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Alleviation of salt-induced adverse effects in eggplant (*Solanum melongena* L.) by glycinebetaine and sugarbeet extracts

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ABSTRACT

Two eggplant cultivars, Dilnasheen and Bemisal, were selected to assess whether pure GB and sugarbeet extract could effectively ameliorate the harmful effects of salt stress on eggplant (*Solanum melongena* L.), under saline conditions. Salt stress markedly suppressed the growth, yield, photosynthetic capacity, internal CO_2 level, transpiration, and stomatal conductance in both cultivars. Potassium (K^+) and Ca^{2+} contents and K^+/Na^+ ratios of both root and leaf were also reduced, while GB and proline in leaves, and Na^+ and Cl^- contents in roots and leaves were significantly enhanced. Exogenously applied glycinebetaine and sugarbeet extracts significantly counteracted the salt-induced adverse effects on growth, yield, various gas exchange characteristics, GB and leaf K^+, Ca^+, Cl^- and Na^+. However, GB and sugarbeet extract showed differential effects on photosynthetic rate, stomatal conductance and transpiration, internal CO_2 level, C_i/C_a ratio, leaf K^+, Ca^{2+}, and Cl^- contents, and K^+/Na^+ ratio. Sugarbeet extract proved better than the GB in improving growth, photosynthetic rate, transpiration, stomatal conductance, yield and GB accumulation. Since, sugarbeet extract contains a substantial amount of GB along with a variety of other important nutrients so it was found as effective as pure GB in improving growth and some key physiological processes in eggplant under salt stress. Thus, it can be used as an alternative cheaper source of GB for its use as an ameliorative agent for protecting plants against the hazardous effects of salt stress.

1. Introduction

Glycinebetaine, an amino acid derivative, is naturally found in microorganisms, plants, and animals (Sakamoto and Murata, 2002; Makela, 2004). It occurs abundantly in many crop plants including spinach (Weigel et al., 1986), wheat (McDonnell and Wyn Jones, 1988), alfalfa (Wood et al., 1991), and bean (Gadallah, 1999). However, many important crop plants, like maize, potato, tomato, and eggplant are unable to accumulate glycinebetaine (Zwart et al., 2003). Glycinebetaine was first discovered in sugarbeet (*Beta vulgaris*) juice at the rate of 100 mmol kg^{-1} plant tissue (Mack et al., 2007). It physiologically acts as an osmolyte to protect cells or as a catabolic source of methyl groups to facilitate various biochemical processes (Craig, 2004). It can protect the plant cells against osmotic inactivation and increases the water retention of cells (Liu and Bolen, 1995; Sakamoto and Murata, 2002; Makela, 2004; Ashraf and Foolad, 2007). However, exposure to salt stress triggers glycinebetaine synthesis in cell chloroplasts of most plant species (Rathinasabapathi et al., 1997; Park et al., 2007) and glycinebetaine-induced improvement in plant is widely reported (Ashraf et al., 2008; Mahmood et al., 2009; Nawaz and Ashraf, 2009). For example, with exogenous application of GB, improvement in salt tolerance had been achieved in different crops, e.g., tomato (Mäkela et al., 1998), cotton (Naidu, 1998), rice (Rahman et al., 2002), *Triticum aestivum* (Raza et al., 2007; Mahmood et al., 2009), and maize (Nawaz and Ashraf, 2009). In almost all studies wherein GB has been shown as an effective ameliorating agent against salt or other stresses reported so far in the literature, pure GB procured from the known chemical manufacturing companies has been used.

Vegetable crops are very important due to their higher yield potential, low production cost and higher nutritional value (Mukerji, 2004; Noreen and Ashraf, 2009a,b). Eggplant (*S. melongena* L.), known as brinjal, aubergine or Guinea squash is cultivated on more than 1.5 Mha in the world (Kantharajah and Golegaonkar, 2004). Nutritionally, eggplant is as tomato, because both are a rich source of vitamins and minerals (Kalloo and Bergh, 1993). However, eggplant is moderately sensitive to salinity (Heuer et al., 1986; Savvas and Lenz, 1996; Akinci et al., 2004). Extensive research has been carried out to examine salt-induced morphological, biochemical and physiological changes in eggplant (Chartzoulakis and Loupassaki, 1997; Hamdy et al., 2002; Akinci et al., 2004). However, information on the alleviation of salt-induced adversaries by foliar applied GB in eggplant is lacking. Of many naturally GB-accumulating plants (Ashraf and Foolad, 2007), sugarbeet is known

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to accumulate a substantial amount of GB (100 mmol/kg plant tissue) (Mack et al., 2007), or 0.2–0.3% (Korteniemi, 2007). In addition to it, 100 g sugarbeet extract contains a variety of other inorganic nutrients and organic compounds such as fiber (2.8 g), carbohydrates (9.56 g), calcium (16 mg), potassium (325 mg), magnesium (23 mg), phosphorus (40 mg), vitamin E (0.3 mg), and vitamin C (4.9 mg) (http://www.botanical-online.com/commonbeet.htm). However, information on the exogenous application of this natural GB source in alleviating the salt-induced adverse effects cannot be deciphered from the literature.

So, we hypothesized that foliar applied pure GB or GB-enriched sugarbeet extract could effectively minimize salt-induced harmful effects on growth of eggplant, naturally a GB-non-accumulator. Thus, the two principal objectives of carrying out this study were: (i) whether or not exogenous application of GB could protect eggplant against salt stress, and (ii) how effective the sugarbeet extract is in regulating growth and some key physiological processes involved in salt tolerance of eggplant as compared to the pure GB.

2. Materials and methods

A greenhouse experiment was conducted in the Botanical Garden, University of Agriculture, Faisalabad, to examine the plausible role of foliar-applied glycinebetaine and sugarbeet extract to minimize the growth loss in eggplant (*S. melongena* L.) caused by NaCl. During experimentation, two eggplant cultivars, Bemisal and Dilnasheen were subjected to two NaCl treatments, i.e., 0 mM (control) and 100 mM. Eight seed of each of the two cultivars were sown in sand (10 kg) contained in plastic pots. Two liters of Hoagland's nutrient solution (full strength) were applied weekly to each pot. Experiment was arranged in a completely randomized design with four replicates. Ten days after seed germination, plants were thinned to five in each pot/replicate, and after 30 days, NaCl treatments were begun. Different concentrations of GB (0, 50 mM pure GB, prepared in 0.1% (v/v) Tween-20 and sugarbeet extract containing 50 mM GB were used for exogenous application. Glycinebetaine (M. wt. = 117.1) of Sigma–Aldrich, Japan was used. For the extraction of sugarbeet juice, fresh sugarbeet roots were procured from the local market. After washing well the roots, they were extracted using an electric extractor (Model Ju-209, Guang Dong, China). The extract was filtered using a fine sieve (0.3 mm) and stored it at −20 °C for further use. GB in the sugarbeet extract was estimated as described by Grieve and Grattan (1983). The GB concentration determined was 50 mmol/kg. The juice was extracted 1 day before its foliar application. Sugarbeet extract or pure GB was applied foliarly at the vegetative stage. Two plants were harvested after 20 days of foliar application of sugarbeet extract or pure GB. After properly washing the plant parts, they were weighed for recording data for shoot and root fresh masses and then dried in an oven at 65 °C for 1 week for recording dry masses. Data for different yield related attributes were recorded at maturity.

2.1. Gas exchange characteristics

Instantaneous measurements of gas exchange attributes such as photosynthetic rate (A), internal CO_2 concentration (C_i), transpiration (E), and stomatal conductance (g_s) were performed on a fully expanded third leaf (from top) of one plant from each replicate using a portable infrared gas analyzer (Model LCA-4; ADC, Hoddesdon, England). The other adjustments/specifications of the leaf chamber were as follow: atmospheric pressure (P) 97.9 kPa, leaf surface area 6.25 cm^2, leaf chamber temperature (T_{ch}) varied from 29.3 to 35.5 °C, gas flow rate of leaf chamber volume (V) 296 mL min^{-1}, atmospheric CO_2 content (C_{ref}) 369 μmol mol^{-1}, molar gas flow rate of leaf chamber (U) 400 μmol s^{-1}

2.2. Determination of glycinebetaine

The method described by Grieve and Grattan (1983) was employed to determine GB in leaf tissues. Optical density of the organic layer was measured at 365 nm using a spectrophotometer (Hitachi-U2001, Tokyo, Japan).

2.3. Determination of proline

Proline in leaf tissues was estimated following the protocol as described by Bates et al. (1973). The absorbance of the chromophore containing toluene was read at 520 nm using a spectrophotometer (Hitachi-U2001, Tokyo, Japan). Proline concentration was calculated using the following formula:

$$\mu\text{mole proline g}^{-1}\text{ fresh weight} = \frac{\mu\text{g proline mL}^{-1} \times \text{mL of toluene}/115.5}{\text{g of sample}}.$$

2.4. Mineral nutrients

2.4.1. Na^+, K^+ and Ca^{2+}

Sodium (Na^+), K^+ and Ca^{2+} in the dried ground leaf and root tissues were determined following Allen et al. (1986). The sample so digested was diluted up to 50 mL in a volumetric flask and filtered. The filtrate was used for the determination of Na^+, K^+ and Ca^{2+} using a flame photometer (Jenway, PFP-7).

2.4.2. Determination of Cl^-

Chloride in the plant samples was extracted by heating the material in water. Dried ground leaf or root sample (0.1 g) was taken in a test tube and 10 mL of distilled water were added to it, and then incubated it overnight at 25 °C. The tubes were then heated at 80 °C in a digestion block until the volume in the test tubes remained half of the original volume. After cooling, distilled water was added to each test tube to maintain the volume up to 10 mL again and Cl^- concentration in the leaf and root extracts determined using a chloride analyzer (Model 926, Sherwood, Cambridge, UK).

2.5. Statistical treatment of the data

Analysis of variance of the data for each parameter was computed using the statistical software COSTAT version 6.303 (Cohort Software, Monterey, CA). Least significance difference (LSD) was calculated following Steel and Torrie (1980) to appraise the significant difference among the mean values within each attribute.

3. Results

Salt stress applied through the root medium (100 mM of NaCl) considerably ($P \leq 0.001$) reduced the shoot and root fresh and dry weights of both eggplant cultivars, Bemisal and Dilnasheen. Under saline regime, cv. Dilnasheen was significantly higher in shoot fresh and dry masses, while cv. Bemisal was better in root fresh and dry weights under both control and saline conditions. Of both GB sources, sugarbeet extract was very effective in promoting growth of both eggplant cultivars under salt stress (Table 1 and Fig. 1).

Salt stress markedly suppressed the shoot and root lengths of both eggplant cultivars. Cultivar Bemisal was relatively better in these attributes than cv. Dilnasheen. Both GB sources (natural and pure) had a significant effect in improving shoot and root lengths in both cultivars. Shoot length was higher at sugarbeet extract while root length at 50 mM of pure GB under saline conditions (Table 1 and Fig. 1).