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Effect of foliar applied glycine betaine on growth performance of broccoli (*Brassica oleracea* var. Italica)

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Abstract

Glycine betaine performs a crucial role, like osmoprotectant and cytoplasmic osmotic solute specifically in the members of family Gramineae and Chenopodiaceae. *Brassica oleracea* var. *Italica* subvariety PALMIRA 2 hybrid due to the presence of glucosinolate plays a vital role for humans. A pot experiment was performed at the Old Botanical Garden, University of Agriculture, Faisalabad. 35 days seedlings were transplanted to pots filled with 7 kg of soil for two weeks to get them acclimatized to pot soil conditions. Foliar application of glycine betaine (0, water spray, 5, 10, and 15 mM) was given after 2 months of transplantation. Data regarding the growth parameters, reactive oxygen species, antioxidant enzymes, mineral ions determination, nutrient use efficiency, nutrient uptake, total soluble proteins, and photosynthetic pigments were collected after 15 days of glycine betaine application. Application of GB increased shoot fresh and dry weight, shoot length, root dry weight, shoot calcium, potassium, nutrient uptake of sodium, calcium, potassium, SOD, peroxidase, and total soluble proteins of Broccoli (*Brassica oleracea* var. Italica). Implementation of GB showed non-significant results of parameters such as root fresh weight, photosynthetic pigments (chlorophyll *a, b, a/b*, carotenoids, and total chlorophyll), nutrient use efficiency of sodium, and catalase activity.

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Introduction

Glycine betaine performs a crucial role, like osmoprotectant or as a well-suited cytoplasmic osmotic solute specifically in the members of family *Gramineae* and *Chenopodiaceae*. Glycine betaine is zwitterionic, N-methyl derivative present in many higher plants, microorganisms, and animals (Saeed *et al.*, 2016). In plants improved the action of antioxidants, proteins, enzymes, and photosynthetic activity of plants (Banu *et al.*, 2009).

Synthesis of glycine betaine is not the same in different plants for example some plants like barley and spinach gather comparatively high levels of GB as compared to *tobacco* and *Arabidopsis thaliana* in their chloroplasts. Application of glycine betaine protect plants from stress, and it is introduced in higher plant and microorganisms by genetic engineering which shows the importance of GB.

It keeps the cell membrane integral in its nature and enzyme equilibrium takes part in the removal of ROS from the cell (Sakamoto and Murata, 2002).

In agriculture from an economical and defensive perspective, foliar-applied glycine betaine is very important. Due to its ameliorative nature to enhance crop production and reduces toxic environmental stresses. Besides GB improves the activities of antioxidant enzymes in rice seedlings. Accumulation of glycine betaine protects plant plasma membrane from disruption, high temperature (Hasanuzzaman *et al.,* 2014).

Broccoli variety PAALMIRA 2 hybrid member of *Brassicaceae* family and grows up to 90cm in length. It is found around the Mediterranean region and southwestern Europe. It is an old vegetable native to Turkey and its cultivation starts there, later it is found in the USA, England.

In 1923 its industrial cultivation started (Decoteau, 2000). Broccoli is composed of sulforaphane, glucosinolates, selenium, polyphenols, and secondary metabolites, A, C, and E vitamins are also present. Isothiocyanates present in broccoli, help in the prevention of cancer, its leaves are also utilized in the treatment of skin-related diseases. The presence of glucoraphanin sulforaphane in broccoli high concentration completely hinder the chemical induction of breast cancer in rats (Meyer *et al.*, 2008).

The sprouts of broccoli are famous because of rich in vitamin contents, minerals, and glucosinolate secondary metabolites act as phenolic compounds (Baenas *et al.*, 2012).

Objectives

To evaluate the effect of foliar application of glycine betaine on broccoli variety PALMIRA 2 hybrid.

To observe the changes in physiological and morphological characters of broccoli by foliar spray of glycine betaine.

Materials and methods

The present research activity was performed to understand the morphological biochemical and photosynthetic parameters of Broccoli (*Brassica oleracea* var. Italica) variety PALMIRA 2 hybrid under the exogenous application of glycine betaine. A pot experiment was arranged to check the effect of glycine betaine on broccoli at Old Botanical Garden, University of Agriculture Faisalabad. Five levels of GB as 0 (control) 5, 10, 15 mM, and water spray were applied exogenously. A completely randomized design was used. Plastics pots were filled with 7kg of soil. 35days of seedlings were transplanted into pots, and after 105 days foliar application of glycine betaine was applied, or after 120 days data were collected, one-way ANOVA with CRD used.

The following parameters were examined.

Measurement of growth parameters

Shoot Fresh Weight (g):

Shoot Dry Weight (g):

Shoot Length (cm):

Root fresh weight (g):

Root Dry Weight (g):

Photosynthetic Pigments

Chlorophyll *a*

Chlorophyll *b*

Chlorophyll *a/b* ratio

Total chlorophyll

Carotenoids

For the determination of Chlorophyll, *a, b* total Chlorophyll protocol was proposed by Arnon (1949).

The following formulas were employed for calculating the concentrations of chlorophyll and carotenoids.

$$\text{Chl a (mg/g f.wt.)} = \frac{[12.7\ (\text{OD } 663) - 2.69\ (\text{OD } 645)\ \times\ V}{1000\ \times\ W}$$

$$\text{Chl b (mg/g f.wt.)} = \frac{22.9\ (\text{OD } 645) - 4.68\ (\text{OD } 663)\ \times\ V}{1000\ \times\ W}$$

$$\text{Chl}\ \frac{a}{b}\ \text{Ratio} = \frac{\text{Chl a}}{\text{chl b}}$$

$$\text{Total Chlorophyll}\left(\frac{\text{mg}}{\text{g}}\text{fresh weight}\right) = [\frac{20.2(\text{OD}645 - 8.02(\text{OD } 663) \times V}{1000 \times W}]\ \text{Chlorophyll } a + \text{Chlorophyll } b$$

Whereas,

OD= Optical density

V= volume of sample

W= Fresh weight of the sample

Carotenoids (mg/g f. wt.) = A^{car}/Em × 100

A^{car} = OD 480 + 0.114(OD 663) – 0.638 (OD 645) and Em = 2500

Photosynthetic attributes

Enzymatic antioxidants

Catalase (Units/mg protein)

Superoxide dismutase

Peroxidase

Mineral nutrient ions

Ion analysis

Sodium (mg/g dry weight)

Potassium

Calcium

Nutrient Use Efficiency (g^2mg^{-1})

Sodium Na^+ (g^2/mg d. wt.)

Calcium Ca^{+2}

Potassium K^+

Similarly, Nutrient use efficiency and nutrient uptake of Sodium, potassium, and calcium ions were analyzed.

The following formula was used for the determination of nutrient uptake

$$\text{NUE}\ (g^2mg^{-1}) = \frac{1}{\text{nutrient concentration}\ (\frac{mg}{g})} \times \text{shoot dry weight (g}$$

Nutrient uptake (mg)

Sodium Na^+ (g^2/mg d. wt.)

Calcium Ca^{+2}

Potassium K^+

For the analysis of nutrient uptake of Na^+, K^+ Ca^{+2} following formula was used.

$$\text{NU} = \text{nutrient concentration}\ (\frac{mg}{g}) \times \text{shoot dry weight (g)}$$

Statistical analysis

At the end of the experimental period, the data analyzed statistically using analysis of variance ANOVA with Co- STAT software (Steel and Terrie, 1986).

Results

To analyze the effect of glycine betaine, an experiment was conducted on broccoli (*Brassica oleracea* var Italica) subvariety PALMIR 2 hybrid. The experiment was carried out in the Botanical Garden, University of Agriculture, Faisalabad. In each pot 2 plants of broccoli seedlings when it 38 days grown transported. Pots filled with the 7kg of soil. One water spray level, control, and three-level of glycine betaine (0 and 5 and 10 mM) were foliar applied. Growth related attributes, and some biochemical parameters and activity of superoxide dismutase, peroxidase, catalase, total soluble proteins, mineral nutrients, and nutrient water use efficiency, nutrient uptake was recorded. Implementation of GB decreased or showed non-significant results such as chlorophyll *a, b, a/b*, total chlorophyll and carotenoids, root fresh weight, nutrient use efficiency of sodium, and catalase (CAT) of Broccoli. Treatment of glycine betaine enhanced shoot fresh and dry weight, shoot length, root dry weight, shoot calcium (Ca^{2+}), potassium (K^{+}), nutrient uptake of sodium, calcium, potassium, superoxide dismutase, peroxidase, and total soluble proteins of broccoli. Glycine betaine increased uptake of ions such as potassium and calcium which play a fundamental role in plant growth. Foliar applied GB showed significantly increased all attributes.

Table 1. Growth parameters of Broccoli (*Brassica oleracea* Var. Italica) when 95 days old plants were subjected to foliar-applied glycine betaine.

Parameters	NS mM	WS mM	5 mM	10 mM	15 mM	F-value
Shoot fresh weight	21.88	18.84	24.66	29.16	34.49	379.788***
Shoot dry weight	2.42	4.60	3.36	3.68	4.45	7.815***
Shoot length	27.70	28.74	28.98	28.22	28.06	2.674***
Root fresh weight	4.07	4.07	3.24	3.60	3.46	1.386ns
Root dry weight	14.02	14.06	15.06	16.17	17.61	1.824***

*= Significant, ***= Highly Significant, Ns= Non-Significant.

Table 2. Photosynthetic pigments Growth parameters of Broccoli (*Brassica oleracea* Var. Italica) when 95 days old plants were subjected to foliar-applied glycine betaine.

Parameters	NS mM	WS mM	5 mM	10 mM	15 mM	F-value
Chlorophyll *a*	12.05	12.87	12.66	12.92	13.40	0.002ns
Chlorophyll *b*	14.46	14.93	14.98	15.23	15.63	0.00718ns
chlorophl *a/ b* ratio	2.84	2.65	2.15	2.91	2.57	1.634ns
Total CHL	9.48	9.71	9.61	10.16	10.53	0.005ns
Carotenoids	10.57	10.85	10.89	11.48	11.95	0.0004ns

*= Significant, ***= Highly Significant, Ns= Non-Significant.

As the level of GB increased from 5, 10, 15 mM, its enhanced growth of shoot fresh weight. Water spray plants show less growth as compared to control plants, similarly, 15mM showed an increase in shoot fresh weight in comparison to control (table 1) (Sakamoto and Murata, 2002).

Table 3. Mineral ions of Broccoli (*Brassica oleracea* Var. Italica) when 95 days old plants were subjected to foliar-applied glycine betaine.

Parameters	NS mM	WS mM	5 mM	10 mM	15 mM	F-value
Shoot Na+	9.88	10.20	10.06	10.54	10.82	85.036ns
Shoot Ca+	9.45	9.67	9.54	10.06	10.30	2.754***
Shoot K+	8.45	8.62	8.45	9.03	9.23	0.027***

*= Significant, ***= Highly Significant, Ns= Non-Significant.

Exogenously applied glycine betaine also increased shoot dry weight and showed a considerable result. Water sprayed plants showed more growth as compared to other treatments of GB. Similar results were observed in soybean (Rezaei *et al.,* 2012).Implementation of GB showed non-significant results for the root fresh weight of broccoli (*Brassica oleracea* var. Italica subvariety PALMIRA 2) hybrid. It does not affect the root fresh weight (table 1). In thecurrent study present result was in contrast with past findings studied in rice seedlings (Rahman *et al.*, 2002). Photosynthetic pigments such as chlorophyll *a, b, a/b* ratio, total chlorophyll, and carotenoids all showed no reaction to glycine betaine (table 2).

Table 4. Nutrient uptake and Nutrient use efficiency of Broccoli (*Brassica oleracea* Var. Italica) when 95 days old plants were subjected to foliar-applied glycine betaine.

Parameters	NS mM	WS mM	5 mM	10 mM	15 mM	F-value
Nutrient use efficiency Na+	9.59	9.83	9.74	10.28	10.57	85.036 ns
Nutrient use efficiency Ca+	9.34	9.58	9.45	9.98	10.23	2.754***
Nutrient use efficiency K+	9.21	9.42	9.29	9.84	10.08	0.027053ns
Nutrient uptake Na+	9.15	9.36	9.23	9.78	10.03	0.052ns
Nutrient uptake Ca+	9.32	9.55	9.43	9.97	10.23	129.20***
Nutrient uptake K+	9.25	9.48	9.35	9.89	10.14	327.91***

*= Significant, ***= Highly Significant, Ns= Non-Significant.

However, glycine betaine resulted in a significant impact on Potassium, and calcium. It increased due to uptake of water and presented similar findings was finds in rice that plants treated with GB had a significantly high level of K^+ and Ca^{2+} level (table 3) in the shoot (Lutts *et al.*, 1999). Nutrient uptake of sodium, calcium, potassium ions of broccoli all increased underexposure of glycine betaine due to uptake of nutrients and showed the significant (table 4).

Table 5. Photosynthetic parameters and Total Soluble protein of Broccoli (*Brassica oleracea* Var. Italica) when 95 days old plants were subjected to foliar-applied glycine betaine.

Parameters	NS mM	WS mM	5 mM	10 mM	15 mM	F-value
Total Soluble protein	9.23	9.45	9.33	9.87	10.12	511.09***
Catalase	9.24	9.46	9.33	9.88	10.13	0.0001ns
SOD	9.26	9.49	9.36	9.90	10.16	9.991***
POD peroxidase	9.25	9.47	9.34	9.89	10.14	1278.39***

*= Significant, ***= Highly Significant, Ns= Non-Significant.

The present research showed the non-significant activity of catalase (table 5). Current results were like the past study as in mung bean (Jabeen *et al.,* 2016). Superoxide dismutase, peroxidase, and total soluble proteins all showed a significant level, and our result following past results as demonstrated in two lettuce cultivars (Khalifa *et al.*, 2016).

Fig. 1. Broccoli (*Brassica oleracea* var. Italica).

Conclusion

Glycine betaine applied possessed several beneficial effects as it protects the overall machinery of plant and improves growth or mitigate the harmful effects. Hence, it is concluded that glycine betaine increased morphological parameters with or without stress. However, in those plants which cannot accumulate GB naturally, exogenous application enhanced the level of glycine betaine. It also plays a role to improve the activities of essential enzymes. Finally, given the above results, deleterious effects can be alleviated when GB was exogenously applied to plants.

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Abbreviations

SOD Superoxide dismutase, POD peroxidase.