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ROLE OF PROLINE IN MITIGATING THE DELETERIOUS EFFECTS OF HEAT STRESS IN CHILLIES

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SUMMARY

Chilli is a spicy crop which belongs to family Solanaceae. As a vegetable crop, it is considered as one of the major cultivated crops in the world seriously affected by the climatic changes, including elevation in temperature. Therefore, in this research various morpho-physiological characteristics of chilli were studied for heat tolerance under the influence of exogenous application of proline. This research was carried out in a growth room of the Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan. Four chilli genotypes were evaluated under the applied conditions. Seeds were grown in plastic pots. Heat stress (40/32 °C day and night temperature) was applied 30 days after the emergence. Foliar spray of proline (0, 5 and 10 mM) was applied during heat stress, and after 7 days of stress the plants were harvested. The results revealed that the inhibition of chilli growth by heat stress was successfully mediated by proline application. Morphological attributes such as the number of leaves per plant, root and shoot length, plant fresh and dry weight were reduced in response to heat stress, and physiological attributes such as photosynthetic rate, water use efficiency and chlorophyll contents were also reduced in response to heat stress, except transpiration rate and stomatal conductance, which increased under heat stress and showed recovery by proline application. Exogenous application of proline promoted tolerance against heat stress in chilli genotypes and enhanced growth. All the observed traits exhibited recovery in response to proline stimulus, indicating the role of proline in mitigating the consequences of heat stress. Recovery also depends on genetic capability of various cultivars.

Key words: chilli, morpho-physiological characteristics, heat stress, proline

INTRODUCTION

Pungent peppers, commonly known as chillies, are a vegetable species widely cultivated in the world, including Pakistan. Chilli holds a high rank among vegetables due to its higher cost and demand value. Two of its varieties – *Capsicum annum* and *Capsicum frutescens* – are prominent in Pakistan. Chilli (*Capsicum frutescences* L.) is a spicy crop of family Solanaceae. As a vegetable, chilli is considered as one of the major crops which is cultivated all over the world. Chilli accounts for 1.5% of Pakistan's GDP. In Pakistan, it was cultivated on 65.1 thousand hectares with production of 148.1 thousand tones (GoP, 2017). Environmental stresses (abiotic and biotic factors) are the main cause of crop yield reduction all around the globe, tumbling typical yields of nearly all vegetables by more than fifty percent. The climatic changes have increased the harshness of environmental stress exerted on vegetable crops. Furthermore, the rise in temperature, salt stress and water deficiency are the main restricting factors in sustaining and increasing vegetable productivity (Abewoy, 2018). The rise in temperature, chemical noxiousness and oxidative stress seriously impede the agriculture all over the world (Mantri et al., 2012). It is the time and there is dire need to put scientific effort to overcome and identify the harshness of abiotic extremes for the vegetable crops which are

affected by abiotic stress. Production of chilli is reduced by increased temperature caused by the climate changes. Chilli is listed as a heat sensitive crop. The economic yield of chilli is reduced by heat stress in all phases of its development i.e. seed germination, vegetative stage and reproduction stage (Erickson & Markhart, 2002). Chilli requires the optimum temperature of 20-30 °C for better growth, while its growth is mainly delayed at below 15°C or over 32°C (Berke et al., 2005; González-Zamora et al., 2013). According to scientific predictions, it is estimated that by 2100 the increasing temperature (1.5 – 5.8 °C) caused by the climate changes will create unfavorable conditions for agricultural crops (IPCC, 2012). Heat stress usually affects the plant-water relationship. Heat stress may lead to disturbance of water uptake and transpiration imbalance, functioning of stomata, distribution of sap and obstruction in biosynthesis of chlorophyll, and eventually cause photosynthesis decline and damage the thylakoid membrane, and thus increase membrane leakage (Prasad et al., 2008; Ristic et al., 2007). Leaf water potential is also affected by heat stress, resulting in decreased water potential. The problems in the plant-water relations caused by various kinds of stresses trigger proline accumulation. During stress when proline was applied exogenously in *Vicia faba*, leaf water potential substantially increased (Gadallah, 1999). Many chemicals have important roles against heat stress (Ashraf & Foolad, 2007). Proline plays a prominent role in plant protection against salt, high temperature and water deficiency (Ashraf & Harris, 2004). It is accumulated in various plants especially in leaves of plants under drought and salt stress (Rhodes & Hanson, 1993; Verbruggen & Hermans, 2008) and helpful to tolerate that stresses (Mattioli et al., 2009). The biosynthesis and accumulation of proline widely studied in plants. Foliar spray of proline reduced the growth inhibition caused by heat stress in various crops, such as tomato (Makela et al., 1998). Furthermore, foliar use of proline enhanced the growth and plant tolerance in many crops under different stress regimes (Ashraf & Foolad, 2007). Gathering of proline looks likely to be linked with temperature stress. Both at high temperature (Ashraf et al., 1994) and low temperature (Tarnizi & Marziah, 1995; Wang & Cui, 1996) the concentration of free proline increased and in unfavorable growth condition proline might assist as a stress gauge in plants. In bean seeds, proline was used as an indicator of heat stress (Neto et al., 2004).

Keeping in view the status of chilli and its high potential for export, the current study was planned with the following objectives: to study the growth and physiological characteristics of chilli under heat stress and to study the heat mitigation effects of proline in chilli genotypes.

MATERIAL AND METHODS

The planned experiment was conducted at the growth room, Institute of Horticultural Sciences, University of Agriculture, Faisalabad, in 2019. The experiment was conducted according to completely randomized design (CRD) with two-factor factorial arrangements and four replications. This study was conducted on four genotypes of chilli (Zard, UK-101, CW-03, Kaka-01). Seeds of the chilli genotypes were sown in plastic pots which were filled with sand media. Water was provided according to demands of the plants by observing the moisture level of the sand. After germination of the seeds, half strength Hoagland solution was applied for the nourishment purpose. Heat stress was provided four weeks after emergence of the seedlings. The temperature was raised gradually to avoid any sudden damage of the seedlings; it was raised 2°C daily until the required temperature was acquired (40/32 °C day and night temperature). Plants were kept under heat stress for seven days. Foliar spray of proline was applied once at the concentration of 0 mM for the control (T0), 5 mM (T1) and 10 mM (T2) during heat stress according to the treatment. One week after heat stress, the plants were harvested. Morphological attributes (number of leaves per plant, root and shoot length, plant fresh and dry weight) and physiological attributes (photosynthetic rate, transpiration rate, stomatal conductance, water use efficiency, chlorophyll contents) were measured by a standard procedure, described by Butt et al. (2016).

Analysis of variance (Steel et al., 1997) was used to analyze the recorded data of chilli genotypes under heat stress and different proline concentration. Treatment means were compared using Tuckey's HSD test at 5% probability level.

RESULTS

Plant shoot length (cm)

Shoot length was severely affected by heat stress. The results showed that all genotypes significantly differed from each other, indicating the existence of variations among genotypes. The results suggested that severe effects of heat stress were recovered by exogenous application of proline. Chilli genotypes under heat stress showed positive response to proline application and shoot length increased due to exogenous application of proline. Treatment with 10 mM proline showed the maximum overall shoot length (Fig. 1a).

Figure 1. **a, b, c, d, e** - the effect of proline on the morphological traits (shoot length, root length, number of leaves per plant⁻¹, fresh weight and dry weight, respectively) of chilli genotypes under heat stress conditions; **f, g, h, i, j** - the effect of proline on the physiological traits (photosynthetic rate, transpiration rate, stomatal conductance, water use efficiency and chlorophyll contents)

Plant root length (cm)

The results presented in Table 1. indicated significant variations among the genotypes and treatments. Root length was increased in response to proline under heat stress. The maximum root length was observed under 10 mM proline treatment for all genotypes (Fig. 1b).

Table 1. Mean squares from analysis of variance for the traits of four chilli genotypes under control and applied proline conditions

Source of Variation	DF	SL	RL	LP ⁻¹	FW	DW
Treatment	2	66.09**	742.99**	259.02*	364.11**	1218.34**
Variety	3	12.95**	211.13**	411.48**	933.00**	415.77*
Treatment × Variety	6	3.56**	3.03*	8.54**	17.72**	5.29*
Source of Variation	DF	PR	TR	SC	WUE	CC
Treatment	2	1436.79**	800.92**	1705.84**	2764.33**	1275.77*
Variety	3	730.07*	274.46**	9553.75*	1981.08**	1010.77**
Treatment × Variety	6	38.20**	4.34**	100.03*	217.17**	43.13**

Legend: *Significant at 5% probability level; ** Significant at 1% probability level; DF= Degree of freedom, SL= Shoot length, RL= Root length, LP⁻¹= Number of leaves per plant, FW= Plant fresh weight, DW= Plant dry weight, PR= Photosynthetic rate, TR= Transpiration rate, SC= Stomatal conductance, WUE= Water use efficiency, CC=Chlorophyll contents

The number of leaves per plant

The number of leaves was affected by heat stress. The results showed variations among the genotypes. The results presented in the table revealed that severe effects of heat stress were recovered by exogenous application of proline. Chilli genotypes under heat stress showed positive response to proline application and the number of leaves increased by exogenous application of proline. The genotype which showed the least reduction under heat stress showed higher recovery by proline application (Fig. 1c). Overall, UK-101 performed better.

Plant fresh weight (g)

Plant fresh weight was decreased by heat stress. The table showed that all genotypes were significantly different from each other under the applied conditions, suggesting the existence of variations among the genotypes. Fresh weight was increased in response to exogenous application of proline. Treatment with 10 mM proline showed the highest overall plant fresh weight. The results determined that fresh weight which was reduced by heat stress was significantly increased by proline application, so proline helped in mitigating the deleterious effects of heat stress. The genotype which showed the least reduction against heat stress showed higher recovery after proline application (Fig. 1d).

Plant dry weight (g)

Plant dry weight was reduced by heat stress. The results indicated that all genotypes were significantly different from each other, indicating variations among the genotypes. Chilli genotypes under heat stress showed positive response to proline application and plant dry weight increased by exogenous proline application. Application of 10 mM proline showed the highest overall plant dry weight (Fig. 1e).

Photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)

Photosynthetic rate in chilli genotypes was decreased by exposure to heat stress and there were variations among genotypes. Under heat stress, photosynthetic rate value significantly varied from genotype to genotype. The lowest value of photosynthetic rate under heat stress conditions was noted in Kaka-01, while the maximum value was observed in Zard. After proline application, Zard showed the maximum value for photosynthetic rate (Fig. 1f).

Transpiration rate ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)

Transpiration rate was increased in chilli genotypes by exposure to heat stress and there were variations among the genotypes. Under heat stress conditions, the highest value of transpiration rate was observed for Kaka-01, while the lowest value was observed for UK-101. After proline application, UK-101 showed the maximum value for transpiration rate (Fig. 1g).

Stomatal conductance to water ($\text{mmol m}^{-2} \text{ s}^{-1}$)

In the current study, stomatal conductance was increased by heat stress in all observed chilli genotypes. Stomatal conductance declined by application of proline. The recovery in stomatal conductance was the highest in treatment with 10 mM proline, followed by 5 mM proline treatment. All genotypes showed different reactions to proline application. The levels of proline made significant impact on stomatal conductance, especially the higher proline value (Fig. 1h).

Water use efficiency

Water use efficiency in all genotypes was reduced under heat stress, while the maximum reduction was observed in Kaka-01, and the minimum reduction was in UK-101. The impact of heat stress on genotypes was significant and

water use efficiency decreased in all genotypes under heat stress. However, genotypes showed recovery after application of proline. The recovery was maximum in 10 mM proline treatment, while it was the least in 5 mM proline treatment. UK-101 genotype showed the maximum recovery under proline application, followed by Zard (Fig. 1i).

Chlorophyll contents (SPAD Value)

Chlorophyll contents were greatly affected by heat stress. The results indicated that all genotypes were significantly different from each other under the applied conditions, revealing variations among genotypes. The results presented in the table suggested that the strong effects of heat stress were recovered by exogenous proline application. Chilli genotypes under heat stress showed positive response to proline application and chlorophyll contents increased by exogenous proline application. Zard showed the maximum recovery under proline application (Fig. 1j).

DISCUSSIONS

Heat stress is the leading abiotic factor that severely disturbs chilli germination and growth (Sood et al., 2009). It causes undesirable changes in plant morpho-physiological as well as biochemical reactions (Sung et al., 2003). Introduction of osmolytes under heat stress is one of the effective methods to overwhelm heat stress and identify best recovered genotypes. However, there is inadequate evidence for many horticultural crops, including chilli crop. Such valuations may help in increasing heat tolerance of chilli genotypes in breeding programs, and it can be appropriate for more tolerant genotypes under high temperature areas. Proline is a free amino acid produced from glutamate, and for achieving osmotic adjustment it can perform as a compatible solute (Lehmann et al., 2010; Szabados & Savoure, 2009). Proline plays a vital part in integrity of the cell membrane, in destruction of reactive oxygen species and sustaining homeostasis in the cell (Hayat et al., 2012; Szabados & Savoure, 2009).

Compatible solutes such as proline play a part in osmotic modification of several crops and against the situations of biotic stresses (Rhodes & Hanson, 1993; Heuer, 1994). The main role of proline is to insulate the plant cell from harmful effects of heat stress by conserving osmotic balance and the composition of the main proteins such as rubisco, by defending the mechanisms of photosynthesis and by acting as free oxygen radicals scavengers. Transgenic plants have lower amount of osmolytes accumulated against the stress, but moderate tolerance was confirmed (Bajaj et al., 1999). Therefore, exogenous application of osmolytes is suggested as a substitute or supplementary approach to mitigate the effect of stress on crops and improve tolerance in plants against stress (Itai & Paleg, 1982).

Heat stress significantly reduces root and shoots length of chilli, as indicated by the results. Muslu & Ergun (2013) obtained similar results, reporting reduction in root length in stress conditions. Proline plays a crucial role in the recovery process, as confirmed by Mukhtar et al. (2016), Murmu et al. (2017) and Usman et al. (2015). Nawaz et al. (2010) also reported that proline plays an important role in enhancing the resistance of chilli against abiotic stresses. Plant fresh and dry weight are harshly affected by heat stress (Zhou et al., 2017). The results showed that all genotypes were significantly different from each other under the applied conditions, indicating variations among genotypes. It was revealed that severe effects of heat stress were recovered by exogenous proline application. Similar findings were reported by Heuer (2003) and Muslu & Ergun (2013).

Heat stress caused increased water loss through transpiration (transpiration rate increased due to high temperature), so to cope with increased water loss plants manufacture various metabolites. Gathering of these metabolites stabilizes the turgor pressure of the cell and as a consequence lowers the water potential level of the cell. Heat stress increases the respiration level which eventually requires higher carbon fixation for preservation and enhancing of the development (Crafts-Brandner & Salvicci, 2000). The salient source of metabolic activity is carbohydrate, which is reduced under heat stress situations and that is a limiting factor in the plant routine (Sweeney et al., 2001). Therefore, this limitation of carbohydrate availability in plants under heat stress leads to decrease in photosynthetic activity and increase in dark respiration activity (Liu & Huang, 2000). The results in this study indicated decreased photosynthetic rate under heat stress. This limitation is caused by reduction in Rubisco activity and increase in photoinhibition. Chaum & Kirdmanee, (2010) presented similar results.

Heat tolerant varieties displayed improved ratio of chlorophyll contents compared to heat sensitive varieties, showing certain positive association of photosynthetic constraints with heat tolerance (Wahid & Ghazanfar, 2006). Usman et al. (2015) reported that heat stress negatively affected chlorophyll contents in chilli. Proline played a role in mitigating the deleterious effects (Nawaz et al., 2010). Ghai et al. (2016) in their studies indicated that heat stress greatly reduced the chlorophyll contents values in plants. Transpiration rate increased in response to heat stress (Cui et al., 2006) and showed recovery upon exogenous proline application. Sharma et al. (2015) reported similar

findings. Exogenous application of proline improves development of different plant cells and during stress conditions it decreases lipid membrane oxidation and maintains the metabolism (Jain et al., 2001).

Stomatal conductance directly modifies plant water relations and photosynthesis. Stomatal conductance increased with cumulative temperature despite the reduction in leaf water potential, rise in transpiration rate, intensification in intercellular CO₂ concentration and was decoupled from photosynthesis (Urban et al., 2017). Chlorophyll contents decreased under rising temperature, but the exogenous proline application improved the chlorophyll contents (Nawaz et al., 2010). Water use efficiency (WUE) was improved by proline application in heat stress situations. Water status of heat-affected plants was modified by proline application. These consequences could have been due to water efflux inhibition via the effects of solutes on membrane stability and condensed transpiration via effects on stomatal cells (Ali et al., 2007).

Foliar spray of proline exerted positive effects and mitigated venomous effects of stress such as heat stress, salt stress and water deficiency. Butt et al. (2016) performed an experiment to investigate the effects of proline against salt stress when proline is exogenously applied on two chilli genotypes to assess their response to the stress. The results indicated that stress adversely affected the morphological and physiological attributes, such as root and shoot length, dry and fresh weight of plants and transpiration rate, photosynthesis, respectively. Proline was applied as foliar spray on 30 days old seedlings. Various levels of proline were applied. The results indicated that improvements occurred in the morphological, biochemical and physiological attributes. Furthermore, it was evaluated that among all the proline concentrations, 0.8 mM concentration proved to be more effective in terms of the morphological, ionic and physiological traits. It can thus be assumed that exogenous application of proline on chilli under heat stress can also be effective.

The improving properties of proline were reported by different scientist for various crops, including maize (Ali et al., 2007), olive (Ahmed et al., 2011), chilli (Butt et al., 2016) and rice (Nounjan et al., 2012). Exogenous application of proline minimizes the lethal outcomes caused by high temperature. The genotypes which performed better compared to others for all observed morpho-physiological parameters are Zard and UK-101.

CONCLUSION

Analysis of all observed parameters revealed that Zard and UK-101 overall performed better compared to two other chilli genotypes under heat stress and showed better recovery in response to proline application. It can be concluded that recovery occurs in response to proline and also depends on genetic capability of various genotypes. Heat stress adversely affected all morphological and physiological attributes and it is clear from the results that proline played an important role in mitigating the deleterious effects of heat stress on chilli genotypes.

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