The Bioscan 10(4): 1585-1589, 2015
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

EFFECTS OF SALICYLIC ACID TREATMENTS ON HEAT TOLERANCE, CATALASE AND POLYPHENOL OXIDASE ENZYME ACTIVITY IN CHICKPEA CV. ICCV 10

S. GAYATRIDEVI[2], S. K. JAYALAKSHMI[1] AND K. SREERAMULU[1]*
[1]Department of Plant Pathology,
College of Agriculture, University of Agricultural Sciences, Raichur, Gulbarga - 585 103, INDIA
[2]Department of Biochemistry, Gulbarga University, Gulbarga - 585 106, INDIA
e-mail: ksramu@rediffmail.com

KEYWORDS
Catalase
Chickpea
H_2O_2
Polyphenol oxidase
Salicylic acid

Received on :
07.08.2015

Accepted on :
21.11.2015

***Corresponding author**

ABSTRACT
The effects of different treatments of salicylic acid on growth, H_2O_2 content, catalase, polyphenol oxidases and protein profile in seedling of chickpea cv ICCV10 were studied before and after the heat stress treatments at different time intervals. Salicylic acid at concentration of 0.8 mM was found to be an optimum for maximum thermo-tolerance. Chickpea plants subjected for heat shock at 45°C caused a significant increase in endogenous H_2O_2 and reduced catalase activity. However PPO activity was increased as compared with the controls. Further, expression of some new proteins including heat shock proteins were observed in both of the treatments. In conclusion, SA protects chickpea seedling from heat shocks at 50ºC.

INTRODUCTION

Salicylic acid (SA) is a natural signal molecule, which plays an important role in regulating a number of physiological processes in plants (Singh and Usha, 2003 Patel and Hemantarajan, 2013; Jaiswal *et al.,* 2014). It has been known for many years that exogenous SA is involved in the defense against pathogen attack, and more recently its role has been widely investigated in both biotic and abiotic stresses. Previous studies have also shown that SA could ameliorate the damaging effects of heavy metals on membranes in rice (Mishra and Choudhuri, 1999), reduce low temperature stress in maize and banana (Janda*et al.,* 1999; Kang *et al.,* 2003) and improve thermo tolerance of tall fescue seedlings (He et al., 2002). It has been reported that heat stress increases salicylic acid concentration in leaves of cucumber seedling (Ma *et al.,* 1998).

Out of the various abiotic stresses, high temperature is the second most important stress, which can strike crop at any times and impose many limitations on growth and development. Plants have evolved various ways of coping with their changing surroundings. Adaptive responses are directly regulated by genetic and biochemical characteristics, which may be manipulated. An understanding of the biochemical changes involved in plant-stress responses will enable the development of genetically engineered plant material with enhanced resistance to biotic and abiotic stress.

Because high temperature is one of the major abiotic stresses limiting plant yield and distribution in many regions of the world (Ong and Baker, 1985; Criddle*et al.,* 1994), it has been the focus of much research, particularly since the discovery and characterization of heat hock proteins (HSPs).

Induction of protein synthesis or altered protein formation may be one of the several mechanisms of adaptation to high temperature (Teeri, 1980). Plant responds to heat stress by changing their metabolic pathways. Under heat stress, synthesis of most proteins is repressed and some proteins which are called heat shock proteins, starts to be synthesized (Vierling, 1991).

Generation of active oxygen species, particularly H_2O_2, during abiotic stresses has also been proposed as part of the signaling cascade leading to protection from these stresses (Doke*et al.,* 1994; Prasad *et al.,* 1994; Foyer *et al.,* 1997). SA also accumulates during exposure to ozone or UV light (Yalpani*et al.,* 1994; Sharma *et al.,* 1996), whereas pretreatment of leaves with SA can protect them from paraquat-induced oxidative stress (Strobel and Kuc, 1995). Therefore, it is interesting to explore whether SA and H_2O_2 may be involved in the induction of protective mechanisms involved in tolerance to abiotic and biotic stresses.

According to our knowledge, there are no reports on the effects of exogenous SA enhancing chickpea tolerance to heat stress. This work focuses on the influence of different SA treatments

on heat tolerance of chickpea. The objectives of this study are to investigate if SA is involved in the regulation of catalase and polyphenol oxidase during heat stress and to determine what concentration of SA has maximum effect on heat tolerance.

MATERIALS AND METHODS

Treatment

All the chemicals and reagents used in this study were of analytical grade.Chickpea seeds cv ICCV 10 were obtained from Agriculture Research Station, Gulbarga. Seeds were surface sterilized with 0.1% mercuric chloride for 2 min and then washed thoroughly with double distilled water and grown for 10 days in a growth room on 24/18ºC day/night cycle.

Heat treatment

Plants were sprayed with a range of (0.2, 0.4, 0.6, 0.8 and 1.0 mM) of salicylic acid (Sigma-Aldrich) concentrations. All spray solutions were including the water control, were adjusted to pH 7.0. For acclimation temperature, plants were exposed to a non lethal temperature (45ºC) for 1, 2, 3 and 4 h to induce heat shock, plants were exposed to 55ºC for 1 h.

Assessment of heat tolerance

Survival was assessed by the capacity of the seedlings to grow after the heat-shock treatment. Thermo tolerance was assessed from the percentage survival in each sample of 60 plants, 6 days after heat shock. Surviving plants often showed signs of damage such as leaf bleaching but their apices and stems remained green.

H_2O_2 estimation

As described earlier, estimation of H_2O_2 was carried out by the method of Noreen and Ashraf (2009). Fresh sample of shoot and root were homogenized in 2 mL of 0.1 % (w/v) TCA in a pre-chilled pestle and mortar. The homogenate was centrifuged at 12,000× g for 15 min and the supernatant was collected. Absorbance of the reaction mixture consisting of 0.5 mL supernatant, 0.5 mL sodium phosphate buffer (pH 7.0) and 1 mL of 1 M KI was read at 390 nm. The H_2O_2 content was determined by using an extinction coefficient of 0.28 iM cm^{-1} and expressed as μM g^{-1} FW.

Catalase enzyme assay

The activity of catalase was determined by Rao *et al*. (1997) by following the consumption of H_2O_2 at 240 nm for 1 min in 1 mL reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0) and 10 mM of 10% substrate. One unit of activity was defined as the amount of enzyme catalyzing the decomposition of H_2O_2 in mmol min^{-1}.

PPO enzyme assay

Enzyme activity (PPO) was determined by measuring the increase in absorbance at 420 nm using catechol as a substrate ((Mayer *et al.,* 1965). One unit of PPO activity was expressed as the amount of enzyme that causes an increase in absorbance of 0.001 mL^{-1} min^{-1}.

Proteins and its profiling

The protein concentration of each sample was determined according to Lowry *et al*. (1951) method using bovine serum albumin as a standard. Protein profiling was carried out by SDS-PAGE according to the method of Laemmli (1970). Equal amounts of protein (30 μg) extract were subjected to SDS-PAGE using 12% polyacryamidegel under denaturing conditions.

RESULTS AND DISCUSSION

Heat treatment on survival percentage

A period of 1h after spraying was adopted for assessment of the concentration dependant of the SA treatments. SA solutions between 0.1 to 0.8 mM significantly increased thermotolerance to heat shock inchickpea plants as comparison with controls sprayed with water (Fig 1). In contrast, spraying with SA concentration of 0.8 mM improved the survival of heat shock at 50°C for 1 h (Fig. 2). Spraying of SA (0.8 mM) significantly improved their tolerance to subsequent heat shock. The effects of SA on thermotolerance were dependant on its concentration, at higher concentrations of SA (above 1 mM), no thermotolerance was observed. Similar type of heat acclimation by SA was reported in mustard seedlings (Dat*et al*., 1998 a and b).

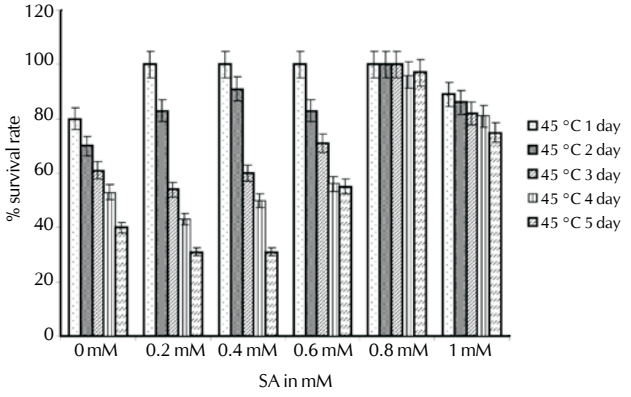

Figure 1: Survival percentage of chickpea plant after 1h heat treatment at 45ºC after spraying with increasing concentrations of SA. SE or SD error bars were indicated

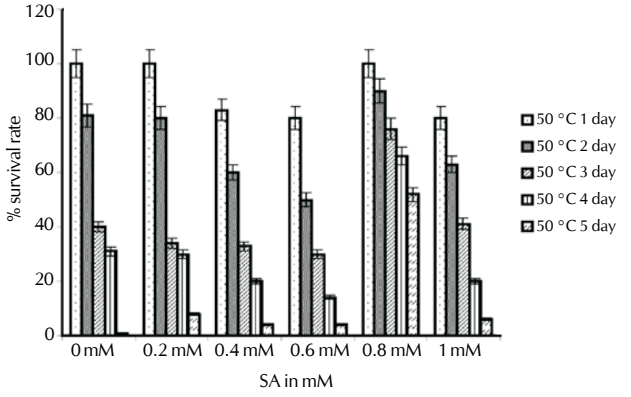

Figure 2: Survival percentage of chickpea plant after 1 h heat treatment at 50ºC after spraying with increasing concentrations of SA. Please provide legend X information. Also, SE or SD error bars were indicated

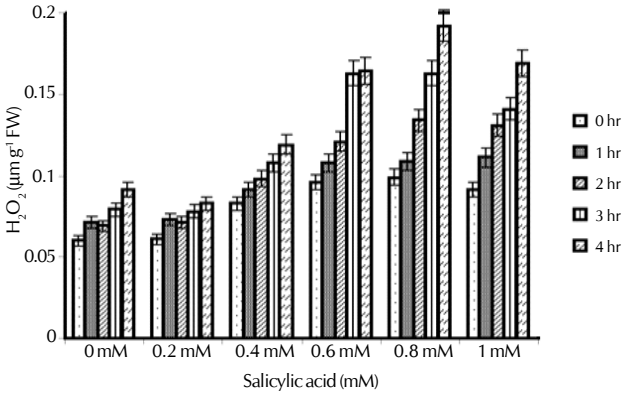

Figure 3: H_2O_2 content expressed in μM g^{-1} FW in chickpea plant subjected to heat shock temperature at 45°C for 1, 2, 3 and 4 h after pretreated by different concentrations of salicylic acid. SE or SD error bars were indicated

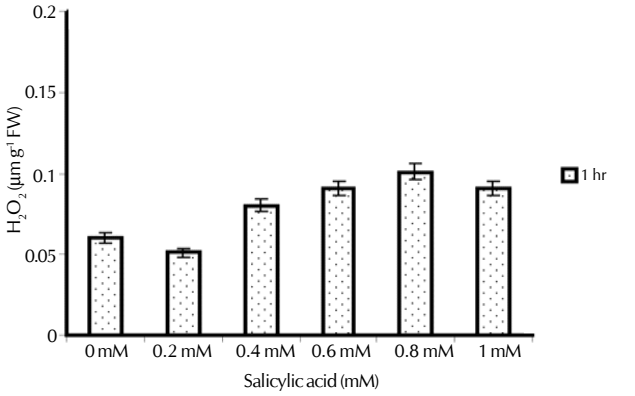

Figure 4: H_2O_2 content expressed in μM g^{-1} FW in chickpea plant subjected to heat shock temperature at 50°C for 1 h after pretreated by different concentrations of salicylic acid. SE or SD error bars were indicated

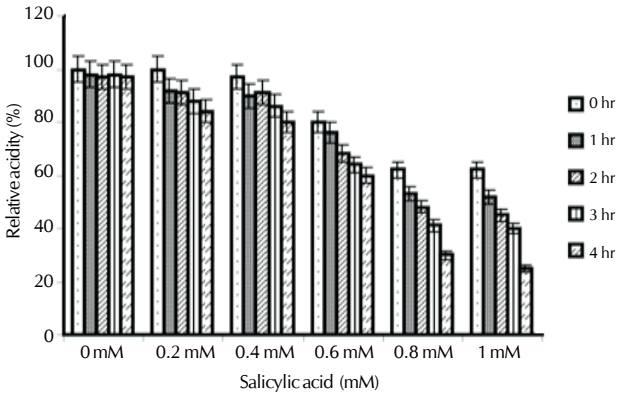

Figure 5: Activity of catalase in chickpea plant subjected to heat shock temperature at 45ºC for 1, 2, 3 and 4 h after pretreated by different concentrations of salicylic acid. SE or SD error bars were indicated

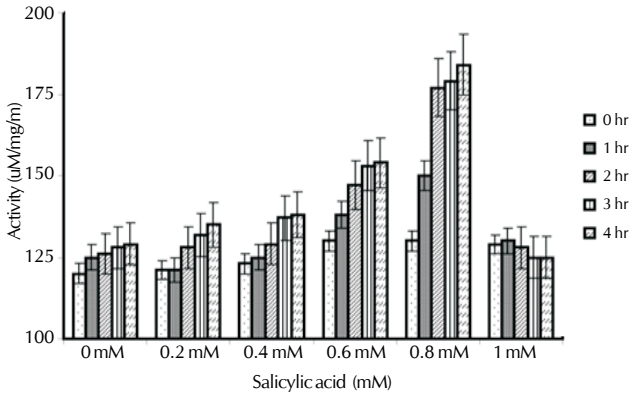

Figure 6: Activity of PPO in chickpea plant subjected to heat shock temperature at 45ºC for 1, 2, 3 and 4 h after pretreated by different concentrations of salicylic acid. SE or SD error bars were indicated

H_2O_2 level

The data showed that heat stress at different time intervals, induced a significant increase in H_2O_2 content when compared to negative control. Application of 0.8 mM SA in both heat stress treatments showed a significant increase in H_2O_2 content (Fig 3). Maximum H_2O_2 content was observed in plants treated with 0.8 mM SA at 45°C for 4 h incubation. Increasing accumulation of H_2O_2 was also observed in seedlings treated with 0.8 mM at 50°C for 1 h (Fig 4). Accumulation of H_2O_2 in mustard seedlings treated with SA at different temperature was reported (Dat*et al.*,1998a and b). The increase in H_2O_2 following heat shock could be explained by the model (Dokee*t al.*, 1994, Doke, 1997) in which abiotic stresses are accompanied by an oxidative burst similar to that involved in signaling during plant pathogen interactions.

Catalase (CAT) and PPO activities

Heat stress significantly decreased CAT activities (Fig 5) while it induced a significant increase in PPO activities (Fig 6) when compared to negative control. There were several reports of decreased activities of key antioxidant enzymes following heat shock, the antioxidant defense may thus be impaired by heat shock and leads to increased oxidant concentrations (Foyer *et al.*, 1997; Polle, 1997). A significant increase in the PPO activities indicates the formation of large amounts of polyphenol complexes as a result of heat shock in presence of SA. There is possible association between PPO activity and growth of seedlings due to high temperature. He et al. (2005) reported that SA-induced heat tolerance could be related to higher O_2^- and H_2O_2 scavenging potential due to higher CAT and PPO activities under heat stress.SA has been reported in protecting against heat stress induced damagein Kentucky bluegrass and creeping bent grass (Larkindale and Knight, 2002;Larkindale and Huang, 2004).

Protein profile by SDS-PAGE

Treatment with SA at 0.8 mM at 45ºC for 1 to 4 h and at 50ºC for 1h, resulted in the synthesis of many new proteins at the low molecular weight range as evidenced by lane 2 to 5 in Fig 7. Plants show the heat tolerance by the virtue of synthesis of heat shocked proteins (HSPs). The expression of HSPs has been investigated in a number of different plants and positive correlation was found between high temperatures. Chakraborty and Tongden (2005) observed a low molecular

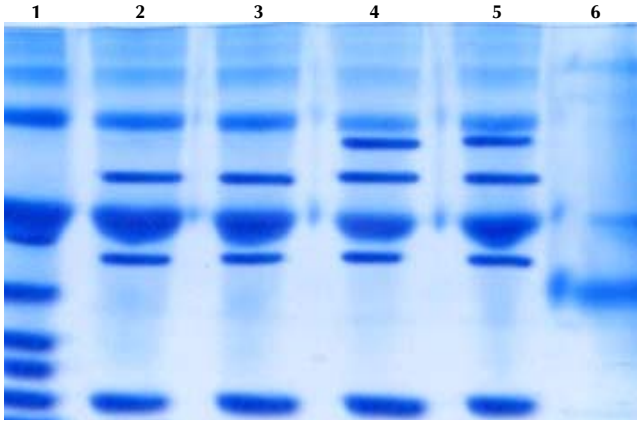

Figure 7: Electrophoresis protein banding pattern of 0.8 mM salicylic acid treated ICCV-10. Lane 1-Control, lane 2- 45°C (1 h), Lane 3- 45ºC (2 h), Lane 4- 45ºC(3 h), Lane 5-45ºC (4 h), Lane 6-50ºC (1 h)

weight 36 kDa in SA pretreated plants challenged with lethal temperature. In the present investigation, heat acclimatized seedlings showed denovo synthesis of some of the low molecular proteins and, which confirm that chickpea seedlings protect themselves against heat stress by expressing different HSPs.The results of the present investigation suggest that the pretreatment with the SA at 0.8 mM is successful in induction of thermotolerance and protects the chickpea seedling from heat shocks at 50ºC.

ACKNOWLEDGEMENT

This research was supported by research grants to KS from DST and UGC-SAP, the Government of India, New Delhi. GS thanks Gulbarga University, Gulbarga, UGC and CSIR for financial assistance in the form of student fellowship during this work.

REFERENCES

Chakraborty, U. and Tongden, C. 2005. Evaluation of heat acclimation and salicylic treatments as potent inducers of thermotolerance in *Cicer arietinum* L. *Curr Sci*. **89:** 384-386.

Criddle, R. S., Hopkin, M. S., McArthur, E. D. and Hansen, L. D. 1994. Plant distribution and the temperature-coefficient of metabolism. *Plant Cell Environ*. **17:** 233-243.

Dat, J. F. Lopez-Delgado, H., Foyer, C. H. and Scott, I.M. 1998a. Parallel changes in H_2O_2 and catalase during thermotolerance induced by salicylic acid or heat acclimation in mustard seedlings. *Plant Physiol.* **116:** 1351-1357.

Dat, J. F. Foyer, C. H. and Scott, I. M. 1998b. Changes in salicylic acid and antioxidants during inducedthermotolerance in mustard seedlings. *Plant Physiol.***118:** 1455-1461.

Doke, N., Miura, Y., Leandro, M. S. and Kawakita, K. 1994. Involvement of superoxide signal transduction: responses to attack by pathogens, physical and chemical shocks and UV radiation. *In* CH Foyer, PM Mullineaux, eds, Causes of Photooxidative Stress and Amelioration of Defense Systems in Plants. *CRC Press, Boca Raton, FL*, pp 177-197.

Doke, N. 1997. The oxidative burst: roles in signal transduction and plant stress. *In* J Scandalios, ed, Oxidative Stress and the Molecular Biology of Antioxidant Defenses. *Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY,* pp. 785-813.

Foyer, C. H., Lopez-Delgado, H., Dat, J. F. and Scott, I. M. 1997. Hydrogen peroxide and glutathione-associated mechanism of acclamatory stress tolerance and signaling. *Physiol Plant*. **100:** 241-254.

He, Y. L., Liu, Y. L., Chen, Q. and Bian, A. H. 2002. Thermo tolerance related to antioxidation induced by salicylic acid and heat hardening in tall fescue seedlings. *J. Plant Physiol Mol. Biol.* **28:** 89-95.

He, Y., Liu, Y., CAO, W., Huai, M., Xu, B. and Huang, B. 2005. Effect of salicylic acid on heat tolerance associated with antioxidant metabolism in Kentucky bluegrass. *Crop Sci*. **45:** 988-995.

Jaiswal, A., Pandurangam, V. and Sharma, S. K. 2014. Effects of salicylic acid in soybean (glycine Max L. Meril) under salinity stress. *The Bioscan.* **9(2):** 671-676.

Janda, T., Szalai, G., Tari, I. and Paldi, E. 1999. Hydroponic treatment with salicylic acid decreases the effects of chilling injury in maize *(Zea mays* L.) plants. *Planta.* **208:** 175-180.

Kang, G. Z., Wang, C. H., Sun, G. C. and Wang, Z. X. 2003. Salicylic acid changes activities of H_2O_2-metabolizing enzymes and increases the chilling tolerance of banana seedlings. *Environ Exp. Bot.* **50:** 9-15.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. **227:** 680-685.

Larkindale, J. and Kinght, M. R. 2002. Protection against heat stress induced oxidative damage in *Arabidopsis* involves calcium, abscicic acid, ethylene and salicylic acid. *Plant Physiol*. **128:** 682-695.

Larkindale, J. and Huang, B. 2004. Thermotalerance and antioxidant system in *Agrositisstolonifera:* Involvement of salicylic acid, abscisic acid, calcium, hydrogen peroxide andethylene. *J. Plant Physiol*. **161:** 405-413.

Lowry, O. H., Rosebrough, N. J. Farr, A. L. and Randall, R. J. 1951. Protein measurement with folin-phenol reagent. *J. Biol. Chem*. **193:** 265-275.

Ma, D. H., Pang, J. A., Li, S. J. and Huo, Z. R. 1998. Effects of temperature stress acclimation onsome physiological characters in leaves of cucumber seedlings. *Acta Hort. Sin.* **25:** 350-355.

Mayer, A. M., Harel, E. and Shaul, R. B. 1965. Assay of catechol oxidase a critical comparison of methods. *Phytochemistry*. **5:** 783-789.

Mishra, A. and Choudhuri, M. A. 1999. Effects of salicylic acid on heavy metal induced membrane deterioration mediated by lipoxygenase in rice. *Biol Plant*. **42:** 409-415.

Noreen, Z. and Ashraf, M. 2009. Change in antioxidant enzymes and some key metabolites in some genetically diverse cultivars of radish *(Raphanussativus*L.). *Environ Exp. Bot*. **67:** 395-402.

Ong, C. K. and Baker, N. R. 1985. Temperature and leaf growth.*In* NR Baker, WJ Davies, CK Ong, eds, Control of Leaf Growth. Seminar Series, Society for Experimental Biology, No. 27. Cambridge University Press, Cambridge, UK, pp. 175-200.

Patel, P. K. and Hemanatarajan, A. 2013. Differential sensitivity of chickpea genotypes to salicylic acid and drought stress during pre-anthesis: Effcts on total chlorophyll, phenolics, seed protein and protein profiling. *The Bioscan*. **8(2):** 569-574.

Polle, A. 1997. Defense against photooxidative damage in plants. In: Oxidative stress and the molecular biology of antioxidant defense, Scandalios J, (Ed.).Cold spring Harbour Laboratory Press, Cold Spring Harbour, NY, pp. 785-813.

Prasad, T. K., Anderson, M. D., Martine, B. A. and Stewart, C. R. 1994. Evidence of chilling induced oxidative stress in maize seedlings and a regulatory role for hydrogen peroxide. *Plant Cell.* **6:** 65-74.

Rao, M. V., Paliyath, G., Ormond, D. P., Dennis, P. O., Murr, D. P. and Watkins, C. B. 1997. Influence of salicylic acid on H_2O_2

production oxidative stress and H_2O_2 metabolizing enzymes. *Plant Physiol.* **115:** 137-149.

Sharma, Y. J., Leon, J., Raskin, I. and Davis, K. R. 1996. Ozone-induced responses in*Arabidopsis thaliana*: the role of salicylic acid in the accumulation of defense related transcriptsand induced resistance. *Proc Natl AcadSci USA*. **93:** 5099-5104.

Singh, B. and Usha, K. 2003. Salicylic acid induced physiological and biochemical changes in wheat seedlings under water stress. *Plant Growth Regul*. **39:** 137-141.

Strobel, N. E. and Kuc, A. 1995. Chemical and biological inducers of systemic acquiredresistance to pathogens protect cucumber and tobacco from damage caused by paraquat and cupric chloride. *Phytopathology*. **85:** 1306-1310.

Teeri, J. A. 1980. Adaptation of kinetic properties of enzymes to temperature variability. InTurner NC, Kramer PJ (Eds.), Adaptation of Plant to Water and High Temperature Stress. *Wiley-Interscience, NY,* pp. 251-260.

Vierling, E. 1991. The roles of heat shock proteins in plant. *Ann.Rev. Plant Physiol. Plant Mol. Biol*. **42:** 579-620.

Yalpani, N., Enyedi, A. J. Leon, J. and Raskin, I. 1994. Ultraviolet light and ozone stimulate accumulation of salicylic acid, pathogenesis-related proteins and virus resistance in tobacco. *Planta*. **193:** 372-376.