2. LITERATURE REVIEW

Plants maintain a complex relationship with various environmental factors and any changes in these abiotic (high and low temperatures, salinity, drought and flooding, change in pH, strong light, UV and heavy metals) and biotic (pest, insect and disease effects) factors adversely affect plant growth and development (Shao *et al.* 2008, Soussana *et al.* 2010). Agricultural sector is one of the vulnerable sectors, facing detrimental impact of environmental stresses. Unfavourable environmental conditions have adverse effect on crop production, which is likely to create problems for food security, particularly in tropical regions. Boyer in the early 1980s predicted that as much as 70% of crop productivity may be restricted by various environmental factors (Boyer, 1982). Bray *et al.* (2000) further substantiated that the average yield loss in cereal crops like wheat, barley and maize due to abiotic stresses to be about 70-80% of the total yield. In changing climate scenario constantly rising temperature is one of the most severe form abiotic stresses affecting the production of crop plants worldwide. Global average temperature is predicted to rise by 1.8 to 4 ^{0}C by the end of this century and will have destructive and harmful consequences on organisms, environment, agricultural productivity and food security (Swaminathan and Kesavan, 2012).

Wheat (*Triticum aestivum* L.), a member of family Poaceae is the main staple food for people from different parts of world and fulfilling the calories demands of growing population. It is a good source of nutrition with 12.1% protein, 1.8% lipids, 1.8% ash, 2.0% reducing sugars, 6.7% pentosans, 59.2% starch, 70% total carbohydrates and provides 314 K cal/100 g of food. Wheat also contains minerals and vitamins like, calcium (37 mg/100 g), iron (4.1 mg/100 g), thiamine (0.45 mg/100 g), riboflavin (0.13 mg/100 g) and nicotinic acid (5.4 mg/100 mg). Worldwide climate change predicted to have negative impact for wheat production. Rapid acceleration in population is only adding to this problem. According to 2015 UN projection world's population is predicted to attain a staggering 9.7 billion by 2050 and to fulfil demands of this rising world population 60% increase in wheat production is needed by 2020 (Edgerton, 2009). On the other hand rise in earth mean average temperature upto 3^{0}C by the end of this century (Swaminathan and Kesavan, 2012) will have destructive impacts and harmful consequences on people, environment and agricultural productivity. With each degree of temperature increase wheat production is assessed to fall by 6% for each

worldwide (Gourdji *et al.* 2013). India is the third leading producer of wheat following the European Union and China. Ideal temperature for wheat cultivation is between 15^0C to 25^0C. In India, wheat is generally sown in the month of November, December and harvested in between March to April with slightly elevated temperatures during grain filling and harvesting. Climate change had a greater impact on nationwide wheat production in India, especially in northern, western and eastern part of India with maximum temperatures crossing beyond physiologically decisive thresholds of around 38^0C to 40 °C (Koehler *et al.* 2013). A decade ago, 40˚C during March was unusual in the Indo gangetic plains. Now such temperatures occur often even before March 30, on average, around one week earlier than normal affects wheat growth and yield in different parts of India.

2.1. Effect of high temperature on plant development and yield

High temperature causes multifarious modifications which are often unfavourable for plant growth, development, physiological processes, and yield (Iqbal *et al.* 2017). Elevated temperature mainly restrains growth and yield of plants growing in tropics. Heat stress hinders various stages of plant development. Morphological signs of high temperature during early development include germination inhibition to sunburn, scorching, wilting of leaves and twigs at seedling stage and abnormalities in floral part, fruit discoloration at later stage of development followed by reduced yield at post harvest period (Vollenweider and Günthardt-Goerg, 2005). Omae *et al.* in 2012 reported wilting and drying of leaves especially leaf margins, leaf tip area and necrosis in sugarcane due to heat stress. Essemine *et al*. 2010 reported high temperature as high as 45°C accelerated cell death and embryo development which can inhibit wheat germination and reduce seedling establishment rate. Heat stress significantly lessened relative growth rate, shoot dry mass and net assimilation rate in maize, sugarcane and pearl millet (Ashraaf and Hafeez, 2004; Wahid, 2007). Long exposure to high temperature decreased seed germination rate and radical plumule growth of germinating seedlings leading to poor seedling vigour in various cultivated crops (Kumar *et al*. 2011). Koini *et al.* (2009) reported plant-water relations, and shoot growth and extension morphophysiological characteristics such as phenology, partitioning, are seriously hampered by heat stress in common bean (*Phaseolus vulgaris*). Plant height, number of tillers, chlorophyll content and total biomass were reduced in rice and mung

bean cultivars in response to high temperature (Mitra and Bhatia, 2008; Kumar *et al.* 2011). In wheat green leaf area and productive tillers/plant were drastically reduced under heat stress (30/25°C, day/night) (Dias and Lidon, 2009). High temperature during reproduction hampers fertilization process in a variety of crops. Even a short exposure to elevated temperature led to significant decrease in floral buds and flowers abortion and hampered meiosis in both male and female organs, impaired pollen and stigma development consequenced anomalous fertilization processes and unfertilized embryo (Foolad, 2005; Cao *et al.* 2008). High temperature during grain filling and seed hardening stage adversely affects crop quality and yield (Wahid *et al*. 2007; Essemine *et al.* 2010). Even a small (2°C) rise in temperature influence the grain quality and yield predominantly through affecting phenological development processes in rice (Wu *et al.* 2016). Many researchers documented yield loss in various cash crops including cereals (e.g., wheat, rice, sorghum, barley, maize), oil yielding crops (mustard, canola) and pulse (e.g., chickpea, cowpea) due to high temperature (Hasanuzzaman *et al.* 2013). It is well documented wheat, cultivated during winter season in India is highly affected by heat stress. Every 1°C rise in average temperature over 17°C to 24°C during grain filling resulted in above 4% to 10% reduction in grain yield (Acevedo *et al.* 1991;Wang *et al.* 2012). Temperature over these ranges (28 to 32°C) even for short periods cause more than 20% yield losses in wheat. Heat stress changed the early dough and maturity stage, shorten the kernel desiccation period and cause grain yield loss in wheat (Sohail *et al.* 2014). High temperature over 35–40°C the reduced grain weight by 7.0%–7.9% , grain length and width by 2% and increased spikelet sterility (61%) in heat sensitive rice cultivars (Mohammed and Tarplay, 2010). In maize and sorghum, seed weight and seed size were reduced over 50% due to heat stress (Suwa *et al*. 2010; Hasanuzzaman *et al.* 2013). Heat also reduced the single kernel weight causing yield loss canola and maize (Kutcher *et al.* 2010; Sinsawat *et al.* 2004). In barley grain continuous heat stress increased concentration of maltose and several proteinogenic amino acids, whereas the total non-structural starch, carbohydrates, raffinose and fructose, lipids content decreased due to high temperature (Högy *et al.* 2013). Loss of productivity in heat stress is chiefly related to which is due to reduced photosynthesis, altered membrane stability, assimilatory capacity reduction and enhanced maintenance respiration costs reduction in radiation use efficiency (Cicchino *et al.* 2010; Zhang *et al.* 2013). Reduction in photosynthetic rate, impaied assimilates translocation process and reduced carbon gain

ultimately end in distorted growth and abnormal reproduction which ultimately declined yield and heat stress affected plants produce of inferior quality of cereals (Vacca *et al.* 2004, Iqbal *et al.* 2017). Thus it is very necessary to be acquainted with about various physiological and biochemical alterations which ultimately hinder plants growth and developmental process of various plants at high temperature.

2.2. Effect of high temperature on biochemical and physiological changes

High temperature often related to reduced water availability and tries to impair the tendency of maintaining cell turgidity of tissue by upsetting water balance with tissue (Mazorra *et al*. 2002; Simoes-Araujo *et al.* 2003). In tomato plants hydraulic conductivity and water relation is perturbed by elevated temperature (Morales *et al.* 2003). Similarly in sugarcane in spite of sufficient water supply and optimal relative humidity condition leaf water potential and root hydraulic conductivity was severely affected by high temperature (Wahid and Close, 2007). Less availability of water during various abiotic stresses induces the production of different kinds of ROS (Reactive oxygen species) including both free radicals such as hydroxyl radicals (.OH), superoxide ($O_2^{\cdot-}$), alkoxy radicals (RO.) and perhydroxy radical (HO_2.) and non-radical forms, that is, singlet oxygen ($^1O_2$), and hydrogen peroxide (H_2O_2) (Foyer, 2005; Hasanuzzaman *et al.* 2012). Production of excess ROS due to heat stress leads to oxidative stress in plants (Hasanuzzaman *et al*. 2012). High temperature causes inactivation of enzymes in chloroplast and mitochondria, inhibition of protein synthesis, protein denaturation and aggregation, and increased fluidity of membrane lipids and ROS production causing membrane injury and even cell death (Howarth, 2005). Membrane dynamics is very sensitive to high temperature. Heat damages secondary and tertiary structure of membrane proteins and increases electrolyte leakage and reduces membrane integrity due to ROS accumulation (Wahid *et al.* 2007). Therefore cell membrane thermo stability has long been utilized as indirect measure of thermo tolerance in many crops, like cotton, soyabean, barley, wheat (Foolad, 2005; Zhang *et al.* 2013). In wheat heat exposure for two days resulted root growth inhibition which was correlated with powerful oxidative stress as evidenced by a significant increase (68%) of $O_2^{\bullet-}$ production in root cells. The MDA content also increased by 27% after exposure at the early stages of seedling development, and this trend also continued during the later stages of development (Camejo *et al.* 2005). Effect of heat stress on

membrane stability depends on various plants species, plant developmental state and seed hardening stage. High temperature induced ROS accumulation and membrane electrolyte leakage has been reported in a variety of crops including sorghum, mungbean, tomato, potato wheat, cotton, barley, rice etc. (Wahid & Shabbir, 2005; Dias and Lidon, 2009; Egorova *et al.* 2011; Kumar *et al.* 2011; Mohammed & Tarpley, 2010; Kaushal *et al*. 2016). ROS produced due to heat stress have tremendous negative impact on plant physiological and metabolic processes. However they have also assumed to act as signalling molecules to trigger the heat shock responses in and develop of heat tolerance in plants (Asada, 2006; Kaushal *et al.* 2016)

2.3. Effect of heat stress on photosynthesis

Photosynthesis is one of the most temperature sensitive physiological processes and any variation in photosynthesis can hamper plant development and thus any changes in photosynthetic attributes can be a good marker for thermo tolerance as it directly correlated with plant growth (Crafts-Brandner *et al*. 2002). Exposure to high temperature reduces leaf water potential, leaf area and pre-mature leaf senescence during heat stress have negative impacts on photosynthetic performance of plants (Greer and Weedon, 2012). In chloroplast, thylakoid lamellae and stroma are the principal sites of heat injury (Wang *et al.* 2009). During heat stress ROS is generated in Photosystem I and II of chloroplast and in other organelles such as peroxisomes and mitochondria. ROS production increased electron leakage from the thylakoid membrane consequencing reduction of PSII activity located in thylakoid membrane (Bavita *et al.* 2012). High leaf temperature persuades photon flux density and regulates thermotolerance of plants through adjustment of PSII (Marchand *et al.* 2005). High temperature dissociates oxygen evolvivg complex, manganese (MN)- stabilizing 33 k Da protein in PSII resulting in release of MN atom disrupting electron flow from oxygen evolving complex towards PSII and PSI untimately reduces photosynthetic efficiency of plants (De Ronde, 2004 Hasanuzzaman *et al.* 2013). Other parts of reaction centres such as D1 and D2 protein are also get affected by heat. In wheat and barley high temperarure and light damaged PSII which sequentially inferred various recovery pathways and block oxygen evolution (Toth *et al.* 2005). Adverse effect of heat on photosynthetic apparatus seems to be associated with reactive oxygen species (Camejo *et al.* 2006; Gou *et al.* 2015). Lipid peroxidation in thylakoid membranes due

to heat stress is also a cause of chlorophyll reduction in sorghum (Djanaguiraman *et al.* 2010). Greer and Weedon (2012) observed that average rates of photosynthesis of *Vitis vinifera* leaves reduced significantly when temperature increased from 25°C to 45°C. Reduction in stomatal conductance and chlorophyll fluroscence (Fv/Fm) ratio was also observed in rice (Yin *et al.* 2010). Heat stress causes degradation of chlorophyll a and b in developing and developed leaves (Wahid *et al.* 2007). According to Camejo *et al.* (2005) decreased chl:carotenoid ratio and increased chlorophyll a:b ratio in tomato and sugar cane plants under high temperature stress attributed themotolerance. Usually C3 plants are more vulnerable to high temperature stress than C4 plants (Yang *et al.* 2006, Wahid *et al.* 2007). High temperature alters Rubicase activity and affects RUBP generatiion (Craft Brandner, 2002). Exposure to high temperature for prolong period decreases, soluble proteins like, Rubisco binding proteins (RBP), large-subunits (LS), and small-subunits (SS) of Rubisco in darkness, photosynthetic pigments and all these together reduces stomatal conductance and net photosynthetic rate (Salvucci and Craft-Brandner, 2004; Sumesh *et al.* 2008). Heat stress significantly increased electrolyte leakage, membrane peroxidation and reduced the membrane thermostability by 28% and 54% in wheat (Savicka *et al.* 2010). Oxidative stress marker, H_2O_2 level increased significantly in perennial ryegrass (*Lolium perenne* L.) when exposed to moderate (36°C) and severe heat stress (40°C) and it was responsible for physiological damage of PS II, cell membrane stability and caused lipid peroxidation (Soliman *et al.* 2011).

2.4. Molecular and antioxidative changes in response to high temperature

In order to annul detrimental effects of heat stress, plants are continuously modifying biochemical and physiological processes which include scavenging of ROS, antioxidants production, compatible osmolyte accumulation, mitogen-activated protein kinase (MAPK), calcium-dependent protein kinase (CDPK) cascades and last but not the least chaperone signalling and transcriptional activation. All these mechanisms at the molecular level together enable plants to thrive under heat stress. Heat stress effects are notable at various levels, including plasma membrane and biochemical pathways operative in the cytosol or cytoplasmic organelles. Prelimarily heat stress affects plasma membrane which shows extra fluidity of lipid bilayer under stress. This leads to the initiation of Ca^{2+} influx and cytoskeletal reorganization, resulting in the upregulation of mitogen activated protein kinases (MAPK) and calcium dependent protein kinase

(CDPK). Initiation of signalling cascades at nuclear level induce production of compatible osmolytes and antioxidants redox and osmotic adjustment (Sung *et al.* 2003).

High temperature generates different types of ROS in plant tissues, which includes superoxide radical (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-) are (Liu and Huang, 2005). Production of ROS in chloroplast and mitochondria is of great importance for signalling as well as production of antioxidants (Bohnert *et al.* 2006). These ROS cause the autocatalytic peroxidation of pigments and membrane lipids thus increase loss of membrane permeability and modifying its functions (Xu *et al.* 2006). Superoxide radical is the first ROS to be synthesized in the chloroplast and mitochondrion during heat stress. Superoxide dismutase (SOD) scavenges of O^{2-} which results in the production of H_2O_2. Ascorbate peroxidase and glutathione reductase are part of ascorbate glutathione cycle facilitates H_2O_2 scavenging. APX is the key enzyme in which detoxifies hydrogen peroxide from chloroplasts and cytosol of higher plants using ascorbate as a substrate. Ascorbic acid being a part of is ascorbate glutathione cycle is effective in scavenging superoxide (O^{2-}), hydroxyl (OH') radicals and singlet oxygen and removes H_2O_2. Dehydroascorbate reductase helps to maintain higher ascorbic acid pool in are in the ascorbate-glutathione cycle (Allakhverdiev *et al.* 2008). Either ascorbate peroxidase (APX) or catalase (CAT) converted H_2O_2 to H_2O and oxygen (Sairam and Tyagi, 2004). Activities of different these antioxidant enzymes enhance with increasing temperature are believed to be correlated with better thermotolerance. Balla *et al.* (2009) reported the significance of the antioxidant enzyme system in thermotolerance. Hasanuzzaman *et al.* (2012) observed heat treatment at 38 °C for 24 h and 48 h increased activity of the antioxidant enzymes—APX, GR, GPX and GST activities in *T. aestivum* seedlings. Thus thermotolerance of the wheat cultivars appeared to be correlated with the higher antioxidant level. The activity of the enzymes such as, glutathione S-transferase (GST), APX and CAT was more in thermotolerant cultivars. Edreira *et al*. (2012) observed expression of maximal increase in CAT, APX and GR activity in *Z. mays* plants compared to *O. sativa* plants while no disparities existed for superoxide dismutase at 45/40 °C. In addition, considerably better levels of non-enzymatic antioxidants like acorbic acid and total glutathione were maintained at 45/40 °C in maize than in *O. sativa* cultivars. Kumar *et al.* (2012) observed that the activities of SOD, POX, CAT, APX and GR increased under heat stress (45.0±0.5 °C)

in two of *B. juncea* genotypes but the increase was significantly high in tolerant genotype NPJ-119 as compared to susceptible genotype. The basal level of all antioxidative enzymes apart from CAT was found more in tolerant genotype. After recovery, SOD and CAT started decreasing but activity of POX and GR remained much higher in both the genotypes however, APX showed inconsistency in behavior post heat stress recovery. APX activity remained very high in tolerant genotype and started decreasing in susceptible genotype during recovery period. Chakraborty and Pradhan (2011) observed that ascorbate peroxidase (APX,) catalase (CAT), and superoxide dismutase (SOD) increased initially before declining at 50°C, while peroxidase (POX) and glutathione reductase (GR) activities turn downed at all temperatures ranging from 20 to 50°C in soybean. Antioxidant metabolites like acorbic acid (AsA), total glutathione (GSH), tocopherol and carotene also protect plants against various abiotic stresses (Sairam *et al.* 2000). Exposure to high temperature increased GSH content and activity of the enzymes involved in GSH synthesis in wheat (Kocsy *et al.* 2002). Enhanced synthesis of AsA and GSH lower of ROS production in turf grass under heat stress (Xu *et al.* 2006). Harsh *et al.* (2016) reported short term heat treatment at 42^0C significantly increased CAT and SOD activity in moth bean (*Vigna aconitifolia*).

2.5. Heat shock proteins

High temperature induces transcription and translation of a new set of heat inducible genes; known as "heat shock genes" (HSGs) which encode heat shock proteins (HSP) which are extremely heterogeneous and dynamic in nature. In plants, based on their approximate molecular weight, well-characterized HSPs can be classifed into five different families: HSP100 (or ClpB), HSP90, HSP70 (or DnaK), HSP60 (or GroE) and small HSPs (15-30 kDa) (Swindell *et al.* 2007; Wang *et al.* 2014). Small HSPs family has inimitable importance in plants stress tolerance due to their unusual abundance and diversity. Constitutive expressions of most of these proteins provide stability, protect other functional intracellular proteins from denaturation through protein folding and thus act as chaperones (Baniwal *et al.* 2004). Heat stress tolerance is pretty much dependent upon induction of heat shock proteins like, HSP90, HSP101, HSP60 and HSP70 (Young *et al.* 2001). The HSP60 and HSP70 are amongst the most highly conserved proteins in nature, consistent with an elementary role in response to high temperature and active products of these genes are very much required for plant's

survival under lethal heat stress (Chang *et al.* 2007). HSP70 takes parts in ATP-dependent protein assembly/disassembly reactions and it checks protein denaturation during heat stress (Iba, 2002). High temperature reduced chloroplastic HSPs accumulation in bentgrass (Wang and Luthe, 2003). Conserved heat shock elements (HSEs) binds to heat shock genes and triggering transcription of HSPs in response to heat stress. These heat shock cis-acting elements having 5'-AGAANNTTCT-3'palindromic sequence give recognizable binding site for heat shock transcription factors. Khurana *et al.* (2013) mentioned probable role of imperfect CCAAT-box element or some novel cis-element of sHsp 26 promoter with respect to high temperature stress. In most cases plants have been shown to have numerous copies of these genes. For example at least 17 and 21 copies of HSF genes have been reported to be present in tomato and *Arabidopsis* plants respectively. These genes have been grouped into three classes (classes A, B and C) depending on variation of flexible linkers and oligomerization domains. Usually HSFs over expression can increase thermo-tolerance of plants, however expression of these HSFs have hardly any effect on plant survival. In plants, there are a number of non-HSP transcripts that are upregulated by heat (Hasanuzzaman *et al.* 2013). Over expression of these HSFs are linked with a number of other genes. Recent findings suggested that the *Arabidopsis* cytosolic ascorbate peroxidase gene contain a functional heat shock element (HSE) in its 5'-promoter region and has been shown only to be heat up regulated HSE. HSFA1a and HSFA1b up regulation control early response of many heat inducible genes along with other HSFs. Interestingly, in tomato, one particular HSFA1 has been suggested to be the 'master regulator' of the heat shock reaction. Down regulation of HSFA1 decreased HSP production making plants more sensitive to heat stress (Mishra *et al.* 2002). These heat shock regulatory proteins come into the nucleus and form a trimer that can bind with the heat shock elements (Sun *et al*. 2002). Heat shock factor binding with other transcriptional components, resulted in gene expression within minutes under heat stress. Over expression HSF gene turned on almost all HSGs and consequently provides protection against heat stress. Although this basic system is universal to eukaryotic cells, it is highly complicated in plants and appear to have a remarkable ability to finely control the expression of heat induced genes through the HSF system and studies also suggested that there is a positive link between the HSP level within cells and heat stress tolerance (Miroshnichenko *et al.* 2005). Li *et al.* (2013) successfully identified a total 81

differentially expressed proteins by MALDI-TOF/TOF. Out of which HSP 90, HSP 70 and sHSPs (HSP 17, HSP 18.2, HSP 20 and two HSP 23) were the most ubiquitous among HSPs subgroup and ascorbate peroxidase, glucan endo-1,3-β-glucosidase, ubiquitin, thaumatin-like protein, dehydroascorbate reductase, 20 kDa chaperonin, germin-like protein, mitochondrial peroxiredoxin were also found to be expressed during heat stress in Alfalfa (*Medicago sativa L*. cv. Huaiyin). Goswami *et al.* (2014) reported heat-responsive miRNAs regulate expression of transcription factors (HSFs) and majority of the heat stress-associated genes (HSPs). Expression profiling showed over expression of HSF3, HSFA4a, HSP17, HSP70, SOD and CAT genes in HD2985 and NIAW-34-34 wheat cultivars under heat stress (42°C, 2 h).

2.6. Accumulation of osmolyes in response to heat stress

Compatible osmolyte accumulation is one of the crucial adaptive mechanisms in plants grown under abiotic stresses conditions, including salinity, osmotic stress and high temperatures. Accumulation of osmolytes such as proline, sugars, polyamines and sugar alcohols contribute to enhanced stress tolerance of plants (Sairam and Tyagi, 2004).

Proline is an amino acid which accumulates in large quantities in response to various environmental stresses. Accumulation of proline decreases water potential of plant cells and thus accumulated water during any kind of abiotic stress, especially in drought and salinity stress (Kishore *et al.* 2005). Proline enhances the stability of protein and membrane under high temperature or osmotic stress. Similarly, accumulation of soluble sugars under heat stress has been reported in sugarcane, which entails great implications for heat tolerance (Wahid and Close, 2007). Under high temperatures, fruit set in tomato plants failed because of the disturbance in sugar metabolism and proline transport during the narrow window of male reproductive development (Sato *et al.* 2006). Proline acts as free radical scavenger, cell redox balancer, cytosolic pH buffer, and stabilizer for subcellular structures during various stresses, especially osmotic and salt stresses (Kishor *et al.* 2005; Verbruggen and Hermans, 2008; Székely *et al.* 2008). Recently, many worker reported proline accumulation may also play regulatory roles throughout plant growth and flowering (Maggio *et al*. 2002; Mattioli *et al.* 2008). However, the precise physiological role of proline during high temperature is still unclear, and several researchers have hypothesized beneficial functions of various genes involved in the process of proline metabolism rather than to the proline accumulation. The alterations of

proline and pyrroline-5-carboxylate (P5C) in different cellular compartments perhaps involved in regulation metabolic signalling and intracellular redox potential in higher plants during various abiotic stresses (Miller *et al.* 2009). However, there are only a few reports of proline accumulation during high temperature stress. In *Arabidopsis*, proline accumulation was not detected during heat stress (Yoshiba *et al.* 1995). There was insignificant increase in proline content in of barley (*Hordeum vulgare*) and radish (*Raphanus sativus*) leaves exposed to 41°C and proline content showed a slight increase under heat stress (Chu *et al.* 1994). In chickpea (*Cicer arietinum*), heat treatment increased in proline content marginally as compared to control (Chakraborty and Tongden, 2005).

Glycine betaine, an amphoteric quaternary amine, plays an important role as a compatible solute in plants under various stresses, such as salinity or high temperature (Sakamoto and Murata, 2002). Accumulation of glycine betaine due to desiccating high temperature or of osmotic stress was reported in maize (Quan *et al.* 2004) and sugarcane (Wahid and Close, 2007). Levels of accumulated glycine betaine are generally associated with the degree of stress tolerance, and differ greatly among from species to species. Glycine betaine protects of the thylakoid membrane in chloroplast by ROS scavenging thus plays a very important role in the osmotic adjustment and, maintain photosynthetic efficiency (Chen and Murata, 2011; Wang *et al.* 2008). Glycine betaine accumulation in tomato induced tolerance against high temperature (Li *et al.* 2011). Among other osmolytes, trehalose, mannitol and non-protein amino acid -4-aminobutyric acid (GABA) was also reported to be accumulated during various abiotic stress conditions which is consistent with its physiological role in the mitigation of stress. (Kinnersley and Turano, 2000).

2.7. Accumulation of secondary metabolite in response to heat stress

Most of the secondary metabolites are synthesized via phenylpropanoid, methyl erythritol phosphate (MEP), shikimate, or mevalonate pathways (Wahid and Ghazanfar, 2006). Heat stress induces phenolic accumulation such as flavonoids and phenylpropanoids. Phenylalanine ammonia-lyase (PAL) is regarded as the key enzyme of the phenylpropanoid pathway. Increased activity of PAL during high temperature stress is believed to be main acclamatory response within plant cells. Studies suggested that plants subjected to high temperature accumulate soluble phenolics alongwith

increase phenyl ammonia lyase (PAL) and decreased peroxidase and polyphenol lyase activities. Increased accumulation of phenolics in watermelon (*Citrulus vulgaris*) was observed during heat stress (Rivero *et al.* 2001). Carotenoids are extensively known to guard cellular structures in various plant species irrespective of the stress conditions (Wahid and Ghazanfar, 2006; Wahid, 2007). Many components of the xanthophyll cycle - carotenoids, violaxanthin and zeaxanthin play crucial role in photoprotection. Zeaxanthin, mainly situated at the edge of the light harvesting complexes, where it functions to avert ROS triggered peroxidative damage to the membrane lipids. Recent studies have revealed that carotenoids and some terpenoids, such as isoprene or -tocopherol, stabilize and photoprotect the lipid phase of the thylakoid membranes (Velikova *et al.* 2005). Phenolics, flavonoids, anthocyanins and lignins, etc., are the most vital group of secondary metabolites in plants and participate in a variety of roles including heat and abiotic stress tolerance (Chalker-Scott, 2002; Wahid and Ghazanfar, 2006; Wahid, 2007)

2.8. PGPR and growth promotion in wheat

PGPR can mediate growth promotion by a plethora of mechanisms which includes, production of growth hormones like IAA, gibberellic acid, cytokinins ; increase mobilization and availability of nutrients, biological nitrogen fixation providing protection to plants from diseases by producing antibiotics, siderophores, hydrogen cyanide and improving the tolerance to abiotic and biotic stresses like salinity, drought, heat etc. PGPR reduce endogenous ethylene levels in plants by production of the enzyme 1-aminocyclopropane- 1-caroxylate (ACC) deaminase (Medeiros *et al.* 2005; Glick, 2012).

Several PGPR inoculants have been used for plant growth promotion in wheat and others plants for their the active and beneficial on growth and yield at different environment under variable ecological conditions (Ozturk *et al.* 2003; Marques *et al.* 2010; Zhang *et al.* 2012). Significant increase in number of tillers per plant (10–21%), root weight (19–43%), grain yield (15–43%) and straw yield 22–39% was observed in wheat plants inoculated with *Pseudomonas fluorescens* (Shaharoona *et al.* 2008). Narula *et al.* (2005) reported application of PGPR strain *Azotobacter* in field saved 25–30 kg N ha^{-1} chemical fertilizer. Three PGR strains, *Bacillus megaterium* BHU1, *Arthrobacter chlorophenolicus* BHU3 and *Enterobacter* sp BHU5 showed maximum nutrient

acquisition and content of micronutrient viz. Fe, Cu, Mn and Zn in wheat grains and also increased plant growth and vigour thus used as efficient microbial consortium for wheat production. Triple combination of strains *B. megaterium*, *A. chlorophenolicus*, and *Enterobacter* significantly increased plant height, straw yield, grain yield and test weight under pot condition as well as under field condition, respectively (Kumar *et al.* 2014).

Turan *et al.* (2013) reported application PGPR (*Bacillus megaterium* M3, *Bacillus subtilis* OSU142, *Azospirillum brasilense* Sp245 and *Raoultella terrigena*) increased root and shoot dry weight in wheat and barley plants and protected the plants from ice nucleation. PGPR priming alleviated deleterious effects of low-temperature in both plants species tested. The lowest ROS and antioxidant enzyme (SOD, POD, CAT) of wheat and barley were observed *Raoultella terrigena* primed plants. Wheat plants inoculation with (PGPR) strains *Acetobacter pasteurianus* and *Stenotrophomonas* specie provided a significant increase in shoot and root length, and shoot and root biomass. Shoot and root nitrogen content significantly increased PGPR primed plants over the un-inoculated control (Majeed *et al.* 2015). *Pseudomonas moraviensis* and *Bacillus cereus*, isolated from rhizosphere soil of halophytic weed (*Cenchrus ciliaris* L.) when applied to wheat (*Triticum aestivum* L.) by seeds soaking increased the fresh weight, proline contents and activities of antioxidant enzymes significantly over control. Added tryptophan with both PGPR, improved the yield by improving number of seeds/spike and spike length. Effects of PGPR inoculation alone and with tryptophan were more pronounced in pots grown plants (Hassan and Bano, 2015).

2.9. Amelioration of high temperature stress by PGPR

Plants do not live in isolation but constantly interact with an array of microorganisms in the soil and in the atmosphere. This interaction is again affected by various environmental factors, and when the environmental conditions become unfavourable they impose different stresses on the plant. With increasing urbanization and population leading to adverse conditions such as extremes of temperatures, water deficit, salinity, increase of heavy metal pollutants in the soil ways and means are now being sought to make plants more resilient. Use of beneficial microorganisms having various attributes not only for plant growth promotion and disease reduction, but also for mitigation of abiotic stresses is now being considered. Plant growth promoting rhizobacteria (PGPR),

are being evaluated for such multiple uses in sustainable agriculture. All of these mainly act by mechanisms which reduce the effects such as oxidative stress or cellular metabolic disruptions brought about by different stresses.

Abiotic stress management is one of the greatest challenges of agricultural scientists and needs careful consideration. Various strategies to manage abiotic stresses consist of shifting the crop calendars, development of heat and drought tolerant varieties, resource management practices etc. (Venkateswarlu and Shanker, 2009). Development of stress tolerant plants (STPs) is another option being considered and this includes manipulation of stress associated genes and proteins for over expression of metabolites, production of transgenics or conventional plant breeding combined with the use of molecular markers and QTLs.

However, though some of these technologies have shown promise, they are cost-intensive and laboratory oriented. Hence these technologies may not be the solution for the common farmers. In this scenario, recent studies indicate that there is another strategy which has high potential to alleviate stresses in plants, is highly eco-friendly and cost effective. It involves the utilization of multi-faceted traits of several beneficial microorganisms which have been known earlier for their role in plant growth promotion, nutrient management and disease control. Several reports on the use of microorganisms, especially plant growth promoting rhizobacteria (PGPR) for induction of tolerance against abiotic stresses have been forthcoming in the last 2-3 decades. Interestingly, researches in several laboratories have now confirmed that several of these PGPR can also help the plant in withstanding against abiotic stresses (Bashan and de Bashan, 2010). Reports are now accumulating on application of PGPB as elicitors for tolerance to abiotic stresses, such as drought, salt and nutrient deficiency in plants and raising possibility for incorporation of microbial genes into plant and diverse microbial species (Chakraborty *et al*. 2015). The beneficial plant microbial interactions are very frequent in nature, where PGPB help the plants to overcome various stresses. Besides bacteria, beneficial fungi such as endophytes or mycorrhizal fungi also confer tolerance to plants against different abiotic stresses. Microbial communities offer a potentially powerful opportunity for understanding these beneficial interactions. Consequently, changes in the structure or function of microbial communities may have a major impact on ecosystem activities (Khan *et al.* 2011). Yang *et al.* (2009) suggested the term ‘induced systemic tolerance’ (IST) for PGPR-mediated physiological and chemical

alterations within plants that improved tolerance to abiotic stress. Temperature affects different plants differently depending on the optimal requirements of plants. Plants growing in colder climates, when exposed to higher temperatures will suffer from elevated temperature stress whereas the same temperature would be optimal for those growing in warmer climates. Similar is the case with tropical plants which when exposed to colder temperatures suffer from cold stress or in colder conditions, to freezing. With global warming and other related phenomena, earth is now witnessing several extreme temperature conditions varying from very high to very low and is now a major concern in agriculture. Water scarcity also leads to temperature increase. Microorganisms also have optimal temperature conditions for growth and hence, the ability of rhizospheric bacteria to alleviate temperature stress will also vary.

It is clear that temperature and soil type may affect the performance of plant-beneficial bacteria interaction (Egamberdiyeva and Hoflich, 2003). Those bacteria which can themselves grow better at higher / lower temperatures could be of use for application in agriculture under elevated/ low temperature conditions. In addition, bacteria colonizing distinct sites may react differently to different environmental conditions. In studies using several rhizospheric bacteria, it was observed that many bacteria such as *Mycobacterium* sp. 44, a *Pseudomonas fluorescens* and a *Pantoea agglomerans* strain isolated from a semi-continental climate were found to considerably enhance the plant growth of winter wheat at 16^0C compared with that at 26^0C in loamy sand (Compant *et al*. 2010). On the other hand, bacteria isolated from semi arid climate such as *Mycobacterium phlei* strain MbP18 as well as *Mycoplana bullata* MpB46 were not affected by temperature change, indicating genotype-specific preferences for certain environmental conditions. A study with the endophyte *Burkholderia phytofirmans* strain PsJN demonstrated that a temperature increase from 10 to 30^0C reduced the colonization of this strain in the tomato rhizosphere, without having an effect on endophytic abundance (Pillay and Nowak 1997). After successful colonization, rhizosphere as well as endophytic bacteria may alleviate temperature by inducing a systemic response (Yang *et al*. 2009).

Srivastava *et al.* (2008) also isolated a thermotolerant *P. putida* strain NBR10987 from drought stressed rhizosphere of chickpea. The thermotolerance of the strain was attributed to the over expression of stress sigma factor and enhanced biofilm formation at high temperatures. The ability of a thermo-tolerant strain of *Pseudomonas* AKM-

P6 was used to alleviate the heat stress in sorghum seedlings (Ali *et al.* 2009). They reported that inoculation induced the biosynthesis of high molecular weight proteins in leaves under elevated temperature, reduced membrane injury, and improved the level of cellular metabolites like proline, chlorophyll, sugars, amino acids, and proteins. Analysis of proteins from inoculated and uninoculated sorghum seedlings exposed to ambient and elevated temperature revealed the presence of three additional polypeptides in the seedlings exposed to elevated temperature, indicating a possible role of inducible proteins in microbial mediated heat tolerance mechanism.

Ashraf and Foolad (2007) reported that *Pseudomonas putida* strain AKMP7 increases themotolerance of wheat plants by stimulating accumulation of proline, which binds to cell membrane, maintain membrane permeability and direct osmotic balance within tissue. Certain oligosaccharides belonging to raffinose oligosaccharide family, galactinol and stachyose as well as sugars, such as trehalose, glucose and sucrose, are known to be associated with responses to environmental stress (Kaplan *et al*. 2007). The over expression of galactinol synthase gene (GolS), a heat shock factor in *Arabidopsis* under abiotic stress and steady state expression of trehalose phosphate synthase 5 (TPS5) gene in wheat plants (cv.Olivin) after *Bacillus amyloliquefaciens* 5113 treatment clearly indicating their probable contributioto overall fitness of plant againt abiotic stress. Likewise, *P. putida* strain AKMP7 neutrializes negative impact of heat stress on wheat and increase yield, spike length and count, grain size and quality. In another study, wheat seedlings (cv. Olivin and Sids1) treated with two PGPR strains *Bacillus amyloliquefaciens* 5113 and *Azospirillum brasilense* NO40, transcript expression level of several heat response transcription regulator homologs (HsfB1, HsfA3, MBF1c), HSP17.8 , which maintain protein conformation under stressed condition, MSFB1 remained unaffected unlike untreated heat stressed seedlings(Abd El-Daim *et al*. 2014). RT PCR expression analysis of heat stressed bacteria untreated and treated wheat plants using APX1 (forward, 5′-GGAGGCTTCCTGATGCTG-3′ reverse, 5′-CGGCGTAG TCCTTGAAGAAT-3′; AF387739.1) and TmSAMS1 (forward, 5′-GACCCAGGTGACTGTGGAGT-3′ reverse, 5′-AGGCACGCCAATAGCATAAG-3′; EU399630.1) gene specific primer showed reduced expression of these two genes in bacterial primed plants. In addition higher basal levels of other enzymes of ascorbate-glutathione pathway DHAR, MDHAR and GR were observed in bacteria primed plants. Similarly, *P. putida* strain AKMP7 inoculated wheat plants had reduced ROS

production pointing towards preventive measure against ROS production and avoiding expensive adaptations tied to ROS detoxification mechanism (Ali *et al*. 2011).

It is thus obvious that such beneficial bacterial strains have the potential to be used in agriculture under temperature stressed conditions in the era of global warming and changing environmental scenario.

Several plants including maize, soybean, cotton, banana etc are sensitive to temperatures below 10–15 °C and exhibit signs of injury. Frost injury is common in many plants and they exhibit different phenotypic symptoms in response to chilling stress which include reduced leaf expansion, wilting, chlorosis and may lead to necrosis and also reproductive development (Mahajan and Tuteja 2005). Several workers have also reported the ability of cold-tolerant bacteria to induce cold tolerance in plants (Chang *et al.* 2007; Selvakumar *et al.* 2008; Mishra *et al.* 2009). Selvakumar *et al*. (2008; 2012) reported role of novel cold tolerant plant growth promoting bacterial species viz., *Pantoea dispera, Serratia marcescens, Pseudomonas fragi, Exiguobacterium acetylicum amd Pseudomonas lurida* in promoting plant growth at cold temperatures. They attributed the observed effects mainly due to auxin production and phosphate solubilization by bacterial species. Under low temperature, such bacteria can sustain their metabolic processes and aid in plant growth promotion. Since ice nucleation has been recognized as a cause of frost damage of plants, recent development in the use of microbial technology is the identification of naturally occurring phyllospheric bacteria with low ice nucleating activity, so that they can be sprayed on leaves to overcome frost damage (Selvakumar *et al.* 2012).

In order to determine the effect of a PGPR on growth and physiological activity of grapevines at low temperature, *in vitro* inoculation of *Vitis vinifera* cv. Chardonnay explants with *Burkholderia phytofirmans* strain PsJN, was carried out. Bacterization enhanced both growth and physiological activity of grapevine at low temperature. An association of endophytic bacterial colonization of the grapevine plantlets both at low (4^0C) and ambient (26^0C) temperatures and their sensitivities to cold stress was apparent. Root growth and plantlet biomass significantly increased in PsJN inoculated plants. PsJN improved cold tolerance compared with that of the nonbacterized control. Moreover, bacterized plantlets had significantly increased levels of starch, proline, and phenolics in comparison to control unprimed plants. These increases correlated with the enhancement of cold tolerance of the grapevine plantlets (Barka *et al*. 2006). In

Table 1: List of microorganisms reported to be used in alleviation of temperature stress in plants

PGPR	Crop	Temperature stress	References
Bacillus amyloliquefaciens 5113	Wheat	Heat	AbdEl-Daim *et al.* 2014
Pseudomonas lurida	Himalayan plant	Cold	Bisht *et al.* 2013
Pseudomonas putida GR12- 2	Canola	Heat	Glick, 2012
Pseudomonas spp. strain PPERs23	Wheat	Cold	Mishra *et al.* 2009
Pseudomonas sp. AMK- P6	Sorghum	Heat	Ali *et al.* 2009
Pseudomonas putida strain NBR10987	Chick pea	Heat	Srivastava *et al.* 2008
Pseudomonas putida	Rapeseed	Cold	Chang *et al.* 2007
Burkholderia phytofirmans	Grape vine	Cold and heat	Barka *et al.* 2006

another study, Mishra *et al*. (2009) examined the effect of seed inoculation with 12 cold tolerant plant growth promoting *Pseudomonas* strains on wheat growth and physiological changes under green house conditions at10^0C. They reported that bacterization with pseudomonads significantly improved root length, shoot length, dry root biomass, dry shoot biomass compared with nonbacterized control. Further, bacterized wheat plants showed enhanced levels of total chlorophyll, anthocyanin, free proline, total phenolics, and starch contents, while a decrease was observed in the ratio and electrolyte leakage values which indicate enhanced tolerate to cold stress conditions. Inoculation of wheat seeds with *Serratia marscescens*, strain SRM, and

Pantoea dispesa, strain 1A increases the seedlings biomass and nutrients uptake at low temperatures (Milosevic *et al*. 2012).

2.10. Effect of climate change on plant disease development

Global climate change especially rising temperature and CO_2 level not only hamper physiological processes within plants but are expected to modify host susceptibility, alter pathogen development and survival rates which eventually changes in the impact of diseases on crops worldwide. Climate change affect the microclimate surrounding plants which perhaps influences various biochemical changes in such a way that it makes plants more susceptible to pathogens (Burdon *et al.* 2006; Legreve and Duveiller, 2010). Climate change continuously rising temperature as well as inconsistency in weather pattern are appropriate inducers of plant disease epidemics and expected to modify the synchrony between crop phenology and disease patterns as a result that currently economically less important pathogens may turn into potential threats in the future (Duveiller and Sharma, 2009).

Several fungal diseases limit crop production worldwide. Fungal diseases such as stem rust (causal agent- *Puccinia graminis),* powdery mildew (causal agent- *Blumeria graminis*), leaf rust (causal agent -*Puccinia recondita* and *Puccinia striiformis),* spot blotch (causal agent - *Bipolaris sorokiniana)* and Fusarium head or ear blight are among the major biotic constraints in wheat cultivation systems around the world (Juroszek and vonTiedemann, 2013). Many scientists have predicted global warming will increase severity of *Fusarium* foot rot in the United Kingdom and *Fusarium* head blight risk in South America. In European countries leaf blotch, karnal bunt risk incidence will increase rapidly due to weather change (Dumalasova and Bartos 2009; Madgwick *et al*. 2011; West *et al.* 2012; van der Fels-Klerx *et al.* 2012; Gouache *et al.* 2012). The two foremost characteristics of the Indian subcontinent climate are high temperatures and humidity and due to changing climatic situation we are experiencing more shorter and warmer winters where the mean temperature of the coolest month is higher than 17.5˚C (Joshi *et al.* 2007). In India wheat diseases like spot blotch, foliar blight, rust, fusarium head blight is assumed to be more severe in the future because of humidity and elevated temperature particularly in the lack of resistance in wheat cultivars (Kaur *et al.* 2008). Malaker and Reza (2011) reported both stem and yellow rusts particularly occurs in late sown susceptible wheat cultivars and remains a major problem in some of the wheat farming zone around the world especially in Bangladesh. Since warm and humid

weather increase spot blotch severity therefore this disease is increasingly becoming a cause of worry particularly in the warm and moist environments of Indian sub-continent (Duveiller *et al.* 2005). In Indian sub-continent wheat production is already experiencing yield reduction due to terminal heat stress (Juroszek and von Tiedemann, 2013). Sharma *et al.* (2007) reported increasing spot blotch incidence into the cooler, non-traditional irrigated wheat growing areas could be due to rising temperature which aggravates spot blotch severity in these areas. In rising incidences of spot blotch due to high temperature during wheat growing season in non-endemic parts in Pakistan, Nepal and Bangladesh were reported (Shamim *et al*. 2010, Mahto *et al*. 2011, Hossain *et al*. 2013). Around 25 million hectares of land worldwide, covering wheat growing areas of North and Latin America, Africa, China, South East Asia and Indian subcontinent and worldwide have been affected by spot blotch (Joshi *et al.* 2002; Duveiller *et al.* 2005.) In the United States spot blotch has been reported to be a serious foliar disease which causes yield loss of wheat (Wegulo *et al.* 2009). Yield losses of 25–45% have been recorded in Kazakhstan and 41% in Russia (Iftikhar *et al.* 2009). Warmer areas of South Asia are highly affected by *B. sorokiniana* and depending upon the severity of occurrence around 40%-50% yield loss was estimated every year (Sharma and Duveiller, 2004). In Indian subcontinent, under rice-wheat cropping system, there was above 70% rise in spot blight incidence (Sharma *et al.* 2007). In India wheat fields covering eastern gangetic plains, northern, western parts of India mostly affected by spot blotch and around 18-22% yield loss every year was estimated (Joshi *et al*. 2007).

Elevated temperature has become a major concern for agricultural sector worldwide not only because of its adverse affects on growth, development, and productivity of plants but also constantly changing plant pathogen interaction which is complicated and ambiguous. Response to various temperatures is determined by a plant's ability to adapt to different climate regimes most of the mechanism is still elusive and ambiguous.

2.11. PGPR in biotic stress alleviation in wheat

Plant growth promoting rhizobactria (PGPR) also act as biocontrol agents, can operate through various mechanisms, in spite of their role in direct growth promotion, such as production of phytohormone auxin and decrease of plant ethylene levels or nitrogen fixing associated with roots (Glick *et al.* 2007). PGPR produces hydrolytic enzymes like, glucanases, chitinases, lipases and proteases that can lyse pathogenic fungal cells. Various siderophores, antifungal and antimicrobial substances were also produced by

PGPR (Neeraja *et al.* 2010; Maksimov *et al.* 2011),While on the other side induce systemic resistance (ISR) with in plants in response to stress imposed by the infection (Van Loon, 2007). ISR is triggered by PGPR in SA-independent and dependent ways, and somewhat intersects with the JA/ET pathway. SAR is stimulated by necrotizing pathogens and a SA-dependent signaling pathway, and results in enhanced level of SA and activation of various PR proteins (Conrath, 2006). This process protected the plant against other viruses and resulted in the conception of "Systemic Acquired Resistance" (SAR). The activation of defense mechanisms induced by fungi, bacteria, viruses, and nematodes can be achieved by different routes, which may occur alone or concomitantly (Hammerschmidt, 2009). Application of PGPR promotes plant growth, yield and induces disease resistance. Existence of the PGPR in the rhizosphere makes the complete plant, including the shoot and root, more resistant to pathogens (Figueiredo *et al.* 2010). Many Strains of PGPR, be a member of genera *Azospirillum, Azotobacter, Azoarcus, Arthrobacter, Pseudomonas, Bacillus, Enterobacter, Gluconacetobacter, and Serratia*, have been reported to bring out significant reductions in disease severity on a diversity of host crops (Reinhold-Hurek and Hurek, 2000; Kloepper *et al.* 2004).

Pseudomonas strains MRS23 and CRP55b inhibited growth of pathogenic fungi, i.e. *Fusarium oxysporum* f. sp. Ciceri, *Rhizoctonia solani* and *Aspergillus* sp. *in vitro* (Goel *et al*. 2002). Monteiro *et al*. (2005) reported that four of these *Bacillus* strains produced lipopeptides active against *Xanthomonas campestris pv. campestris* during its late growth phase. Lipopeptides can also stimulate ISR in plants, probably by interacting with plant cell membranes and inducing temporary alterations in the plasma membrane which could raise plant defenses (Ongena *et al.* 2009). *Bacillus methylotrophicus* BC79 produced phenaminomethylacetic acid which was reported to have antifungal activity (Shan *et al.* 2013). Culture filtrate of BC79 showed biocontrol efficiency against rice blast. *Bacillus amyloliquefaciens* Ba33, an antiviral agent against tobacco mosaic virus was used in field as a soil disinfector and (TMV) (Shen *et al.* 2012). *Bacillus amyloliquefaciens CM*-2 and T-5 enhanced the growth of tomato seedlings along with the biocontrol of tomato bacterial wilt caused by *Ralstonia solanacearum* (Tan *et al.* 2013).

Natural resistance of wheat to various pathogens is found to be low (Acharya *et al.* 2011). However, there is a possibility of biological control of spot blotch which can occur by inducing resistance to pathogens.There are very few reports of PGPR mediated

biocontrol of *B.sorokiniana*. Hogdes *et al.* (1994) indicated that *Pseudomonas* PSD-42 was antagonistic to *B. sorokiniana* on leaves of *Poa pratensis*. Application of *Pseudomonas chlororaphis*, strain MA 342 reduced spot blotch symptoms to some extent (Johnsson *et al.* 1998). Endophytic *Bacillus subtilis* strain E1R-j was reported to control wheat stripe rust as well as powdery mildew in greenhouse and field trials (Li *et al.* 2013; Gao *et al*. 2015). Application of *B. amyloliquefaciens* KB3 and *B. subtilis* QST-713 reduced wheat leaf rust (Nam *et al.* 2016). Two PGPR strains (JD204 and JC186) of *Pseudomonas putida* promoted wheat growth and JD204 strain had stronger growth-promoting, phosphorus-solubilizing and IAA-producing activities than strain JC186. Natural infection of stripe rust on the JD204-inoculation in winter wheat cultivars significantly reduced strip rust and also increased wheat yield (Pang *et al.* 2015).

There are very few instances of biotic and abiotic stress amelioration at a time by PGPR application. However, very recently Singh *et al.* (2017) isolated *Stenotrophomonas maltophilia* SBP-9 from *Sorghum bicolor* promoted wheat growth under saline condition and enhanced the defense response against fungal pathogen "*Fusarium graminearum*." SBP-9 isolate significantly increased plant growth in terms of various growth parameters such as shoot length/root length (20–39%), fresh weight/dry weight (28–42%), and chlorophyll content (24–56%) in wheat plants. Bacterial inoculation decreased the level of proline, and malondialdehyde, whereas elevated the antioxidative enzymatic activities of superoxide-dismutase (SOD; 28–41%), catalase (CAT; 24–56%), and peroxidase (POX; 26-44%) and maintained the K^+/Na^+ ratio. SBP-9 isolate also increased the expression of defense enzymes, such as peroxidae (PO), β-1, 3 glucanase, polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL), which protect plants from the Fusarium heat blight infection.

Use of PGPR for amelioration various abiotic and biotic stresses is reported by many workers. However PGPR mediated heat stress amelioration and role of the PGPR in inducing plant defense response at high temperature is very less reported especially PGPR mediated thermotolerance mechanisms remains elusive. Thus further exploration is needed to understand the mechanisms underlying the development of heat-tolerance in plants by PGPR application which can be used for crop improvement.