5. DISCUSSION

High temperature is one of the major abiotic stresses which greatly limit plant growth and productivity all over the world. Plants counter heat stress by modifying physiological and biochemical mechanisms. Acclimatization is mediated by either innate ability of plants to endure heat stress due to basal tolerance and or their ability to acquire tolerance during high temperature exposure (Maestri *et al.* 2002). The ability to withstand and to acclimate to elevated temperature results from both prevention of heat damage and repair of heat sensitive components. Adaptation to high temperature is mediated by changes in antioxidative enzymes and gene expression, osmolyte accumulation and array of metabolic alterations which enables plants to reduced heat injury (Rampino *et al.* 2009). In India wheat is the second main food crop, contributing nearly one third of the total food grain production grows optimally in winters thus highly sensitive to high temperature. Therefore, high temperature during wheat growing season would be fatal in the absence of adaptation or acclimation, and thus acquire thermo tolerance capacity is crucial during heat stress (Senthil-Kumar *et al.* 2007). The degree of susceptibility of different wheat cultivars may vary depending on their thermo tolerance level.

In the present study, preliminary screening of eight wheat cultivars was carried out to assess their basal thermo tolerance level and finally two most susceptible wheat cultivars were selected for PGPR application and temperature amelioration studies. Comparative study of basal thermo tolerance in various cultivars was based on heat susceptibility index, cell viability, loss of chlorophyll pigment, changes in membrane stability, generation of reactive oxygen species H_2O_2 and osmolyte accumulation. Heat susceptibility index (HSI) found to be a reliable heat stress responsive marker for rapid screening of wheat cultivars at the seedling stage from % reduction in fresh weight and relative water content on exposure plants to heat stress were utilized to calculate heat susceptibility index (HSI). Hameed *et al.* (2012) in his studies ranked various wheat cultivars for high temperature tolerance on the basis of Heat susceptibility index and established correlation with cell death and antioxidative changes in wheat cultivars exposed to heat stress. In the study, considerable reduction in relative water content as well as fresh weight was observed when plants were exposed to temperatures above 30^0C for 12 h. Based on the heat susceptibility index (HSI) values at 35^0C and 40^0C, C306 and HT 17 were most tolerant, while PBW 550 and HT41 were found to be most

heat sensitive among the cultivars. High temperature changes in lipid composition, affects of cell membrane integrity, reduce cell viability and hampers primary photosynthetic processes (Wahid *et al.* 2007). Thus Cell viability, chlorophyll reduction and electrolyte leakage have been extensively used to asses basal thermotolerance level in diverse plant species (Mullarkey and Jones, 2000; Ibrahim and Quick, 2001; Dhanda and Munjal, 2006; Yıldız and Terzioğlu, 2006; Yildirim *et al.* 2009). Heat tolerance is quantified by cell viability assay based on mitochondrial reduction of MTT (Yildiz and Terz, 2008). In the present study, considerable variation existed among cultivars which were tested for cell viability as well as chlorophyll content. According to findings maximum reduction in cell viability and chlorophyll content upon exposure to 30^0C, 35^0C and 40^0C for 6 h and 12 h were found in case of PBW550, PBW343 and HT 41, followed by HT15, GY and MW where as C306, HT17 were least affected. The reduction in cell viability resulting from heat stress may be because of uncoupling of the electron transport chain through disruption of the inner mitochondrial membrane or inactivation of enzymes of the respiratory pathway (Porter *et al*. 1999). Leaf disc bioassay was also conducted to assess heat sensitivity of eight cultivars. In this assay, extent of changing colour of leaf discs immersed in water from green to yellow after heat treatment was used as an indicator of effect of high temperature on leaf tissues and has been used in the evaluation of basal thermo tolerance in many crops including wheat and *Arabidopsis thaliana* (Burke, 1994; 1998; Burke *et al.* 2000; O'Mahony *et al,* 2000; Dash and Mohanty, 2001; Camejo *et al.* 2005).The result of leaf disc bioassay suggested that at 40^0C – C306, HT17, GY were least affected whereas PBW550, PBW343, HT41 showed high heat sensitivity. Thermotolerance is a complex attribute and controlled by several physiological and biochemical parameters and these parameters are greatly responsible for the plants to achieve a steady stable state similar to untreated plants (Hasanuzamman *et al.* 2013). Like other abiotic stresses, high temperature also alters metabolic pathways which leads to the accumulation of harmful ROS ,like superoxide radical ($O_2^{\cdot-}$), singlet oxygen ($^1O_2$), hydroxyl radical ($OH^{\cdot}$), hydrogen peroxide (H_2O_2) and which are responsible for membrane damage, protein degradation, deactivation of enzyme that reduces the cell viability remarkably (Savicka and Škute, 2010). Hanumantharao *et al.* (2016) suggested changes in various biochemical markers which include relative membrane permeability, H_2O_2, malonaldehyde and proline by and large can depict heat shock tolerance level in various plants after heat treatment. In the

present study exposure of plants at various temperatures for 12 h led to gradual increase in electrolyte leakage, H_2O_2 accumulation and membrane lipid peroxidation in all eight wheat cultivars. Highest change across all cultivars was noticed at 40^0C. However, maximum increase in all three parameters was noticed in PBW550, followed by HT41, PBW343, GY, MW and least was observed in case of C306 and HT 15. Accumulation of osmolytes such as proline, sugar and trehalose, glycine betain, is a recognized adaptive mechanism in plants against abiotic stress including high temperature and positively correlated with stress tolerance (Rasheed *et al*. 2011). Results showed that the osmolyte proline, which believed to be positively related with stress tolerance accumulated in least amount in C306 and HT17, whereas proline accumulation found to be quite high in PBW550, HT41, PBW343. Similar result was also reported by Kumar *et al.* (2013). Hua *et al.* (2011) observed that proline accumulation in *Arabidopsis* under high temperature stress reduced the thermotolerance level, probably by inducing ROS production via Pro/P5C cycle. In contrary, soluble sugar accumulation in heat sensitive PBW550, HT41, PBW343 was found to be lesser across all temperature and duration of treatment whereas maximum increase in soluble sugar content was observed in C306 followed by HT 15 and GY.

Finally hierarchical cluster analysis using the software CLUSTER 3.0 was used to characterize the interrelation among physiological parameters and align various wheat cultivars on the basis of therir basal thermotolerant attributes. Similar type of hierarchial cluster analysis has been performed to evaluate the natural variation of Bermuda grass differing in drought tolerance (Shi *et al.* 2014) and rice cultivars differing in salt tolerance (Chunthaburee *et al.* 2016). For hierarchical clustering, relative fold change values of all biochemical parameters studied were taken into consideration. Based on variation in physiological and biochemical parameters, all eight cultivars were grouped according to their high temperature tolerance that could be interpreted with the aid of the fold change value denoted by colour bars. The relation between the physiological parameters among themselves was also illustrated in cluster analysis. In the present study, following cluster analysis, cultivars were clearly divided into two groups - one of the groups comprising C306 and HT17 and second group further divided into two subgroups. One of the subgroup contains PBW550 and HT 41 and other group having GY, MW, HT15, PBW343. Comparing HSI values with cluster analysis data indicated that the first group containing C306 and HT 17 with minimum HSI Index are the

tolerant ones. The group comprising PBW550, HT41, having highest HSI values can be considered as heat susceptible and other group to be moderately susceptible compared to the former group. Thus among the eight two most susceptible cultivars, PBW550 and HT41 were selected for PGPR application and temperature stress amelioration studies to have an idea of PGPR modulated physiological and biochemical changes associated with temperature stress tolerance.

Exploitation of vast diversity of beneficial rhizospheric microorganisms for betterment of plant health as well as biotic and abiotic stress alleviation is an ecofriendly approach towards sustainable agriculture and to accomplish that plant and microbe compatibility is a necessity leading to constructive outcome such as demonstrated for growth promotion of wheat with certain microbial strains (Nain *et al.* 2010; Anderson and Habiger, 2012**)**. Two previously tested PGPR were selected for the present investigation. *Bacillus safensis* and *Ochrobactrum pseudogrignonense* used in the study have previously been shown to ameliorate drought and salt stress in wheat (Chakraborty *et al.* 2013). *Bacillus safensis* is a gram-positive, rod haped spore-forming bacterium, firstly isolated from a spacecraft in Florida and California (Satomi *et al.* 2006). It colonizes a wide range of niches, many of which are stringent for the survival of some microorganisms. Unique physiological and genotypic characteristics help *Bacillus safensis* to survive in extreme environments. It is closely related to *Bacillus pumilus, Bacillus altitudinis, Bacillus xiamenensis and Bacillus invictae*. *B. safensis* strain VK and W 10 isolated from cumin plant rhizophere in saline desert area and wheat rhizospere respectively were found to induce plant growth promotion and salinity tolerance of plants (Kothari *et al.* 2013; Chakraborty *et al.* 2013). More recently, a marine *B. safensis* strain (JX126862) isolated from mangrove sediments having cadmium solubilizing attributes was identified by Priyalaxmi *et al.* (2014). It is thus evident that *B. safensis* is a multifaceted bacterium with ability to survive in different stressful conditions. Many species under the genus *Ochrobactrum*, such as *O. anthropi, O. cytisi and O. lupine* isolated from diverse habitat from including soil, plants and their rhizosphere, animals were already reported to have plant growth promoting as well as abiotic stress (heat, heavy metal) tolerance abilities. *Ochrobactrum pseudogrignonense* is a gram negative, non spore forming alpha protebacterium belonging to family Brucelleceae. Chakaborty *et al.* in 2013 reported plant growth promoting ability of water stress and salt tolerant *O. pseudogrignonsense* strain IP8; besides, biodegradation

of malachite green by copper tolerant *O. pseudogrignonsense strain GGUPV1* was reported by Chaturvedi and Verma (2015). *In vivo* plant growth promoting ability of these two strains was tested in thermo susceptible cultivars HT41 and PBW550. For this, plant height, root and shoot dry weight were tested in 15 and 30 days after PGPR application. Result showed significant increase in plant height and dry weight in PGPR primed plants. Similar finding was also reported by Chakraborty *et al.* (2013). PGPR have been shown to promote the production of growth promoting phytohormones such as IAA, cytokinin and gibberellins which are perhaps responsible for plant growth promotion (Porcel *et al.* 2014). Recently Park *et al.* (2017) reported production of various endogenous phytohormones and ABA in *Bacillus aryabhattai* SRB02 inoculated soybean plants during heat stress induced plant growth promotion and ameliorated oxidative and nitrosative stress. Compared to other essential macronutrients, phosphorus is the least mobile nutrient element in plant and soil (Gyaneshwar, 2002). Satyaprakash *et al.* (2017) along with many others reported phosphorus deficiency negatively affect plant growth and development. Phosphate solubilising microorganisms release insoluble form of phosphate, is an important aspect of P availability in soils (Khosro, 2012). In the present study increase in root and leaf phosphate content in PGPR primed plants indicates increased mobilization from soil to in PGPR primed plant roots. Plant growth promoting rhizobacteria induced plant growth promotion and development via assembly and secretion of various regulatory chemicals around rhizosphere. Generally, plant growth promoting rhizobacteria facilitate the plant growth directly by either supporting resource gaining (nitrogen, phosphorus and essential minerals) or adjusting plant hormone levels, or indirectly by lessening the inhibitory effects of various abiotic and biotic factors on plant growth and development in the forms of biocontrol agents (Khan *et al.* 2007).

After screening for growth promotion in heat susceptible cultivars, *Bacillus safensis* and *Ochrobactrum pseudogrignonense* were used for temperature stress alleviation. Results showed pre treatment with these two PGPR improved plants capacity to ameliorate high temperature induced oxidative stress, increase cell viability by reducing ROS and cell membrane injury. However, among the two PGPR, *B. safensis* was found to be more effective than *O. pseudogrignonense* in amelioration of temperature stress. This could be because *Bacillus* sp. themselves are known to be able to withstand high temperature in soil due to their ability to produce endospore. These traits are desirable for acquired

thermo tolerance in different plants (Rampino *et al.* 2009; Liu and Huang, 2000; Jiang and Huang 2001; Hameed *et al.* 2012).

High temperature often leads to generation of ROS, like hydrogen peroxide and superoxide, singlet oxygen ensuing oxidative stress (Hasanuzzaman *et al.* 2013). These signalling molecules activate antioxidative response and react with polyunsaturated fatty acid and lipids through peroxidation and interruption of cell membrane stability by disrupting secondary and tertiary structure of protein molecules present in membrane and accelerate movement of molecules across membrane thereby increasing electrolyte leakage (Savchenko *et al.* 2002). In the present observation we found that in HT 41 and PBW550 prolonged heat stress for 12 h gradually increased electrolyte leakage and lipid peroxidation although pre treatment with PGPR protected plants to a degree from these detrimental effects. This in turn protected membrane stability which is considered to be the desirable approaches to alleviate high temperature induced oxidative damage other than calcium-dependent protein kinase (CDPK) signalling as well as initiated of mitogen-activated protein kinase (MAPK) activity (Meena *et al.* 2015).

Chloroplast is the primary site of heat injury (Wise *et al.* 2004) as PSI and PSII are the key location of ROS production other than mitochondria and peroxisomes (Soliman *et al.* 2011). In our observation chlorophyll content decreased significantly in unprimed plants. This may be because high temperature severely affects chloroplast and thylakoid membrane leading to changes in structural organization of thylakoids and grana (Hasanuzzaman *et al.* 2013) which in turn reduced photosynthetic efficiency of plants (Wahid *et al.* 2007). Thus any alteration in chlorophyll content and photosynthetic efficiency can be a good marker of thermo tolerance. In our findings, high temperature distorted cell wall, mitochondria and chloroplast ultrastructure especially grana and thylakoid stacking with membrane damage leading to electrolyte leakage. Formation of large vacuoles within chloroplast and mitochondria could be observed in case of unprimed heat stressed plants exposed to 40^0C for 12 h. Similar findings were also reported in grapes (*Vitis vinifera*) in response to heat stress. Heat stress severely damaged the stroma lamellae in chloroplasts, the contents of vacuoles formed clumps, whilst the cristae were disrupted and mitochondria became empty and hence decreased photosynthetic and respiratory performance (Zhang *et al.* 2005). However, PGPR priming particularly with *B. safensis* lessened chlorophyll reduction and also minimized heat induced ultrastructural damages in chloroplast which may be responsible for

restoration of PSI and PSII system. PGPR primimg also reduced mitochondrial ultrastructural abnormalities in leaf and accumulate greater amount of plastoglobules in chloroplasts. Coupling of plastoglobules and thylakoid membranes allows for the free exchange of lipid molecules, such as plastoquinone, carotenoids, and tocopherol from the thylakoids to plastoglobules protect the photosynthetic apparatus from free radical damage (Austin *et al.* 2006). *Pseudomonas aeruginosa* (strain 2CpS1) was also reported to increase chlorophyll content in wheat under elevated temperature (Meena *et al.* 2015). PGPR primimg also increased accumulation of carotenoid and thus protected chloloplast from photoinhibition during heat stress. Carotenoid quenches superoxide, singlet oxygen and peroxyl radicals, thus minimizes formation of ROS by receiving excess energy from the chlorophyll.

Modulation in protein expression is one of the key responses of plants to various environmental stresses (Wang *et al.* 2004). High temperature up-regulated expression of several heat inducible genes which encode heat shock proteins (HSPs) and these active products are very much essential for plants to withstand lethal heat stress. Heat induced constitutive expression of most of these proteins which acts as chaperones shield intracellular proteins from degradation and retains their stability and function through protein folding (Park and Seo, 2015). In present study heat treatment for 12 h initially increased protein content across all treatment followed by gradual reduction. However in PGPR primed plants, especially *B. safensis* primed plants of both the cultivars higher level of protein content was obtained as compared to control plant subjected to heat stress. Protein profiling by SDS-PAGE showed appearance of new bands in the range of 20 kDa to 67 kDa in plants exposed to high temperature. Appearance of new stress responsive proteins along with expression of existing ones was more in PGPR primed plants; especially *B. safensis* primed plants as compared to untreated plants after heat treatment. Maximum expression of proteins after 12 h of heat stress at 40^0C was observed in *B. safensis* treated plants. These stress responsive proteins were found to be small HSPs, HSP 90, HSP101, and most proteins probably were related to antioxidative enzymes. Kumar *et al.* (2013) observed expression of heat responsive proteins was more in thermotolerant cultivars in compared to susceptible cultivars when exposed to 42^0C for 2 h. Similar expression pattern of heat responsive proteins was also reported in barley (Salekdeh *et al.* 2002). PGPR mediated abiotic stress tolerance has been reported in many crops including beans, maize, groundnuts, pepper etc. (Dardanelli *et al.*

2008; Egamberdiyeva, 2007; Grichko and Glick, 2001; Saravanakumar and Samiyappan, 2007; Lim and Kim, 2013). However, PGPR-mediated heat stress tolerance has been less reported in wheat. Ali *et al.* (2009, 2011) reported that inoculation of *Pseudomonas* sp. strain AKM-P6 and *P. putida* strain AKM-P7 improved thermotolerance of sorghum and wheat plants which was attributable to the synthesis of high-molecular weight proteins and also improved the levels of cellular metabolites. Recently Singh *et al.* 2017 reported *Enterobacter cloacae* SBP-8 mediated expression of different proteins in wheat seedlings involved in protein synthesis, proteolysis, maintenance of cell structure, photosynthesis, defense, fatty acid synthesis, homeostasis, and other metabolic pathways which participate in strengthening and maintenances of cell to prevent cellular damage during salinity stress.

In order to understand the mechanism for the observed better performance of PGPR primed wheat plants under heat stress, we examined the expression of several major HSPs, including HSP101, 90, 70, and small HSPs which include HSP 26.3, 25.6, 17.8 and heat shock factor HSA3. Heat shock proteins are commonly known to play essential roles in heat stress tolerance of higher plants (Wang *et al.* 2014). HSP101, a member of ClpB protein subfamily as well as HSP 90 and HSP 70 promote ATP dependent renaturation of protein aggregates during heat stress allow them to be refolded by other chaperones thus essential for the induction of thermotolerance (Nieto-Sotelo *et al.* 2002. Wang *et al.* 2004, Hsanuzzaman *et al.* 2013). Hsp101 along with HSP70 participated in establishing of thermotolerance in *Arabidopsis* (Queitsch *et al.* 2000). Katiyar-Agarwal *et al.* (2003) further confirmed that improved thermotolerance in transgenic rice seemed to be solely attributable to over expression of *Athsp*101. Grigorova *et al.* 2011 reported over expression of HSP101, HSP70, small HSPs like, HSP 26.3 and HSP 17.8 genes in thermotolerant wheat cultivars after combine application of drought and high temperature. Kumar *et al.* (2013) suggested that elevated HSP90 transcript level along with over expression of antioxidant enzymes and low proline accumulation is a promising target for developing heat tolerance wheat genotypes. RT PCR analysis of these HSP genes revealed that transcript level of HSP 101C, HSP 90 and HSP 70 increased significantly in initial hours followed by sharp decrease at 8^{th} and 12^{th} h of heat treatment. Whereas in *B. safensis* primed plants expression of these genes, particularly HSP 101 and HSP 90 steadily increased and higher transcript level was maintained over a period of time as compared to heat stressed unprimed plants. Similar

trends were also observed in case of HSP 23.5, HSP 17.8 HSP 26.3 and HSFA3. One of the characteristics of plant heat shock tolerance is the expression of large number of small heat shock proteins ranging from 17- 24 kDa (Iba, 2002) which was also detected in case of *B. safensis* primed wheat plants exposed to heat stress for 12 h and transcript expression of HSP 17.8 and 23.5 was found to more in *B. safensis* primed plants in relation to control plants. Hsp17.8 stabilizes chloroplast outer membrane proteins and maintains cell membrane stability thus enhanced thermotolerance in *Arabidopsis* (Kim *et al.* 2012). In another study Jiang and his colleagues (2009) reported RcHSP17.8, of *Rosa chinensis* to be responsible for conferring resistance to a range of stresses to *Escherichia coli,* yeast and *Arabidopsis thaliana*. Higher transcript levels HSP 23.5 in *B.safensis* primed plants supports data reported for cereals in which the presence of multiple small mitochondrial HSPs in maize was associated with higher thermotolerance when compared to wheat and rye, in which only one mitochondrial small HSP was expressed (Wahid *et al.* 2007, Rampino *et al.* 2009). Elevated transcript level of HSP 26.3 in heat stressed plants are in accordance with a number of studies showing the importance of chloroplast small HSPs in thermotolerance. In particular a genetic relationship between acquired thermotolerance and the expression of a plastid-localised HSP 26 was demonstrated using recombinant inbred wheat lines (Joshi *et al.* 1997). Furthermore, analyses of defective mutants in one or more tomato chloroplast small HSPs indicated that genetic variation observed in the production of chloroplast small HSPs may have a determinant role in photosynthetic system and whole plant tolerance (Heckathorn *et al.* 2004). Chloroplast small HSP has been shown to protect photosynthesis during heat induced oxidative and photoinhibitory stress by preventing irreversible protein aggregation and protecting PSII or other aspects of thylakoids by stabilizing chloroplast membranes (Nakamoto *et al.* 2000; To¨ro¨k *et al.* 2001), and possibly as site-specific antioxidants (Hamilton and Heckathorn, 2001, Heckathorn *et al.* 2004). Heat stress transcription factors (HSFs) play a crucial role in the plants adaptation to high temperatures. HsfA3 expression was also increased in the *B. safensis* treated heat stressed seedlings compared to the nontreated heat stressed seedlings. Acoording to Guo *et al.* (2016) heat stress transcription factors A2 (HsfA2) and A3 (HsfA3) modulated heat regulation pathway in *Arabidopsis* and confers thrmotolerance. In *Arabidopsis* HsfA3 enhances the production of several HSPs (Ikeda *et al.* 2011) and is regulated by two independent pathways suggesting an active role in stress

management (Liu and Charng 2013). The HSFA3 binds to target genes, such as HSP25.3 (CP-sHSP*),* Hsp101, ascorbate peroxidase and stimulates their transcription (Nishizawa *et al.* 2006; Schramm *et al.* 2006, Kotak *et al.* 2007). Because of the complexity of the plant HSF network, our understanding of the heat-stress response and acquired thermotolerance is still inadequate.

Phenolic compounds including phenylpropanoids and flavonoids play crucial roles in mitigating the adverse effect of biotic and abiotic stresses in plants. Phenolic compounds protect cells from potential oxidative damage and increase cell membrane stability (Burguieres *et al.* 2006). In the present study high temperature increased total and O dihydroxy phenol significantly in all plants across all treatments. However, in *B.safensis a*nd *O. pseudogrignonense* primed plants high level of total and O dihydroxy phenol contents were maintained under in heat stressed conditions.

Phenylalanine ammonia-lyase (PAL) is the principle enzyme in the phenylpropanoid metabolism pathway. In present study heat stress induced PAL gene expression across all treatments and in *B. safensis* primed plants transcript level was higher than unprimed plants and remained unchanged even after 12 h of heat stress. Induction of PAL gene expression during heat stress was reported by Zhang *et al.* (2013). According to Obaid *et al.* (2016) increased PAL expression in *Rhazya stricta* leaves in response to high temperature is a component of main acclimatory response prevailing within plants where the enzyme induced the biosynthesis of other phenolics in the pathway. Chalcone synthase is the key enzyme responsible for orchestration of flavonoid and anthocyanin biosynthesis. This enzyme is responsible for formation of proanthocyanidins, a precursor of anthocyaninne which protects plants fron UV radiation during the daytime (Rienth *et al.* 2014). Enhanced expression of chalcone synthase was also across all treatments during high temperature stress may give protection from heat injury by anthocyanine synthesis (Obaid *et al.* 2016).

Production of ROS within plants tissues turns on different enzymatic and non-enzymatic signaling pathways. Antioxidative enzymes contributing to stress signaling such as peroxidase, catalase, ascorbate peroxidase, glutathione reductase and superoxide reductase (Lee and Lee, 2000). Superoxide dismutases is the first enzyme to initiate ROS scavenging mechanism participate in dismuting of $O^{2.-}$ radical to molecular O_2 and H_2O_2. Hydrogen peroxide is then converted to H_2O and O_2 either by CAT and

peroxidase or in ascorbate-glutathione cycle by APX and glutathione reductase help to regenerate glutathione pool resulting detoxification of H_2O_2 (Foyer and Noctor, 2003). Non-enzymatic antioxidants such as glutathione, tocopherol, ascorbic acid and carotenoid play an important role in protecting the plant from stress-induced oxidative injury by performing photoprotective role and by inhibition of lipid peroxidation (Gill and Tuteja, 2010; Ashraf and Foolad, 2007). In PGPR primed plants there was significant increase in activity of antioxidative enzymes like APX, SOD, GR associated with ascorbate-glutathione cycle in comparison to control after heat stress and over expression of these redox enzymes probably facilitates the plants towards better stress tolerance mechanism (Abd El-Daim *et al.* 2014). Sharp increase in SOD and APX activity in control and PGPR primed seedlings at initial hours of heat stress signifies role of SOD and APX as first line of defense during high temperature stress. Abd El-Daim *et al.* (2014) reported over expression of APX1 transcripts along with MDHAR, DHAR, GR during high temperature stress in B. *amyloliquefaciens* 5113 and *A. brasilense* NO40 treated wheat seedlings. PGPR seed priming significantly enhanced APX and SOD activity in heat treated plants and this could be considered as marker of higher acquired thermo tolerance (Almeselmani *et al.* 2006). This may be attributable to its ability to protect chloroplasts from ROS and scavenge superoxide after exposure of plants to heat and drought (Koussevitzky *et al.* 2008). GR activity was also considerably higher in PGPR treated plants especially in *Bacillus safensis* treated plants in comparison to control heat treated plants. Enhanced activity of GR and in addition initial increase in ascorbic acid and total glutathione may add to glutathione and ascorbate regeneration system ascorbate-glutathione cycle to accentuate APX activity (Abd El-Daim *et al.* 2014). However, elevated activity of enzymes like APX and SOD is not sufficient to scavenge all ROS, especially H_2O_2 produced from APX and SOD activity which are toxic for cells. High temperature also increased activity of other enzymes like catalase and peroxidase significantly. Over expression of these redox enzymes enhance abiotic stress tolerance in plants (Singh and Grover, 2008) by increasing ROS detoxification. Since POX and CAT activity in PGPR treated plants are significantly higher than control over time during high temperature treatment it is possible that PGPR treated plants can scavenge greater amount of H_2O_2 out of the cell than heat stressed unprimed plants.

Plants under different abiotic stress including heat stress, salinity, drought accumulate various compatible osmolytes, like proline, sugars, polyols and glycine betain as a part of adaptive mechanism (Wahid *et al.* 2007). However in our observation high temperature treatment significantly increased proline concentration in unprimed HT 41 and PBW550 although PGPR priming reduced proline accumulation in both the cultivars. An increase in proline level during heat stress is not always helpful for plants unlike other stresses particularly in case of osmotic stress, where proline accumulation plays a positive role in stress mitigation (Lv *et al.* 2011). Rizhsky *et al.* (2004) reported proline can amplify the inhibitory consequences of temperature stress on seedling growth in *Arabidopsis*. Kumar *et al.* (2013) reported exposure to high temperature leads to generous accumulation of proline in heat sensitive cultivar PBW 343 than tolerant cultivar C306 indicating higher basal thermo tolerance level when proline content is low in tissues. Proline accumulation is controlled by transcriptional alterations of proline biosynthesis and degradation occurring at the inner mitochondrial membrane. At first glutamate is converted to Δ1-pyrroline-5carboxylate (P5C) by Δ1-pyrroline-5carboxylate synthetase (P5CS) followed by Δ1-pyrroline-5-carboxylate reductase (P5CR) catalyzed reduction of P5C to proline. In the next step proline is oxidized to P5C by proline dehydrogenase (PDH) (Mattioli *et al.* 2009, Lehmann *et al.* 2010). 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. One interesting finding is that the PDH expression significantly increased with time in *B. safensis* primed plants in comparison untreated plants. In case of unprimed plants PDH expression was even down regulated after 8^{th} and 12^{th} hour of heat stress. Xue *et al.* (2009) have indicated that stress-induced accumulation of proline in rapeseed results from the activated biosynthesis and also the inhibited proline degradation. Over expression of PDH genes in *B. safensis* primed plants turned out to be responsible for comparatively low level proline than untreated control plants. There are very few reports regarding discrepancies in proline accumulation during heat stress. Larkindale and Vierling (2008) reported that higher proline content apparently reduced PDH activity, showed reduced tolerance to heat treatment in *Arabidopsis thialiana*. Lv *et al.* (2011) found proline accumulation through the Pro/P5C cycle and inhibition of ethylene

and ABA biosynthesis in plants exposed to high decreased the thermotolerance, possibly by increased ROS accumulation. The reason behind this discrepancy is not clear. One possibility is that instead of proline, other osmolytes like, glycine betain, polyamines and sugar play active role in heat stress tolerance. Rizhsky *et al.* (2004) reported that during a combined application of drought and high temperature, sugar rather than proline accumulated and play protective roles during heat stress. Similar trends were also found in this study. In the present study, another osmolyte polyamines biosynthesis related genes ADC1, ADC2 and SAMDC2 were found to be over expressed during heat stress in both unprimed and *B.safensis* primed plants exposed to heat stress. ADC1 and SAMDC2 transcript level found to be relative high at 8^{th} and 12^{th} h in unprimed plants in comparison to *B. safensis* primed plants. Polyamines are rapidly induced by different abiotic stresses in plants, including drought, salinity, chilling, hypoxia, UV irradiation, heavy metals. Arginine decarboxylase (ADC1 and ADC2) catalysed decarboxylation of arginine to form primary amine putrescine. In subsequent reactions SAM decarboxylase converts putrescine to spermidine and subsequently spermine (Alcázar *et al.* 2006). Kumar *et al.* (2014) reported exogenous application of putrescine induced thermotolerance of wheat at pre-anthesis stage. Roy and Wu in 2001 reported over accumulation of spermidine and spermine in thermotolerant rice cultivar exposed to high temperature. Later in a number of studies it has been showed that polyamines act as ROS scavenger and reduced which induced cellular damage in transgenic plants of rice, *Arabidopsis*, tobacco, pears and through the over-expression of key biosynthetic enzymes in the polyamine biosynthetic pathway (Pathak *et al.* 2014). SAMDC2, ADC1 and ADC2 genes were induced during heat shock treatment at 35 °C for 1 h in *A. thaliana*. Over expression of polyamines protect plants from heat shock damage by the expression of heat shock protein genes like HSP101, HSP 90, and HSP70 (Roy Pathak *et al.* 2014). There are several reports about increased endogenous polyamine level following PGPR application. Recently, Zahedi and Abbasi in 2015 reported application consortium of PGPR- *Rhizobium japonicum*, *Azotobacter chroococcum* and *Azospirillum brasilense* enhanced osmotic stresss tolerance in soybean plants by increasing endogenous in phytohorhome and polyamine level.

Glycine betain, a quaternary amine has an important role during high temperature stress (Sakamoto and Murata, 2002). Our results showed that seed bacterization facilitates augmentation of more glycine betain in plant tissues than unprimed plant during heat

stress. Tian *et al.* (2017) suggested high level of glycine betain in wheat leaf stabilizes lipid and protein complexes of thylakiod membrane and improve photosynthesis during salinity stress. Accumulation of glycine betain during heat stress was also reported in sugarcane and maize (Quan *et al.* 2004; Wahid, 2007). Total sugar content significantly increased in PGPR primed plants under heat stress. According to Sairam and Tyagi (2004), accumulation of soluble sugar might contribute to heat tolerance. Increasing concentration of soluble sugar was reported in sugarcane (Wahid, 2007). Gou *et al.* (2015) also reported *Klebsiella variicola, Pseudomonas fluorescens* and *Raoultella planticola* application increased glycine betain and improve drought tolerance in maize. In the present study accumulation of osmolytes like sugars, glycine betain and polyamines probably play positive in PGPR induced heat stress amelioration.

High temperature throughout grain filling phase is an important yield limiting factor in wheat (Zhao *et al.* 2016). Even a small (1.5°C) rise in temperature adversely affects crop yields (Warland *et al.* 2006). In the present study, effect of elevated temperature on seed quality in late planted unprimed and PGPR primed plants were evaluated. Result revealed that heat stress significantly reduced spike length, grain number and grain weight in unprimed heat stressed plants. Our study is in agreement with that of Baloch *et al.* (2016). Total protein and starch content also reduced in heat stressed seeds. Reduced grain quality and yield in late sown wheat plants might be linked to grain insubstantiality due to high temperature injury during reproductive growth (Wardlaw, 2002). However, there was less reduction in spike length, grain number and grain weight in *B.safensis* primed plants as compared to unprimed plants. Protein and starch content was relatively high in *B.safensis* primed plants in comparison to unprimed 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. During terminal heat stress thermal denaturation decreased ADP –glucose pyrophosphorylase, starch sucrose synthase and starch branching enzyme activities resulting in low starch granules synthesis. These weakens sink strength and restricts grain filling and ultimately produced shrinking of kernels and in due course form grains with less weight and thus reduces yield (Kumar *et al.* 2016; Baloch *et al.* 2016). 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. Kumar *et al.* in 2013 suggested

high SOD activity and over expression of certain heat shock proteins like HSP 90, 70 and small HSPs protect starch sucrose synthase and other enzymes involved in starch synthesis in wheat plants under heat stress thus improved seed quality in thermotolerant cultivars. In the present study increased expression of SOD and HSP 90 along with other sHSP in *B.safensis* primed heat stressed plants might have helped to protect enzymes involved in starch synthesis from heat shock and thus improve seed quality.

Changing weather in terms of elevated temperature and CO_2 with more frequent extreme weather events, modify physiological and biochemical processes within plants which profoundly affect plant-pathogen interactions. An elevated growth temperature often hampers plant immunity and renders plants more vulnerable to pathogens (van Maanen and Xu, 2003, Zhu *et al.* 2010). High atmospheric temperature and relative humidity increase the occurrence of spot blotch (Aggarwal *et al.* 2004, Viani *et al.* 2013). In this study elevated temperature significantly increased the susceptibility of wheat plants to spot blotch causing pathogen *Bipolaris sorokiniana*. Maximum disease incidence was observed in the range of 35^0C to 38^0C and with relative humidity 70% and a further rise in temperature beyond 38^0C significantly reduced disease incidence. Our result was in accordance with finding of Sharma *et al.* (2007). They found increased spot blotch incidence in wheat under high night temperature (30^0C – 35^0C). Viani *et al.* (2017) proposed a spot blotch forecasting system and predicted favourable temperature (18-34°C) and RH-duration (15 h or above) facilitates spot blotch infection and once infection takes place subsequent progress of the disease is largely dependent on temperature.

Temperature sensitivity in plant disease resistance is a phenomenon reported as early as 1969 and observed in various plant-pathogen interactions (Wang *et al.* 2009). Elevated temperature modifies cellular metabolism and membrane properties in such a way that ultimately which negatively affect plant defense responses. Perhaps some common mechanism exists for temperature sensitivity and various disease resistance systems since many of them share similar signalling molecules and use similar signalling cascades (Wang *et al.* 2007; Zhu *et al.* 2010). Results of the present study showed that with the onset of disease electrolyte leakage, H_2O_2 accumulation and lipid peroxidation significantly increased over the period of time in infected plants. High temperature during disease commencement changed membrane permeability in terms of electrolyte leakage and lipid peroxidation making the plants even more vulnerable to pathogen

attack. Therefore, dual application of heat stress and *B. sorokinana* intensify membrane damage many folds and altered antioxidative defense response. With onset of disease and pathogen recognition an oxidative burst of reactive oxygen species (ROS) such as hydrogen peroxide and superoxide occurs which on one side are important signals interceding defense gene activation (Sharma *et al.* 2012) and activates induced systemic response (ISR) while excess amount of ROS is toxic for cell and causes lipid peroxidation and membrane damage (Lamb and Dixon, 1997; Montillet *et al.* 2005). Pathogen invasion and oxidative damage in plant cells activate various defense enzymes, phenolics and hormones which in turn induce overall disease resistance capability of host plants (Torres *et al.* 2006). In PBW 550 leaves *B. sorokiniana* infection induced accumulation of defense enzymes -chitinase, glucanase, phenylalanine ammonia lyase, peroxidase along with phenolics and osmolytes which are perceived to be one of the coordinated and multifaceted defense mechanisms. However, high temperature during disease commencement adversely affects defense enzyme activity as well as protein, phenolics and osmolyte accumulation. Time course accumulation of all of these components during prolonged 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. Sharma *et al*. (2007) reported prolonged higher mean temperatures for more than a 6 year experimental period was associated with heightened susceptibility to the fungus *Cochliobolus sativus* causal agent of root rot in wheat. Similar findings were reported by many scientists (Bale *et al.* 2002; Luck *et al.* 2011; Madgwick *et al.* 2011). Non-acclimation to high temperature causes more susceptibility of plants to pathogen. For example, instant exposure of ornamental plant roots to 45°C increased severity of root rot causing agent *Phytophthora infestans* (MacDonald, 1991). In *N. tabacum* and *A. thaliana* high temperature compromised hypersensitive response (HR) and resistance (*R*)—gene mediated defense responses to *P. syringae* pathovars (causal agent of brown spot in thale cress) and viral elicitors Wang *et al.* (2009). Temperature-dependent inhibition of host resistance has been reported for *Tomato spotted wilt virus* (TSWV; causal agent of spotted wilt in tomato) and *Tobacco mosaic virus* (TMV; causal agent of mosaic disease in tobacco) and TMV is able to overcome the *N*-gene mediated resistance at temperatures above 28°C in *N. tabacum* while TSWV is able to suppress TSW, a dominant gene-mediated resistance in *Capsicum chinense* plants at high temperatures

(Király *et al.* 2008). Thus, heat stress generally leads to suppression of host defense responses along with the other metabolic processes, thereby increasing their susceptibility to pathogens.

Exploitation of symbiotic association of microbes with plants has currently become an important tool to protect the plant health in eco friendly manner (Gupta *et al.* 2015). PGPR improve plant health and growth promotion by array of mechanisms that include formation of soil structure, disintegration of organic matter, reprocessing of essential elements, mineral nutrients solubilisation and producing copious amount of plant growth regulators which act as stimuli of root growth. These increase soil fertility and also protect plants from broad spectrum of soil and seed borne pathogens by induced systemic resistance (Sivasakhti *et al.* 2014). Jasmonate and ethylene signalling along with a range of defense enzymes and phenolic compounds induce systemic resistance within the plant and incite the host plant's defense reactions against a range of plant pathogens (Glick, 2012). In this study *B. safensis* and *O .pseudogrignonese* were used to ameliorate spot blotch. Both these PGPR, especially *B.safensis* was found to be more effective in amelioration of heat stress in heat susceptible PBW550 and HT41cultivars. Therefore, in this study we also tried to determine 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.* The antagonistic activity of *B.safensis* and *O.pseudogrignonese* against *B.soroikiniana* were pre screened *in vitro*. Both these PGPR effectively inhibit mycelial growth in soild media. Ethyl acetate fraction of cell free culture filtrate of *B. safensis* and *O. pseudogrignonese* were further characterised by GC/MS analysis. The analysis confirmed the presence of several compounds. 10-Octadecenoic acid (retention time 18.88 min) and Pyrollo pyrazine (retention time 25.858) were most abundant in ethyle acetate fraction of *B. safensis* whereas in case of *O. pseudogrignonense* Pyrollo pyrazine (retention time 25.858) was most abundant. Deepthi *et al.* (2016) reported the antifungal activity of *Lactobacillus plantarum* MYS6 against *Fusarium proliferatum* MYS9. They have reported 10-Octadecenoic acid, methyl ester as one of the major antifungal compounds produced by the isolate by its volatile substances. Chemoprofiling of this volatile substance secreted from root endophyte *Pseudomonas putida*BP25 indentified Pyrrolo [1,2-a] pyrazine-1,4-dione, an antifungal compound inhibited broad range of pathogens such as *Giberella*

moniliformis, Phytophthora capsici, Pythium myriotylum, Rhizoctonia solani, Colletotrichum gloeosporioides, Athelia rolfsii, and plant parasitic nematode*, Radopholus similis* (Sheorn *et al.* 2016).There are no previous reports of these two PGPR species as antagonists of *Bipolaris sorokiniana* . However antagonistic activity of other species of *Bacillus* (*Bacillus amyloliquefacians* , *Bacillus pumilus*, *Bacillus subtilis*) against *B. sorokiniana* were reported by Kilic-Ekici and Yuen (2004). Different species of *Ochrobactrum*, such as *Ochrobactrum lupini KUDC1013* induced systemic resistance (ISR) in tobacco against soft rot disease caused by *Pectobacterium carotovorum subsp. Carotovorum* (Sumayo *et al.* 2013) and *Ochrobactrum anthropi* BMO-111 against blister blight disease of tea (Sowndhararajan *et al.* 2012).

In the present study PGPR priming significantly reduced disease severity in *B.sorokiniana* infected plants *in vivo*. At high temperature both the PGPR was not as effective in induction of resistance as at normal temperature. However, among the two, *B. safensis* was more effective in reducing disease severity in comparison to *O. pseudogrignonense at* elevated temperature. Initial interaction between host plant and *B. sorokiniana* leads to biochemical alterations which eventually provide resistance to *B. sorokiniana* within plant tissue and treatment with PGPR modulate biochemical changes within host to induce this resistance. Inception of disease within leaf triggered accumulation of oxidative stress as evident by enhanced accumulation of of H_2O_2. Pre-treatment with the PGPR decreased the accumulation of H_2O_2 markedly. H_2O_2 in low concentrations act as secondary messenger and triggers hypersensitive response around infection zone (Orozco-Cardenas *et al*. 2001); High concentration of H_2O_2 can start off a free radical chain response and cause numerous disruptive effects such as increasing membrane peroxidation by-products such as MDA, ROS localization, and in severe cases even causes cell death (Zlatev *et al*. 2006). In present study both the PGPR significantly reduced lipid peroxidation in infected plants which in turn 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.

Plant phenolics and proteins are directly or indirectly involved in various metabolic pathways and play vital role in disease resistance. Increased protein expression perhaps involved in modulation of enzyme expression, signal transduction, degradation of plant

metabolism, transport, biocontrol (Yaoyao *et al.* 2017). 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. 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. Chloroplastic Hsp70 induced JA-dependent signal transduction pathway which plays a key role in induction of defense responses in stripe rust infected wheat plants (Duan *et al.* 2011; Yu *et al.* 2015). Hsp70 is important for non host resistance to *P. chicorii* in *N. benthamiana* and it is a part of immune complex with SGT1 and Hsp90 (Jelenska *et al.* 2009). HSFA3, a heat shock factors –a member of HSF A gene family as well as HSP17.8 were stongly upregulated may possibly to shut-off of the heat shock response in healthy and infected PGPR primed plants. Kumar *et al*. (2009) reported that HsfB1 and HsfB2b family interacted with class A-Hsfs and upregulates many heat shock proteins including Hsp17.6, Hsp70, Hsp83.1, and Hsp101 and induced resistance against many pathogens (*P. syringae, P. infestans, B. cinerea, A. brassicicola*).

Fungal infection accelerates phenolic metabolism and lignin synthesis. Phenolic compounds, including phenolic acids and cell wall bound ferulic acid, can give mechanical strength to the cell wall to guard against infection, ferulic acid crosslinking of phenylpropanoid esters directs to the formation of lignin-like polymers, such hydroxycinnamic acids and their derivatives (Maurya *et al.* 2007). In this study 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 overtime in infected leaf tissues.

To impede pathogen attack PGPR modulate expression of an array of defense enzymes which are already present in plants as intrinsic defence against any pathogen attack. Among the defense enzymes POX is one of the key enzymes countering pathogen attack and provide defense against plant pathogens (Anand *et al.* 2007). Different isoforms of POX provide resistance against plant pathogens by lignin and suberin

deposition, polymerization of hydroxyl proline rich glycoprotein on cell walls guarding cells against fungal hyphae invasion and also reduce oxidative damage by H_2O_2 scavenging (Hammond-Kosack and Jones, 1996; Yoshida *et al.* 2003; Maksimov *et al*. 2014). Other than POX, application of two PGPR significantly increase expression of PR proteins, such as β 1,3 glucanase (PR 2) and chitinase (PR 3) in infected plants at the same time reduce disease incidence. Chitinase and β 1,3 glucanase accumulate around necrotic region. These two enzymes destroy fungal hyphae and restrict hyphae penetration within cells (Armijo *et al.* 2016).

Another vital enzyme and part of phenyl propanoid pathway PAL activity also increased significantly after PGPR application. PAL is the principal enzyme in the phenylpropanoid metabolism and has important function in the production of several defense-related secondary compounds such as lignin and phenols (Tahsili *et al*. 2014; Hemm *et al.* 2004). Less PAL activity affects phenolic biosynthetic pathways and reduces phenolic compound production (Jayaraj *et al.* 2010). Boominathan *et al.* (2013) reported *B. megaterium* (AUM72) mediate induction of defense related enzymes against rhizome rot causing fungus *Pythium aphanidermatum*. Plant growth promotion in wheat by rhizosphere bacteria with multi-functional traits was reported by Chakraborty *et al.* (2013). Chakraborty *et al.* (2016) also reported the role of PGPR as plant growth promoter in tea and biocontrol agent against root rot pathogens. Elevated expression defense-related genes, namely puroindoline protein, β-1,4-glucanase and chitinases in wheat during *Tilletia indica* infection was reported by Tripathi *et al.* (2013). Overexpression of pathogenesis related (PR) proteins like peroxidase, β-1,3-glucanase and chitinase, polyphenol oxidase (PPO), phenylalanine ammonia lyase -an integral part of phenyl propanoid pathway, phenols and also chlorophyll contents induced systemic resistance (ISR) in infected plants (Sundaramoorthy *et al.* 2013; Graham *et al.* 2003). β-1,3-glucanase and chitinase can break down the cell wall components of pathogens, PAL and peroxidase was found to induce defense responses in many crops under fungal attack. Coordinated accumulation of these enzymes systemically and locally are linked to the development of systemic acquired response (SAR) (Ferreira *et al.* 2007; Kasprzewska, 2003; Santos *et al.* 2004). Investigation expression patterns of involved genes in production of these metabolites e.g. PAL and CHS, for a better understanding of defence mechanisms towards various stresses appears significantly useful. 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.* Enhanced expression of PAL and CHS genes mainly triggered an array of signalling cascade resulting in production of phenolics and flavoinois which induce resistance against pathogen attack. According to Compant *et al.* (2010) application of PGPR increased accumulation of phenylalanine ammonia lyase, peroxidase, phytoalexins, polyphenol oxidase, and/or chalcone synthase. Induction of these plant defense compounds (e.g., chalcone synthase) perhaps triggered by the the chemical PGPR use for intraspecific signaling (Mathesius *et al.* 2003; Compant *et al.* 2010)

From the overall experiments it is apparent that plants protect themselves from pathogen attack by modulating series of biochemical changes and elevated expression and various defense enzymes and plant phenolics. Application of PGPR decreased disease susceptibility of plants by inducing systemic resistance in terms of over expression of defense enzymes and phenolic content and by reducing membrane injury and disease induced oxidative damage.

Glycine betain content increased insignificantly at normal temperature grown infected plant. Significant increase in glycine betain content was observed in both healthy and infected plants only when exposed to elevated tempetutes pointing towards less important role of glycine betain in inducing resistance against *B. sorokiniana*. Accumulation of another osmolyte proline during disease establishment has been reported by many scientists. In the present study spot blotch infection increased proline content across all treatments both normal and elevated temperature; however PGPR priming was able to retain high proline level at high temperature even 72 h after disease commencement. Plant enduring hypersensitive respose due to pathogen attack accumulate proline to protect the plants from oxidative injury and cell death (Fabro *et al.* 2004). Increased level of proline in plants tissues during hypersensitive response was most likely due to upregulation of the key enzyme in proline biosynthesis, P5CS, at and around the sites of HR (Deuschle *et al.* 2004). In the present sudy RT PCR analysis revealed *B. safensis* primimg significantly increased 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.

Biosynthesis of the polyamines putrescine, spermidine, and spermine is induced in response to pathogen infection of plants (Lou *et al.* 2016). Polyamine biosynthesis related genes ADC1, ADC2 and SAMDC2 gene inceased in infected plats at normal temperature. ADC1and ADC2 whose expression increased many folds in heat stressed healthy plant were found be down regulated during ineraction between heat stress and *B.sorokinina* infection. On the contrary, SAMDC2 expression found to increase in infected plants exposed to heat stress. This is consistent with ODC and ADC2 induction being part of the coordinated defence response to *Fusarium* head blight. (Gardiner *et al.* 2010), Egg plants expressing the oat ADC gene exhibited tolerance to the wilt-causing fungus *Fusarium oxysporum* (Prabhavathi and Rajam, 2007).These results were similar to others found in barley, where the levels of free PUT and SPN and conjugated forms of PUT, SPD and SPN were increased following inoculation with the powdery mildew (Cowley and Walters, 2002). *Ustilago maydis* induced accumulation of putrescine in maize leaves (Rodríguez-Kessler and Jiménez-Bremont, 2009). Chinese cabbage with turnip yellow mosaic virus caused a significant increase in the levels of S-adenosylmethionine and the polyamines, spermidine, spermine and putrescine in the leaves (Torgate *et al.* 2005). Comparing results of this study with previous studies it can be assumed that *B.safensis* priming effectively increases tolerance to *B. sorokinina* by increasing expression of polyamine synthesis related genes.

Seed priming with *Bacillus safensis* and *Ochrobactrum 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 to some extent. 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.

Elevated temperature significantly increased the susceptibility of wheat plants to spot blotch causing pathogen *Bipolaris sorokiniana*. High temperature adversely affected anti oxidatidative defense response within plants making plants more prone to spot blotch. However, *B. safensis* and *O. pseudogrignonese* priming helped in induction of resistance biochemical response of wheat plants against spot blotch causing pathogen, *B. sorokiniana.* Use of PGPR for amelioration of abiotic and biotic 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.