed plants were used. In water stressed plants, fluorescence was measured 2 days after withholding water (leaf water potential between −0.8 and −1.0 MPa) and before the apparent symptoms of wilting could be observed. The fluorometer probe was placed on the adaxial leaf surface between the midrib and margin of leaflets that had been dark adapted for 5 min. The fluorescence was measured with excitation at 5.2 μm−2 for 10 s.

2.6. CO₂ uptake

Gas exchange measurements were made on the leaves of plants growing in the greenhouse with a Portable Photosynthetic System, LI-6200 (LI-COR, NE) equipped with a 0.25-l clamp-on cuvette (leaf area, 15 cm²). Measurements were made on fully expanded leaves that had been exposed to light for at least 8 h at 23°C at a mean light intensity of 800 μmol m⁻² s⁻¹. The water stressed samples were similar to those used for the fluorescence study (leaf water potential between −0.8 and −1.0 MPa).

2.7. Growth measurements

Plant growth characteristics were measured in plants subjected to a regime of long-term water stress. The growth characteristics measured included shoot biomass, plant height, leaf area, leaf number, number of pods, and pod yield. Approximately 10 weeks after planting, plants were harvested, and pods were separated from the shoots. The dry weights of shoots and pods were determined after drying in an oven at 85°C for 2 days.

3. Results

3.1. Leaf CO₂ uptake and the fluorescence

The rate of CO₂ uptake by the leaves decreased considerably in response to water stress. The reduction in the CO₂ absorption rate caused by water stress was prevented fully by the glycine betaine treatment (Fig. 1). Thus, the CO₂ absorption rate in water stressed plants receiving glycine betaine was similar to that in well-watered control plants.
The endogenous level of glycine betaine in bean leaves increased by ~26% in response to water stress (Fig. 1). As expected, exogenous application of glycine betaine to plants increased the leaf glycine content by over 58% in well-watered and water stressed plants.

3.2. Leaf water relation

There was no significant decrease in the leaf water potential for up to 6 days of water withholding both in glycine betaine-treated and control plants. The leaf water potential of unstressed glycine betaine-treated did not differ from that in the untreated control plants. However, the leaf water potential of untreated plants began to decrease more rapidly than that of glycine betaine-treated plants after 7 days into the water stress treatment (Fig. 3). Furthermore, the untreated plants showed initial symptoms of wilting ~7 days after water stress treatment whereas such symptoms typically did not occur until 11 days in glycine betaine-treated plants. When the wilting reached a severe stage (leaf water potential ~ −1.9 MPa) in the untreated plants, typically after 13 days of water stress treatment, both the untreated and glycine betaine-treated plants were watered to observe their ability to recover from wilting. While the untreated plants failed to recover, indicating that they had reached the permanent wilting point, the glycine betaine-treated plants fully recovered although some leaves appeared to exhibit partial scorching or drying (Fig. 4). Thus, the results suggest that the delay in the onset of wilting in the glycine betaine-treated plants appear to be due to a better water status during the water stress treatment. However, the exogenous glycine betaine treatment of bean plants did not lead to an osmotic adjustment as indicated by no significant change in the leaf osmotic potentials (~1.4 MPa) in the glycine betaine-treated plants.

3.3. Growth characteristics

Exogenous glycine betaine treatment increased the overall growth of plants as indicated by improvements in all the growth characteristics measured, including pod yield (Table 1). On the other hand, water stress treatment significantly reduced the plant growth. For example, shoot biomass and pod dry weight in plants decreased by ~32 and
Fig. 3. Effect of exogenous glycine betaine on leaf water potentials during water stress in bean plants. Leaf water potentials were measured in glycine betaine-treated and untreated plants during water stress. Glycine betaine treatment was as outlined in Fig. 1. The values expressed as means ± SD are shown (n = 10).

Fig. 4. Effect of exogenous glycine betaine on the recovery of water stress symptoms in bean plants. Water stress was induced in glycine betaine-treated (GB+WS) and untreated (WS) plants by withholding water until leaf water potential reached ~ −1.9 MPa in the untreated plants. Plants were subsequently rewatered to observe recovery from wilting symptoms. The glycine betaine treatment was as outlined in Fig. 1.
Table 1
Growth characteristics of bean plants in response to water stressa

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Shoot biomass (g/plant)</th>
<th>Pod number/plant</th>
<th>Pod yield (g/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15.7 ± 3.8</td>
<td>5.8 ± 2.0</td>
<td>6.7 ± 2.2</td>
</tr>
<tr>
<td>Glycine betaine</td>
<td>20.9 ± 4.5</td>
<td>7.2 ± 2.4</td>
<td>10.3 ± 3.0</td>
</tr>
<tr>
<td>Water stress</td>
<td>10.5 ± 2.6</td>
<td>3.4 ± 0.8</td>
<td>4.2 ± 1.8</td>
</tr>
<tr>
<td>Water stress + glycine betaine</td>
<td>11.1 ± 2.6</td>
<td>3.8 ± 0.9</td>
<td>4.4 ± 1.9</td>
</tr>
</tbody>
</table>

a Glycine betaine-treated and untreated plants were subjected to drought cycles over a 4-week period and the growth characteristics, shoot biomass and pod yield on dry weight basis and pod number, were measured when plants were 10 weeks old. Unstressed plants were supplied with water (control) or glycine betaine (GB) solution (10 mM) once in 2 days. Drought cycles were induced in plants by applying water or glycine betaine when leaf water potential reached between −1.0 and −1.2 MPa over a 4-week period. The data are expressed as means ± SD (n = 10).

38%, respectively, in response to water stress compared to the untreated control plants. Similarly, the number of pods/plant also was reduced significantly in water stressed plants. However, glycine betaine treatment in plants subjected to long term water stress showed little or no effect in shoot biomass and pod yield.

4. Discussion

Bean leaves increased the glycine betaine levels in response to water stress. Such an increase in the level of endogenous glycine betaine, a compatible osmolyte, in response to water and salt stress has been shown in a wide variety of plants [1]. When glycine betaine was applied exogenously, uptake by the roots was observed with a significant accumulation occurring in the leaves. Similar observations have been made in tobacco, in which over 98% exogenously applied glycine betaine was taken up readily by the roots in a solution culture [21] and by alfalfa roots to a lesser extent [8]. When glycine betaine is applied exogenously to the growing medium or soil, a possibility exists for interaction with the microflora. Although uptake of glycine betaine by bean plants was significant, how much of the applied glycine betaine was available to the plants and its interaction with microflora are unclear. However, we have not observed an uptake of exogenous glycine betaine by microflora for at least up to 72 h when applied to bean and strawberry plants (unpublished results).

The results indicate that the decrease in the rate of CO₂ uptake in beans caused by water stress was offset by the exogenous glycine betaine treatment. Furthermore, glycine betaine application to unstressed plants also increased the CO₂ absorption rate. Similar results were reported in maize, in which glycine betaine accumulation lines showed less adverse effect of salt stress on CO₂ assimilation rate than did the glycine betaine-deficient lines [15].

Data on chlorophyll fluorescence also suggest that the effect of water stress on fluorescence was reduced by the glycine betaine treatment. \( F_v/F_o \) was drastically reduced in response to water stress and this reduction was partially overcome by the glycine betaine treatment. Chlorophyll fluorescence has been used widely in a number of plants to determine injury or tolerance to various environmental stresses including chilling, freezing, heat, and radiation [20–23]. Typically, the rate and yield of variable chlorophyll fluorescence were reduced by stress conditions. This is consistent with our observations in beans with water stress. Partial recovery of fluorescence yield in response to glycine betaine treatment indicates restoration of function in photosystem II [24]. Using chlorophyll fluorescence in isolated chloroplast, Williams et al. [13] showed that glycine betaine can improve the thermal stability and electron transport in photosystem II. Similarly in spinach chloroplast, glycine betaine was shown to protect the oxygen evolution in photosystem II against salt stress [14]. Glycine betaine also has been shown to protect thylakoid membranes against freezing stress and preserve photosynthetic activity at low water potential [7,25]. Thus, it is likely that less inhibitory effect on photosystem II by water stress may lead to a favorable impact on the photosynthetic activity of plants. We found that glycine betaine treatment had a favorable impact on both CO₂ uptake and chlorophyll fluorescence during water stress.

As water was withheld, the leaf water potentials of plants decreased more rapidly in untreated plants than in glycine betaine-treated plants, indi-
indicating that the development of water stress in leaves was more gradual or perhaps delayed by the glycine betaine treatment. The water status of the glycine betaine-treated plants was slightly better than the untreated plants. For example, the leaf water potential of the untreated plants was −1.06 MPa, while it was −0.775 MPa for the glycine betaine-treated plants. Grumet and Hanson [6] found that endogenous glycine betaine was related closely to osmoregulation in barley leaves under salt stress. We found that the leaves of the glycine betaine treated bean plant did not show osmotic adjustment. The untreated plants developed water stress symptoms (loss of turgor and wilting) much earlier than did the glycine betaine-treated plants. In fact, the untreated plants reached the permanent wilting point after water was withheld for approximately 11 days, whereas no wilting was observed in the glycine betaine-treated plants. When water was withheld for 13 days, the untreated plants were lethally injured as indicated by their lack of recovery after subsequent rewatering. In contrast, glycine betaine-treated plants recovered after this water stress treatment. These observation indicate that glycine betaine application can reduce/delay water stress in bean plants and thereby allowing these plants to survive and function at low moisture availability. This ability can be perhaps attributed to an avoidance of water stress, as indicated by higher leaf water potentials in glycine betaine-treated plants than in the untreated plants during water stress treatment. It is not clear as to how glycine betaine application can improve the water status in plants which can possibly result from a more efficient water uptake by plants or by retarding water loss from plants during water stress, or both.

The growth characteristics of bean plants was consistent with the results from the CO₂ uptake study, in that glycine betaine treatment of well-watered plants resulted in significantly greater growth than in the untreated plants. As expected, the growth characteristics, such as shoot biomass, pod number and yield, were adversely affected by water stress. However, glycine betaine treatment had no apparent effect in offsetting the adverse effect of water stress on the growth characteristics of beans. However, as glycine betaine application to water stressed bean plants can improve the water status of plants, it is reasonable to expect that in turn may lead to a favorable effect on the photosynthetic activity and other growth characteristics. Indeed consistent with this, Saneoka et al. [15] noted that during salt stress, glycine betaine-containing lines of maize maintained higher relative water content and turgor in leaves than the glycine betaine-deficient lines. In addition, they found that some of the growth characteristics were less adversely affected by salt stress in glycine betaine-containing lines of maize, and this was attributed to better water status and higher CO₂ fixation. However, improved or better growth in glycine betaine-treated plants may not be related to glycine betaine as a source of nitrogen in plants [26]. Recently, even contrary to the conventional view of glycine betaine as a compatible solute, some possible negative effects of high levels of exogenous glycine betaine were noted in rape leaf discs [27]. In our study, the effect of increased CO₂ absorption rate caused by glycine betaine treatment was not fully apparent on the shoot biomass accumulation and pod yield of water stressed plants.

In summary, exogenous application of glycine betaine to beans resulted in a favorable water status of plants as indicated by a slower decrease in leaf water potential and delayed wilting during water stress as compared to the untreated plants. The adverse effects of water stress on CO₂ uptake and chlorophyll fluorescence in beans were fully or partly prevented by the glycine betaine treatment. However, the unfavorable effect of water stress on shoot and dry matter accumulation was not affected by the glycine betaine treatment.

References


