ABSTRACT

Plants are continuously interacting with a range of environmental factors. In fast changing climatic condition abiotic factors, such as extreme temperatures drought, salinity, cold, disturb the normal physiological and biochemical processes in plants and can have devastating impact on agricultural yield. Among the changing components of the environment, rising temperature is one of the major constrains which greatly limit plant growth and productivity worldwide. To counter heat stress plants are continuously modifying physiological and biochemical mechanisms which on one side protect plants from heat injury and repair of heat sensitive components, on the other side modify plant pathogen interaction. Heat tolerance of various plants may differ depending on these biochemical and molecular alterations.

Like other countries, agricultural sector in India is also facing negative impact of climate change. Wheat (*Triticum aestivum* L.) is the second main food crop after rice and grows optimally in winters and is very sensitive to high temperature. In India and other tropics wheat plants suffer from exposure to high temperature with heat increases above 35^0C causing stress like conditions which lead to yield reduction. Heat sensitivity of various wheat cultivars is not similar and thus high temperature would be lethal for plants lacking better acclimation capacity. The present study was undertaken to investigate the effect of high temperature on wheat, how inherent antioxidative mechanisms and signalling protect the wheat seedlings from heat induced oxidative stress and the prospect of reducing the harmful consequences of high temperature by use of plant growth promoting rhizobacteria (PGPR) treatment was explored. An attempt was made to know the impact of high temperature and biotic stress (spot blotch) interactions on biochemical and physiological traits and to alleviate temperature stress by application of plant growth promoting bacteria.

Preliminary screening of eight wheat cultivars *viz.* C306, PBW343, PBW550, HT41, HT17, HT15, GY, and MW cultivars was carried out to assess their basal thermo tolerance level. Heat susceptibility indexes (HSI) of all the cultivars were calculated after exposure plants to 25^0C, 30^0C, 35^0C and 40^0C for 6 and 12 hours. Leaf disc bioassay was also carried out to evaluate thermo susceptibility of eight cultivars on the basis of changing colour of leaf discs from green to yellow when immersed in water at

40^0C for 12 hours. Comparative study of basal thermo tolerance in various cultivars has been done by observing changes in eight biomarkers for thermotolerance: relative water content, cell viability, chlorophyll pigment, electrolyte leakage, membrane lipid peroxidation, H_2O_2 content, total sugar and proline content upon exposure to 25^0C, 30^0C, 35^0C and 40^0C for 6 and 12 hours. Finally from hierarchical cluster analysis two most heat susceptible cultivars PBW550 and HT41, having highest HSIs were selected for PGPR application.

For high temperature stress alleviation seeds of PBW550 and HT41 were primed separately with two potential PGPR strains previously isolated and identified by Chakraborty and others (2013) *Bacillus safensis* (JX 660688) and *Ochrobactrum pseudogrignonense* (JX 660689) isolated from the wheat (*Triticum aestivum* L.) and blady grass (*Imperata cylindrica* L.) rhizosphere respectively. Both PGPR were able to retain their plant growth promoting attributes *in vitro* and could grow under elevated temperature conditions, but among the two, *B. safensis* could grow better at 40^0C. *In vivo* plant growth promoting ability of these two strains was also tested. PGPR priming significantly increased plant height and dry weight in wheat plants. Phosphate mobilization from soil to plant through root system was also increased in PGPR primed plants.

After screening for growth promotion *B. safensis* and *O. pseudogrignonense* were used for temperature stress amelioration at 40^0C. Pre treatment with PGPR lessened water loss, improved cell viability and acquired thermo tolerance in HT 41 and PBW550 plants signifying a protective effect by these bacteria especially by *B.safensis* at 40^0C. Heat stress upto 12 hours at 40^0C significantly increased membrane lipid peroxidation, electrolyte leakage and produced ROS such as hydrogen peroxide and superoxide, in unprimed plants. *B. safensis* and *O. pseudogrignonense* priming reduced heat induced oxidative stress in plants by reducing ROS generation and cell membrane injury.

Chloroplast is the primary site of heat injury as PSI and PSII are the key locations of ROS production other than mitochondria and peroxisomes. High temperature significantly reduced chlorophyll content in plants under heat stress for 12 hours at 40^0C. TEM studies revealed high temperature distorted cell wall, mitochondria and chloroplast ultrastructure especially grana, thylakoid stacking and mitochondrial cristae. Formation of large vacuoles within chloroplast and mitochondria can be observed in

case of unprimed heat stressed plants exposed to 40^0C for 12 hours. PGPR priming particularly with B. *safensis* lessened chlorophyll reduction and also minimized heat induced ultrastructural damages in chloroplast which perhaps restored PS I and PSII system. PGPR primimg also reduced mitochondrial ultrastructural abnormalities in leaf and accumulate greater amount of plastoglobules in chloroplasts during heat stress. PGPR primimg also enhanced carotenoid accumulation thus protects chloloplast from photoinhibition during heat stress. Carotenoid quenched superoxide, singlet oxygen and peroxyl radicals, thus minimized formation of ROS by receiving excess energy from the chlorophyll.

Heat stress at 40^0C enhanced protein expression in all plants irrespective of treatments. Appearance of new stress responsive proteins along with expression of existing was more in PGPR primed plants; especially *B. safensis* primed plants as compared to untreated plants after heat treatment. Differential expression analysis of various HSP genes revealed that in unprimed PBW 550 plants transcript level of HSP 101C, HSP 90 and HSP 70 increased significantly in initial hours followed by sharp decrease during heat treatment. Whereas in *B. safensis* primed plants relative expression of HSP101 and HSP 90 steadily increased and higher transcript level was maintained overtime as compared to heat stressed unprimed plants. Over expression of small HSPs such as HSP 23.5 and HSP 17.8 HSP 26.3 and HSFA3 were also observed. Relatively high expression of HSP101C, HSP 90 and small HSPs (HSP 23.5 and HSP 17.8 HSP 26.3) in PGPR primed plants all through heat treatment possibly provide better tolerance to heat injury. *B.safensis a*nd *O pseudogrignonense* primed plants maintained relatively high total and o dihydroxy phenol content plants during heat stress. Enhanced expression of chalcone synthase (CHS) during high temperature stress may give protection from heat injury. Relatively high expression of PAL gene in leaves of *B. safensis* primed plants in response to high temperature perhaps a part of acclamatory response where the enzyme induced the biosynthesis of other phenolics in the pathway.

Activities of antioxidative enzymes contributing to stress signalling such as peroxidase, catalase (CAT), ascorbate peroxidase (APX), glutathione reductase, and superoxide reductase increased across all treatment during initial hours of heat stress at 40^0C. PGPR primimg maintain high level of antioxidative enzyme activity during heat stress. Among the two PGPR *B. safensis* was more effective in retaining high antioxidant activity at

40^0C. PGPR priming also increased accumulation of non enzymatic antioxidants total glutathione and ascorbic acid in PGPR primed especially in *B. safensis* treated plants. Initial increase in ascorbic acid and total glutathione may add to glutathione and ascorbate regeneration system ascorbate-glutathione cycle to accentuate APX activity.

High temperature treatment significantly increased proline concentration in unprimed HT 41 and PBW550 as compared to PGPR primed plants of both the cultivars. RT PCR analysis indicated differential expression of proline biosynthesis related genes P5CS and PDH during temperature treatment might be responsible for different proline level in *B.safensis* primed and untreated plants. In both *B. safensis* and unprimed plants exposed to 40^0C transcript level of P5CS and P5CR increased with time and over expression of PDH genes turned out to be responsible for comparatively low level proline in *B. safensis* primed plants during heat stress. Polyamines biosynthesis related genes ADC1, ADC2 and SAMDC2 were found to be over expressed during temperature stress in both unprimed and *B.safensis* primed plants. Seed bacterization facilitated augmentation of more glycine betain and total sugar in plant tissues than unprimed plant during heat stress. High temperature increased glycine betain production in leaves for all treatments. It was observed that glycine betain and total sugar content in wheat leaf attained much higher level with *B.safensis* than *O. pseudogrignonense* priming.

Elevated temperature significantly reduced spike length, grain number and grain weight. Seed quality also gets affected in late planted plants. Ultrastructural analysis of starch grains revealed presence of flat, deformed starch granules as compared to round, spherical shaped granules observed in control as well as *B.safensis* primed heat stressed plants. Aleuronic layer of *B. safensis* plants was less affected as compared to heat stressed control plants suggesting positive role of *B.safensis* priming in improving seed quality in plants under heat stress.

Rising temperature significantly increased the susceptibility of wheat plants to spot blotch causing pathogen *Bipolaris sorokiniana*. Highest disease incidence was observed in the range of 35^0C to 38^0C and with relative humidity 70% and further rise in temperature for from 38^0C to 40^0C reduced disease incidence. Electrolyte leakage, H_2O_2 accumulation and lipid peroxidation significantly increased over time in infected plants. Therefore dual application of heat stress and *B. sorokinana* intensify membrane damage many folds and altered antioxidative defense responses.

An attempt was made to assess the potential of *B. safensis* and *O. pseudogrignonese* in induction of resistance and compare their performance at normal and elevated temperature in terms of biochemical response of wheat plants against spot blotch causing pathogen, *B. sorokiniana.* Both these PGPR effectively inhibit mycelia growth *in vitro* in soild media. GC-MS analysis showed that *B. safensis* and *O.pseudogrignonense* produced antifungal and antimicrobial compounds in culture. Seed priming with these two bacteria significantly increased growth, modulate antioxidative signalling and induced resistance which eventually reduced disease incidence in wheat plants at optimum as well as elevated temperature. Pre-treatment with the PGPR decreased the accumulation of H_2O_2, lessened lipid peroxidation in infected plants thus reduced electrolyte leakage and membrane damage protecting the plants from adverse consequences of oxidative injury. At high temperature PGPR, especially *O. pseudogrignonense* was slightly less effective in comparison to their performance at normal temperature. Total protein, phenol and O dihydroxy phenol significantly decreased over time in unprimed infected plants exposed to high temperature despite the fact that at normal temperature all these parameters increased in infected plants tissues across all treatment. However PGPR primimg were able to maintain comparatively high level of protein phenol and O dihydroxy phenol content over time in infected leaf tissues.

Spot blotch significantly increased accumulation of proline and soluble sugar in infected plants. RT PCR analysis revealed *B.safensis* primimg significantly increase relative expression of P5CS gene in comparison to PDH and P5CR genes in infected plants as a result proline accumulation increases drastically in PGPR primed infected plants pointing towards positive role of PGPR primimg in inducing hypersensitive response at elevated temperature.SDS PAGE analysis revealed expression of few new bands ranging from 10- 34 kDa in PGPR primed plant which were absent in case of unprimed infected plants both at normal and elevated temperature. RT PCR analysis of various HSPs revealed *B. safensis* seed priming significantly increased expression of HSP70 and HSP23.5, HSP26.3 and HsfA3. Whereas expression of theses HSPs remain more or less similar in unprimed healthy and infected plants during commencement of infection indicating role of these HSPs in *B. safensis* induced resistance spot blotch at high temperature.

In addition to this, time course accumulation of defense enzymes such as chitinase, Phenyl alanine ammonia lyase, Peroxidase and β-1, 3 GLU was determined following challenge inoculation with *B. sorokiniana.* Higher activities of CHT, POX, PAL, β-1, 3 GLU and accumulation of higher phenolic compounds were observed heat susceptible wheat plants. However, high temperature during disease commencement adversely affect of defense enzymes activity as well as protein, phenolics and osmolyte accumulation. Time course accumulation of all of these components indicating prolong exposure at 38^0C decreased defense enzyme activity, phenol and osmolyte accumulation in infected plants which might possibly facilitated pathogen spread and cause susceptibility to the diseases. *B.safensis* and *O. pseudogrignonense* primimg maintain higher level enzyme activity and accumulate osmolyte thus gave protection against *B. sorokiniana* even at high temperature. Results revealed *B. safensis* priming significantly increased PAL and CHS gene expression in infected plants especially during elevated temperature indicate greater amount of phenolics accumulation in *B. safensis* primed plants during disease commencement which helped plants to induced resistance against *B.sorokiniana.*

The findings of the present study provide insights into the PGPR activated response of wheat plants to heat stress. Seed priming with *B.safensis* and *O. pseudogrignonense* not only increased overall plant vigour but also protected plants from high temperature induced oxidative injury. Among the two PGPR, *Bacillus safensis* was most effective and has better potential to ameliorate heat stress. Application of PGPR elicited overall defense mechanism by over expression of antioxidative enzymes, heat shock proteins, osmolyte accumulation and antioxidant. Further, it reduced photosynthetic damage. These positive changes facilitated the plants to acquire thermo tolerance. The study also revealed active biochemical cross talk between elevated temperature and spot blotch disease development and furthermore uncover PGPR mediated array of antioxidative and molecular alterations responsible for induction of resistance against spot blotch disease at elevated temperature. Use of PGPR for amelioration of abiotic stresses especially heat stress appears to be a cost effective eco friendly technique; however all the PGPR strains cannot be used for stress alleviation. Factors such as the ecological niche of the microorganisms selected, specific interactions with the plants and the responses elicited should be taken care of before recommending a particular organism.