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RESEARCH PAPER

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Mitigation of adverse effects of heat stress in chillies by using glycine betaine

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Abstract

Chilli (*Capsicum spp.*) is an important vegetable cum spice crop of the night shade family requiring 20-30 °C optimum temperatures for plant growth and development, usually growth starts retarding below 15°C or above 32°C temperature. Almost all growth stages of chilli plants are influenced by high temperatures ultimately leads to economic yield losses in final crop productivity. The experiment was carried out in growth chamber of mushroom lab, Institute of Horticultural Sciences, University of Agriculture, Faisalabad aiming at identifying the best glycine betaine treatment proved to be useful in coping with adversaries of high temperature stress in chillies. Chilli genotypes named as C-37, Uk-101, H-13 and jawala were grown and sprayed with different concentrations (0, 5, 10, 15 and 20 mM) of glycine betaine at the seedling stage under high temperature stress (40/32°C day and night temperature) in growth chamber provided with controlled conditions. Various physiological attributes of chilli genotypes under heat stress. Glycine betaine has also been proved effective in enhancing the heat tolerance potential under high temperature stress.

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Introduction

Chilli has been classified as a temperature sensitive plant species, since it responds differently to heat stress at each growth stage in contrast to other horticultural crops (Afzal et al., 2014). Chillies require 20-30°C optimum daily temperatures for growth and development and usually growth is retarded when temperature falls below 15°C or increases above 32°C (Berke at el., 2005; Safina et al., 2006; Alberto et al., 2013). Chillies are used either cooked, dried, raw or as additives in foods and their use has also been reported in pharmaceutical industry, due to presence of wide range of antioxidants like capsaicinoids, flavonoids, carotenoids (A vitamin), ascorbic acid (C vitamin) and tocopherols (E vitamin) in its fruit (Howard et al., 2000; Sigge et al., 2001; Marti et al., 2011; Carvalho et al., 2011; Akhund et al., 2017). Antioxidants are incredibly efficient in preventing cardio-vascular diseases and ovarian cancer (Bramley, 2000). Chilli is a profitable and highly valued vegetable (Howard et al., 2000).

Elevation in average temperature due to global warming becomes a major threat for crop productivity around the globe (Hall, 2001). Human race is actually responsible for depositing dangerous greenhouse gases in the environment, thus making the environment unsuitable for living. It is well understood that these greenhouse gases are continuously increasing the global temperature. In last century, the mean earth temperature increased upto 2°C and is expected to into an increament of 5.8°C in the next century. A potential threat for crop production which may lead to severe famines worldwide (IPCC, 2012). High temperature causes heat pressure: a major issue in warm regions of the world causing undesirable and multifarious changes on plant morphology, anatomy as well as physiology (Shaked et al., 2004). Heat stress directly effects plant physical along with its indirect effects on growth and development patterns. Instances are there like, in the long run heat stress may cause delayed germination, hence, leading towards poor appearance and deformation of seeds. Improvement of plants via stress tolerance induction needs the complete awareness about physiological and genetic information of plants. Substantial biotechnological investigations focused on the plant development at molecular level has been made regarding stress tolerance in the past twenty years (Holmberg and Bulow, 1998; Serrano *et al.*, 1999; Kasuga *et al.*, 1999; Hasegawa *et al.*, 2000; Zhu, 2001; Rontein *et al.*, 2002; Prabhavathi *et al.*, 2002).

Accumulations of low molecular weight organic compounds (osmolytes) plays significant role in plant defense mechanisms under a variety of abiotic stresses. Sairam and Tyagi, (2004) found various osmolytes like tertiary and quaternary ammonium compounds (glycine betaine and proline etc) were produced in response to abiotic stresses. Several quaternary ammonium compounds have been identified by plant scientists, among these quaternary ammonium compounds, glycine betaine (GB) is reported to be of much importance mainly accumulated in large quantities in response to abiotic stresses (Venkatesan and Chellappan, 1998; Mansour, 2000; Mohanty et al., 2002; Yang et al., 2003; Delgado-Gayt et al., 2017). Glycine betaine is rich primarily in chloroplasts, where it played an important role in modification and protection of the thylakoid membrane and had also been proved helpful in maintaining photosynthetic effeciency (Genard et al., 1991). In response to heat stress, glycine betaine (GB) is naturally accumulated in numerous vegetables together with chillies, spinach and sugar beet (Chen and Murata, 2002; Yang et al., 2003) and played a key role in osmotic adjustments, commonly known to be an adaptive response of heat, drought and salinity stresses (Subbarao et al., 2001; Chinnusamy et al., 2005; Yang and Lu, 2005). For the reason, a brief study was designed to evaluate the potential of exogenously applied glycine betaine on chilli plants under high temperature stress.

Materials and methods

Planting material and growing conditions

The proposed research work was performed in growth chamber of mushroom lab at Institute of Horticultural Sciences, University of Agriculture,

Faisalabad during Summer season, 2015. Healthy, viable and disease free seeds of four genotypes (C-37, Uk-101, H-13 and jawala) were collected from AARI, Faisalabad and sown in medium sized plastic pots filled with sand media watered according to the irrigational requirement. Experiment was performed with four replications. Hoagland's nutrient solution with half strength (0.5) was used for plant nourishment. Heat stress was applied for four weeks after emergence by increasing 2°C/day temperature upto 40/32°C day and night temperature.After attaining desired high temperature, different levels (0, 5, 10, 15 and 20 mM) of glycine betaine were sprayed on chilli seedlings. Seven days after glycine betaine application, plants were harvested for further analysis of various physiological characteristics.

Transpiration, photosynthesis and stomatal conductance to water

For the analysis of physiological characteristics like rate of transpiration, photosynthetic rate and stomatal conductance to H₂O, two fully developed, young, healthy and uniform sized leaves per plant (from each replication) were selected and kept one by one in portable chamber of a gadget named as IRGA (Infra-Red Gas Analyzer) (LCi- SD, ADC Bio-scientific UK). All the readings of physiological characteristics were determined during day time (10.00 a.m-12.00 p.m) at 99.9 kPa atmospheric pressure, 403.3 mmol m⁻²s⁻¹ molar flow of air per unit leaf area, water vapor pressure in the chamber ranged from 6.0 to 8.9 mbar, photosynthetically active radiation up to 1711 pmol m⁻ ²s⁻¹ at leaf surface, surrounding CO₂ level was 352 pmol mol⁻¹ and atmospheric temperature 40°C.

Water use efficiency

Water use efficiency was calculated by the formula given below,

WUE = A/E

Chlorophyll contents

Chlorophyll meter (CCM-200 plus Bio- Scientific USA) was used for the measurement of chlorophyll contents.

Experimental design and statistical analysis

Experiment was laid out with two factorial arrangements under Complete Randomized Design (CRD). Analysis of variance (ANOVA) and means comparison test (HSD Tuckey test) were determined using Statistix 8.1 computer package. Differences among treatments of glycine betaine and chilli genotpes were considered significant at $p \le 0.05$ after statistical analysis.

Results

Effect of glycine betaine treatments on photosynthesis rate of chilli genotypes

Glycine betaine significantly (P<0.05) enhanced photosynthesis rate of chilli genotypes as compared to control (Fig. 1). Genotypes C-37 and UK-101 gave the maximum photosynthesis rate (14.02 and 13.38 µmol m⁻²s⁻¹, respectively) with foliar spray of glycine betaine @15mM. However, H-13 and Jawala had maximum photosynthesis rate (6.97 and 8.14 µmol m⁻ ²s⁻¹, respectively) at the same concentration of glycine betaine. Minnimum photosynthesis rate (2.05 and 1.57 µmol m⁻²s⁻¹) was observed in H-13 and Jawala, respectively where no glycine betaine was applied.

The interaction between genotypes and glycine betaine application (GB \times G) was significant as depicted in Table 1.

Effect of glycine betaine treatments on stomatal conductance to water in chilli genotypes

Glycine betaine significantly (P<0.05) improved the stomatal conductance of genotypes as compared to control (Fig. 2). The genotypes H-13 and Jawala gave the highest stomatal conductance (2 mmol $m^{-2}s^{-1}$), followed by UK-75 which also showed highest stomatal conductance (1.88 mmol $m^{-2}s^{-1}$) at control. Minimum stomatal conductance (0.40 mmol $m^{-2}s^{-1}$) was observed in UK-101 where glycine betaine was applied @15mM, followed by C-37 which gave 0.45 mmol $m^{-2}s^{-1}$ stomatal conductance at the same of level glycine betaine.

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Transpiration rate

Stomatal conductance to

H₂O

Water use efficiency

Chlorophyll contents

** ×->

NS

** **

* **

**

*

**

| genotypes under high temperatur | re stress. | |
|---------------------------------|---------------------------|----------------------------------|
| Attributes of chilli genotypes | Source of variation (SOV) | Significance level (p- value) |
| Photosynthetic rate | Chilli genotypes (G) | ** |
| - | Glycine betaine (GB) | ** |
| | $G \times GB$ | ** |

Chilli genotypes (G)

Glycine betaine (GB) $G \times GB$

Chilli genotypes (G)

Glycine betaine (GB) G ×GB

Chilli genotypes (G)

Glycine betaine (GB) $\mathbf{G} \times \mathbf{GB}$

Chilli genotypes (G)

Table 1. Analysis of variance (ANOVA) table for effect of glycine betaine treatments on various attributes of chilli

| Glycine betaine (GB) | ** |
|----------------------|----|
| $G \times GB$ | * |

** For highly significant when P<0.01; * for significant when P<0.05; NS for non-significant when P>0.05.

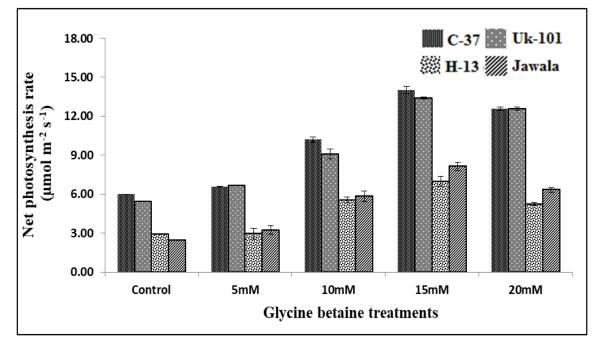


Fig. 1. Effect of glycine betaine treatments on photosynthesis rate of chilli genotypes.

The interaction between chilli genotypes and glycine betaine application ($GB \times G$) was significant as shown in Table 2.

Effect of glycine betaine treatments on transpiration rate of chilli genotypes

Foliar spray of glycine betaine had significant effect on transpiration rate of chilli genotypes as compared to control. Data for transpiration rate is shown in Fig. 3. C-37 and UK-101 gave the minimum transpiration rate (0.98 and 0.78 mmol m⁻²s⁻¹, respectively) with foliar application of glycine betaine @ 15mM. On the other hand, H-13 and Jawala showed maximum transpiration rate (2.36 and 2.58 mmol m⁻² s⁻¹, respectively) at control. Although, data regarding the transpiration rate showed that glycine betaine application found to be useful for chilli genotypes growing under high temperature conditions.

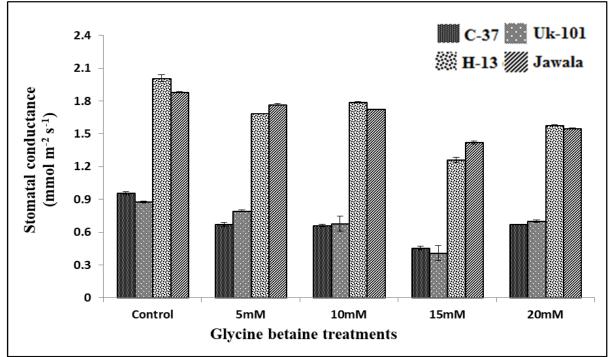


Fig. 2. Effect of glycine betaine treatments on stomatal conductance of chilli genotypes.

The interaction between genotypes and glycine betaine application (GB \times G) was non-significant as depicted in Table 3.

Effect of glycine betaine treatments on water use efficiency of chilli genotypes

Exogenously applied glycine betaine was significantly (P<0.05) enhanced water use efficiency of all the

chilli genotypes over control (Fig. 4). UK-101 gave the maximum water use efficiency (18.08 μ mol CO₂ mmol⁻¹H₂O) when glycine betaine was applied @ 15mM, followed by C-37 which gave 14.76 μ mol CO₂ mmol⁻¹ H₂O WUE at the same level of concentration. The minimum water use efficiency (0.61 μ mol CO₂mmol⁻¹ H₂O) was observed in Jawala, followed by in H-13 (0.86 μ mol CO₂ mmol⁻¹ H₂O) at control.

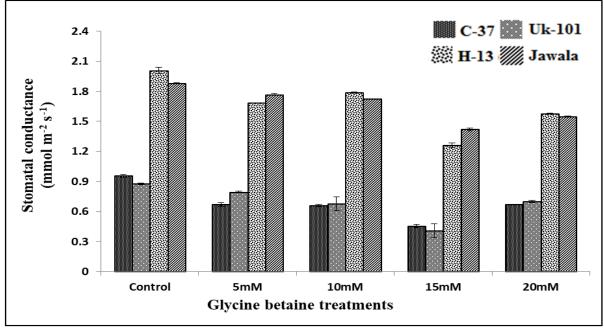


Fig. 3. Effect of glycine betaine treatments on transpiration rate of chilli genotypes.

The interaction between genotypes and glycine betaine application (GB x G) was significant as depicted in Table 4.

Effect of glycine betaine treatments on chlorophyll contents of chilli genotypes

Exogenously applied glycine betaine significantly $(p \le 0.05)$ enhanced seedling chlorophyll contents of genotypes as compared to control. Data regarding chlorophyll contents are represented in Fig. 5. Chilli

genotypes C-37 and UK-101 gave the maximum chlorophyll contents (17.26 and 14.72, respectively) whereas, H-13 and Jawala gave 10.32 and 9.32, respectively, chlorophyll contents @ 15mM level of glycine betaine as compared with control.

On the other hand, chilli genotype H-13 had minimum chlorophyll contents (6.60) at 5mM level of glycine betaine and Jawala had minimum chlorophyll contents (5.15) at control.

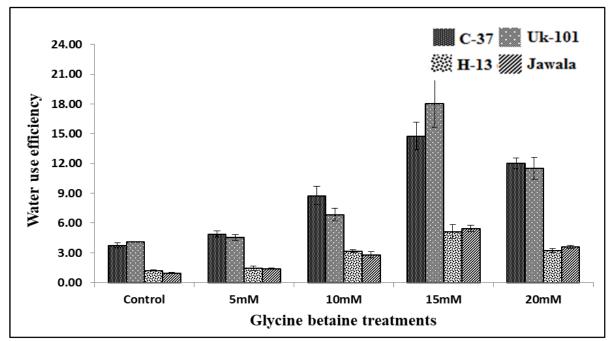


Fig. 4. Effect of glycine betaine treatments on water use efficiency of chilli genotypes.

The interaction between chilli genotypes and glycine betaine application (GB \times G) was significant as depicted in Table 5.

Discussion

Glycine betaine is a key organic osmolyte which is reported to be produced in many agronomic as well as horticultural crop species (i.e. sugar beet, wheat, spinach, barley and sorghum) under changing environment such as extreme temperatures, drought, salinity, heavy metals and UV radiations. It has significant effects on plant physiology under stress conditions (Ashraf and Foolad, 2007; Chen and Murata, 2008). Rhodes and Hanson, (1993) reported that heat tolerant genotypes of several plant species, accumulate greater amounts of glycine betaine than sensitive ones in response to heat stress. The plants which were unable to accumulate glycine betaine naturally, stress tolerance were induced through exogenous applications of osmolytes (Park *et al.*, 2006; Farooq *et al.*, 2008).

All levels of glycine betaine significantly decreased transpiration rate of all the four chilli genotypes in contrast to control where no glycine betaine was applied. Data regarding transpiration rate showed that C-37and UK-10 had lower transpiration rate at glycine betaine level of 15 mM. The higher transpiration rate was found in H-13 and Jawala at glycine betaine @ 5mM. The results of current study were confirmed by studies of Korkmaz *et al.* (2012) who reported the significantly reduced transpiration

rate in pepper seedlings with the application of glycine betaine under stressed environment. Glycine betaine significantly enhanced stomatal conductance of chilli genotypes in contrast to control where no glycine betaine was applied. The genotypes H-13 and Jawala depicted the highest stomatal conductance at control. The lowest stomatal conductance was observed in UK-10 where glycine betaine was foliarly applied @ 15mM, followed by C-37. Makela *et al.* (1998) worked on tomato and turnip under drought and salt stress and reported that glycine betaine significantly decreased the stomatal conductance whereas, higher stomatal conductance was observed in control.

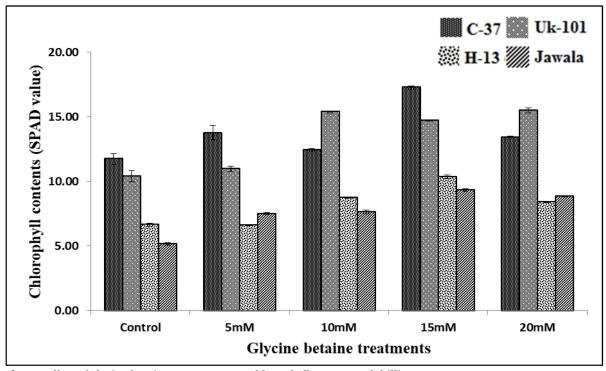


Fig. 5. Effect of glycine betaine treatments on chlorophyll contents of chilli genotypes.

The data regarding photosynthetic rate indicated that C-37 and UK-101 had higher photosynthetic rate @ 15mM of glycine betaine. Minnimum photosynthesis rate was observed in H-13 and Jawala, respectively where no glycine betaine was applied. Moreover, it was found that glycine betaine @ 0.5 mM had very little effect on photosynthesis rate of all genotypes. According to earlier studies, foliar spray of glycine betaine enhanced the numerous plant physiological attributes such as closure of stomata, photosynthetic rate, membraneous stability, ion uptake and finally increases the plant growth (Huve et al., 2005; Taiz and Zeiger, 2006; Surapu et al., 2014). Kormkaz et al, 2012 found that glycine betaine increased the photosynthetic rate and carbon dioxide assimilation rate by increasing dry matter contents. Chen and Norio, (2008) reported the significant role of glycine

betaine in protection of photosynthetic machinery under various types of abiotic stress. Glycine betaine application significantly improved the water use efficiency of chilli genotypes over control. The genotypes C-37 and UK-101 showed the high water use efficiency when glycine betaine was applied @ 15mM. The minimum water use efficiency was observed in H-13 at control treatment. Exogenously applied glycine betaine significantly enhanced the seedling chlorophyll contents of chilli genotypes as compared to control. The results for chlorophyll contents depicted that genotypes C-37 and UK-101 produced maximum chlorophyll contents @ 15mM level of glycine betaine as compared to control. On the other hand, genotype H-13 had minimum chlorophyll contents at 5mM level of glycine betaine and Jawala had minimum chlorophyll contents at control.

Although, data regarding the chlorophyll contents and glycine betaine application was found more useful under stressed conditions but more efficient data were recorded under high temperature stress.

The results are in line with Korkmaz *et al.* (2009) who observed increased chlorophyll contents in stressed pepper seedlings in response to foliar application of glycine betaine.

Conclusion

Glycine betaine at 15mM concentration was found appropriate to alleviate the drastic effects of heat stress. However, glycine betaine was also found much effective in alleviation of heat stress effects in C-37 and UK-101 which considered as heat tolerant chilli as well as in H-13 and Jawala chilli genotypes which considered as heat sensitive.

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