गेहूँ (*ट्रिटीकम एस्टाइवम* एल.) की अन्त ताप प्रतिबल सहनषीलता में सैलीसिलिक अम्ल की भूमिका

ROLE OF SALICYLIC ACID IN TERMINAL HEAT STRESS TOLERANCE OF WHEAT (*Triticum aestivum* L.)

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ROLE OF SALICYLIC ACID IN TERMINAL HEAT STRESS TOLERANCE OF WHEAT (*Triticum aestivum* L.)

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This is to certify that the thesis entitled **"Role of Salicylic Acid in Terminal Heat Stress Tolerance of Wheat (*Triticum aestivum*L.)"** submitted to the Post-Graduate School, ICAR-Indian Agricultural Research Institute, New Delhi, in partial fulfillment of the requirements for the degree of **Doctor of Philosophy** in **Plant Physiology** by Mr. **Shailesh Kumar** embodies the results of *bona fide* research work carried out by him under my guidance and supervision. No part of the thesis has so far been submitted anywhere for the publication or for any other degree or diploma.

All the assistance and help received during the course of the investigation has been duly acknowledged.

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IARI, New Delhi *(Shailesh Kumar)*
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DEDICATED TO
ALMIGHTY GOD

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1. INTRODUCTION

Wheat is one of the most important staple food crops of the world including India in terms of the harvested area, human nutrition and grown primarily for its grain. Wheat is grown in India under sub-tropical environment during mild winter which becomes fairly hot towards grain filling stages of the crop. The global demand for cereal crops is anticipated to increase by 56% (1048 million metric tons) in 2050 since the base year 2000, 26% of this increase is predicted for wheat (Hubert et al., 2010). Most of the wheat growing areas of the world experience many environmental stresses including drought (water stress) and high temperature (heat stress) that adversely affect yield (Lott et al., 2011). Future climates will also be affected by larger variability in temperature and more incidences of hot days (Pittock, 2003). Such elevated temperatures can instigate high temperature stress: a severe menace to wheat production in many countries including India, notably when it happens during reproductive stage.

High temperature above 30°C during the reproductive stage can cause pollen infertility, tissue dryness, decrease CO_2 assimilation and enhance the photorespiration (Saini and Aspinal, 1982). The excess active oxygen species (AOS) generated during high temperature stress leads to oxidative stress in plant system (Hasanuzzaman et al., 2012). Yield reduction in wheat under HT stress is caused by hastened phasic development (Rawson et al., 1979), decrease in photosynthesis (Conroy et al., 1994), fast senescence (Kuroyanagi et al., 1985), enhanced respiration (Berry et al., 1980) and inhibition of starch deposit in emergent kernels (Jenner et al., 1994).

The grain filling in wheat also depends on remobilization of stored carbohydrates from stem and leaf sheath, particularly the dependency on reserved carbohydrates increased, when existing photosynthetic supply from leaf and ear decline during biotic and abiotic stress condition (Blum, 1994; Plaut et al., 2004). The main reserved form of carbohydrates in the stem of wheat is fructan (Goggin and Setter, 2004). The flow of carbon into fructan is mainly governed by enzyme sucrose: sucrose 1-fructosyltransferase (1-SST) and hydrolysis of fructan is catalyzed by fructan exohydrolases (FEHs) during remobilization of stored carbohydrates.

To facilitate survival under heat stress, several changes occur at cellular, molecular and biochemical level like, maintenance of membrane integrity by scavenging the extra AOS generated during stress, increase in production of antioxidants enzymes and antioxidant metabolites, deposition and regulation of compatible solutes, enhanced expression of several HT associated heat shock factors and small and large molecular weight HSPs, and also stimulation of biosynthesis of components of signalling pathways like, stimulation of mitogen-activated protein kinase (MAPK) and calcium-dependent protein kinase (CDPK) cascades. All these changes inside the plant system facilitate or help the plant to survive under high temperature stress condition and these changes mainly regulated at gene or molecular level (Hasanuzzaman et al., 2012). The employment of plant growth regulators is acknowledged to play an essential role in plant response to stress (Chakrabarti and Mukherjee, 2003). Plant growth and development is a composite phenomenon and is ascertained by several endogenous and exogenous aspects. Among the internal factors, hormones occupy a fundamental job in regulating growth and development.

Salicylic acid (SA) has lately been documented as a plant hormone (Hayat et al., 2007). It act as endogenous signalling molecule and play an important role in growth (by inducing the mitotic activity) and development regulation of plant and also involved in interaction with other organisms and responses to several abiotic stresses (Miura and Tada, 2014). Salicylic acid plays miscellaneous physiological roles in plants together with thermogenesis, flower initiation, nutrient uptake, ethylene biosynthesis, stomatal movements, photosynthesis and enzyme actions (Hayat et al., 2007). Disease resistance and abiotic stress tolerance are supplementary roles which are associated to SA (Janda et al., 2007). Latest research illustrate that the SA is synthesised from chorismate via isochorismate in processes catalysed by the isochorismate synthase (ICS) and isochorismate pyruvate lyase (IPL) enzymes, in that order (Wildermuth, 2006). Nevertheless, the IPL encoding gene is not known in plant. However, the role of shikimate pathway in SA synthesis of juvenile pea plants was demonstrated (Szalai et al., 2011). Endogenous basal level of SA differs by several orders of magnitude in the diverse plant species and unfavourable environmental conditions have shown to boost the endogenous SA levels in plants (Pal et al., 2013).

Exogenous treatments with SA have been evidenced to impart defence alongside several kinds of stresses. The function of salicylic acid in imparting tolerance to a several biotic stresses has been exhaustively deliberated; in addition, fresh reports have also confirmed that salicylic acid provide tolerance to several abiotic stresses like salt, drought, chilling, heat, ultraviolet radiation, and heavy metals (Miura and Tada, 2014). Horvath et al. (2007) have reported and also proved in different experiment conducted by several workers that plant response to exogenously applied salicylic acid depends on many aspects, together with the species and growth stage of the plant, the mode and method of application, and the dose of salicylic acid applied and its endogenous level in the specified plant. The exogenous application of salicylic acid improves both acquired and basal thermotolerance in plants has been confirmed by several scientific groups (Dat, 1998a, b; Larkindale and Knight, 2002). One of the probable modes of action of salicylic acid is the inhibition of activity of catalase enzyme, an important hydrogen peroxide scavenging enzyme, thus mild increase in concentrations of hydrogen peroxide in cell, which act as a second messenger and stimulating stress associated genes (Chen et al., 1993). The first paper to validate the consequence of SA on heat tolerance illustrated that the heat tolerance of mustard plants was enhanced by spraying with SA (Dat et al., 1998a). This effect was concentration-dependent. Treatment with SA has been stated to increase photosynthetic rates and stomatal conductance in heat stressed plants (Wang et al., 2010) and boosts the enzymatic activities of antioxidant enzymes. Heat acclimation and exogenous salicylic acid treatment enhance the antioxidant enzymes activity and antioxidant metabolites contents (Wang et al., 2006). Growth-invigorating role of salicylic acid have been proved in several crop plants (soybean, wheat, maize, chamomile etc) by several groups of worker (Kovacik et al., 2009). It has been suggested that the growth-promoting effects of SA may perhaps be correlated to alteration in the hormonal status (Shakirova et al., 2003; Abreu and Munne´-Bosch, 2009) or by enhancement of photosynthesis, transpiration, and stomatal conductance (Stevens et al., 2006). Reports have revealed that heat stress produce a noteworthy reduction in the oxygen evolution complex in PSII and D1 protein in wheat plants (Zhao et al., 2011). ROS impairs D1 protein (Yamamoto et al., 2008) and impedes the restoration of photo-damaged PSII by restraining the *de novo* synthesis of D1 protein (Murata et al., 2007). Correspondingly, Zhao et al. (2011) also communicated, exogenous SA treatment may as well hinder the disintegration of D1 protein and PSII functional damage in heat stress. In cucumber, alike other stresses, low temperature stimulates endogenous SA accumulation in leaves, and this accumulation could be prevented by spraying with an inhibitor of SA biosynthesis (Szalai et al., 2011). On exogenous SA application, the leaf SA concentration of greenhouse raised tomato attained a peak after two days of the application and then progressively declined to a concentration statistically equivalent to that of the check plants after 10 days of application (Guzmán-Téllez, 2014).

The role of salicylic acid (SA) in the control of abscisic acid (ABA) biosynthesis and ABA accumulation is controversial even though both plant growth regulators accumulate in tissues experiencing abiotic and biotic stress conditions (Horváth et al., 2015). So, it is essential to explore the effect of exogenous SA on endogenous level of ABA under high temperature stress. Jiang et al. (2010) have reported an antagonistic interaction of abscisic acid (ABA) with salicylic acid (SA) signalling pathways in rice. Fahad et al. (2012) have also proved that the lower ratio of ABA/IAA is because of the balance between the two phytohormones affected by SA under stress condition in maize. Report also shows that SA treatment prevented the reduction in IAA and cytokinin content absolutely which abridged stress-generated inhibition of plant development in wheat (Sakhabutdinova, 2003).

It is recently reported that the higher dose of salicylic acid (1mM) enhanced expression levels of the fructan biosynthesis pathway gene (1-FFT and 1-SST) (González et al., 2014) in the *Agave* species which induced the accumulation of fructo-oligosaccharides (FOS) in stem. Whether FEH expression is also affected by exogenous application of SA is not known.

Moreover, the heat shock response in plants is also controlled by a group of highly conserved small and large molecular weight heat shock proteins, and the transcription of genes of heat shock proteins is regulated by heat inducible transcription factors, known as heat shock factors (Hsfs). In wheat total 56 TaHaf members have been identified (Xue et al., 2014) and classified into three classes A, B, and C. Among TaHsfs, class A2 and A6 members are classified as dominant member which expressed mainly during high temperature condition. Information related to effect of exogenous SA in regulation of gene expression of large and small molecular weight HSPs and Hsfs under normal and high temperature stress conditions in wheat particularly at reproductive stage or during terminal heat stress condition is not documented.

The outcome of exogenous SA depends on various factors for instance the species and developmental stage of the plant, the mode of application, and the concentration of SA and its endogenous level in the specified plant. Based on background knowledge and prior scientific facts, it is apparent that the effects of SA were studied only at seedling stage or at vegetative stage in wheat and following exposure of short duration of heat stress. On the other hand, wheat crop encounters prolonged heat stress throughout reproductive phase. Consequently, this study was accomplished to elucidate the role of SA in imparting thermal tolerance to wheat crop all through the terminal heat stress. Studies were also conducted to explore the physiological as well as molecular mechanisms by which the SA regulates the thermal tolerance (possibly by interaction of SA with ABA). Furthermore, the role of non-structural carbohydrates of stem in supporting grain filling under heat stress and *vis-à-vis* SA has not been well documented. Keeping the aforementioned background in mind the following objectives are framed:

Objectives:

1. To investigate the role of salicylic acid (SA) on the physiological and biochemical traits in contrasting sets of wheat genotypes under high temperature stress.
2. To understand the physiological and molecular mechanisms of salicylic acid regulated changes in hormonal homeostasis under terminal heat stress response.

2. REVIEW OF LITERATURE

Wheat is one of the most important principal food crop, grown over 200 mha in a wide range of environment all over the globe. India attained extraordinary growth in wheat production through the last 4 decades and is the second leading wheat producer in the world. India documented unprecedented production of 95.85 million tonnes production from area 30 million hectare during 2013-14 (Ministry of Agriculture, India). However, India requires production 100 million tonnes of wheat by 2030 to supply the expanding population, which is a foremost challenge in the varying climatic scenario (annual report DWR, 2012-13). The impetus of progress in wheat production could not maintain pace with the demand in the last 5 years. Total factor productivity (TFP) and yield growth have been lingering in most productive wheat belt of India (Rejesus et al., 1999).

High temperature critically influences seed germination, photosynthesis, respiration, transpiration, membrane stability, fertilization, dry matter partitioning, maturation, quality of seeds, nutrient absorption, protoplasmic movement, transport of materials and also modulated level of hormones and primary and secondary metabolites and crop duration (Chen et al., 1982; Wahid et al., 2007).

In recent years salicylic acid (SA) has been the focus of intensive research due to its function as a signal transducer or messenger under stress conditions and induction of different anti-stress programs (Klessig and Malamy, 1994). The important protective action of SA probably reflects its ability to induce the expression of genes coding not only for PR-proteins, but also induced synthesis of heat shock proteins in tobacco plants (Burkhanova et al., 1999). The influence of exogenous SA depends on several aspects, comprising, the species and developmental stage of the plant, the mode of application, and the concentration of SA and its endogenous level in the specified plant (Horvath et al., 2007; Ashraf et al., 2010; Hayat et al., 2010). The effective protective effect of SA against various environmental stresses was concentration dependent (Horvath et al., 2007).

2.1 Growth, phenological and morphological changes

High temperature stress is one of the major limiting aspect concerning plant growth and crop yield. Ashraf and Hafeez (2004) reported in maize and Wahid et al. (2007a) observed in pearlmillet that net assimilation rate, plants' relative growth as well as shoot dry mass get considerably reduced due to elevated temperatures. Elevated temperature hastens phenological development in wheat and influence grain growth and yield. Increase in atmospheric temperature can disturb plant productivity since duration and rate of grain filling together are controlled by change in temperature. Enhancement in rate of grain development and shortening of grain filling period has been observed in several crops (Wheeler, 2000). Rise in temperature in optimal range curtails time to flowering in soybean (Baker et al., 1989) and dry bean (Prasad et al., 2002). Reproductive processes like gamete formation, germination pollen and development of pollen tube, viability of ovules, receptiveness of the stigma for pollen, the process of fertilization, development of embryo and endosperm were severely influenced by rise in temperature in tomato (Foolad, 2005).

Salicylic acid is known to stimulate flowering in several plants, like ornamental plant African violet and *Carica papaya* (Martin-Mex et al., 2005). Kumar et al. (1999) observed that foliar treatment with salicylic acid improved flower development and pod development in soybean. Khan et al. (2003) corroborated that the leaf area and production of dry matter in soybean and maize was augmented by the application of SA. In plants sprayed with SA the dry matter build up appreciably increased in mustard (Fariduddin et al., 2003), maize (Khodary, 2004) and barley (Pancheva et al., 1996). Nevertheless, higher dose of SA had an inhibitory outcome. Majority of studies have revealed that the SA influence on root development is positive (Wang et al., 2010), however in a few reports like in Sahu et al. (2011) harmful consequences were obtained in root biomass. Sakhabutdinova et al. (2003) observed an improvement in wheat productivity owing to 0.05mM SA treatment as it boosted mitotic division in the root apical meristems. The influence of the exogenous application of SA on vegetative growth depends on the genotypes, developmental stage and the endogenous concentrations of salicylic acid. The growth-inducing impact of salicylic acid has been observed in chamomile (Kova´cik et al., 2009). Soybean plants treated with 100 µM, SA showed 20% and 45% increase in shoot and root development, in that order, 7 d after treatment. Similarly, 50µM salicylic acid accelerates the development of leaf rosettes by 32% and roots by 65% in chamomile, however, greater concentrations (250 µM) produced ambiguous results (Kovacik et al., 2009). The growth inducing influences of salicylic acid might be linked to alterations in the concentrations of plant growth regulators (Shakirova et al., 2003; Abreu and Munne´-Bosch, 2009) or to increase in stomatal conductance, transpiration and photosynthesis (Vicente et al., 2011). Subjecting *Brassica* seedlings to high temperature instigated inhibition of seedling growth drastically. Yet, pre-treatments with heat acclimation and SA assisted the seedlings to recuperate from heat-shock produced damage (Kaur et al., 2009). Kaur et al. (2009) has also observed an enhancement in development of heat stressed mungbean seedlings, pretreated with heat acclimation and SA. Increase in growth may perhaps be associated to better accumulation of fresh/dry weight in pre-treated heat stressed seedlings. The treatment of Indian mustard (*Brassica juncea* L.) seedlings with SA (10^{-5}M) improved the entire growth parameters over the control by 13%, 14.7%, 14.0% and 35.5% for root length, shoot length, fresh mass and dry mass of plants, in that order, and permeated the deleterious results produced by high temperature (Hayat et al., 2009).

2.2 Membrane thermo-stability and lipid peroxidation

2.2.1 Membrane thermo-stability

High temperature stress liberates the chemical bonds within the molecules of biomembranes increasing their permeability. The high kinetic energy renders increase in fluidity of the lipid bilayer by increasing unsaturation or causing proteins denaturation (Savchenko et al., 2010). The reduced thermostability of the cell membrane (CMT) because of the greater loss of solutes due to increased membrane permeability, has been exploited as a parameter of measuring HT tolerance in various crops, including soybean, cotton, sorghum, wheat and barley (Martineau et al., 1979; Ashraf et al., 1994; Marcum, 1998; Blum et al., 2001; Wahid and Shabbir, 2005). Treatment with salicylic acid in rice lessened the decline in yield on account of elevated night temperature by reducing the respiration rates and rising the membrane thermal stability and total antioxidant power of rice plants (Mohammed and Tarpley, 2011). Use of salicylic acid was observed to improve the antioxidant ability thus avoiding injury to membranes, consequently, improving yield by principally impacting spikelet fertility in rice plants (Mohammaed and Tarpley, 2011).

2.2.2 Lipid peroxidation

Lipid peroxidation is a marker of stress as it is coupled with oxidative injury. The increase in lipid peroxidation has been analyzed in heat stressed plant of cotton (Mohan and Mauget, 2005) and lily (Yin et al., 2008) chickpea (Kumar et al., 2011). Wang et al. (2006) observed that spraying with a 0.1 mM solution of SA reduces thiobarbituric acid reactive substances and comparative electrolyte leakage in young grape leaves during heat stress, representing that SA may be able to produce intrinsic heat tolerance in grapevines. Wang et al. (2014) reported that quick enhancement in MDA concentration was found in the wheat plants that were rendered to heat and high light stress, which was 107 % higher than the control. The SA treated stressed wheat plants appreciably reduced the levels of MDA in contrast to the plants exposed to stress alone, even though they were evidently higher than their relevant controls.

2.3 Photosynthesis and pigments

2.3.1 Photosynthesis, photochemical efficiency and its associated parameters

Photosynthesis is an extremely heat stress susceptible physiological mechanism in green plants (Weis et al., 1989). Heat stress can result into dissociation of the oxygen evolving complex (OEC), ensuing in an discrepancy through the electron flow from OEC en route the acceptor side of photosystem II (PSII) (Ronde et al., 2004). Heat stress may also damage additional domains of the reaction center, e.g., the D1 and/or the D2 proteins. Numerous reports illustrate that heat stress impedes electron transport at the acceptor side of PSII (Wen et al., 2005). In high temperature stress the photosynthetic traits which bear association with plant's growth and development prove to be excellent markers for thermotolerance. The growth of the plants gets restricted due to hindrance in photosynthesis elevated temperatures. Wise et al. (2004) observed that the main spots of injury caused by raised temperatures are the light reactions in thylakoids and the stromal carbon metabolism within the plastids. Decline in the rubisco activation state curtails the net photosynthesis and stomatal conductance on moderate heat stress in several crops (Morales et al., 2003). Photosynthesis was repressed when leaf temperatures rose above 38°C and reticence was acute on abrupt rise in temperature, in maize. Crafts-Brander and Salvucci, (2002) reported that the photosynthetic inhibition was not dependent upon stomatal response to heat stress. Generally, the photosynthetic rate declines whereas photo-respiration and dark respiration rates enhance significantly on experiencing heat stress (Wahid et al., 2007). Additionally, biochemical reaction rates are abridged and enzyme denaturation occurs on the rise of temperatures, guiding sharp decrease in photosynthesis (Nakamoto and Hiyama, 1999). The proportion of variable chlorophyll fluorescence to maximum fluorescence (Fv/Fm), and the base fluorescence (F_0) have been observed to associate with heat tolerance (Yamada et al., 1996). Heat and high light stress controlled substantial reduction in Fv/Fm, Fv/Fo, ETR and Pn, which were 19, 59, 29 and 53 % lesser compared to their corresponding controls in wheat (Wang, 2014).

The enhancement of photosynthetic rates subsequent to treatment of SA may not be recurrently associated with high stomatal conductance levels or transpiration rates; yet, the intercellular CO_2 concentrations of plants sprayed with SA were usually lower compared to the control plants. This implies that the improvement in photosynthetic rates pursuing spray applications of some phenolic compounds like SA might be the consequence of increased enzyme activity associated with CO_2 uptake at the chloroplast level, than merely frequent in stomatal opening (Khan et al., 2003). Nevertheless, the precise mechanism of the photosynthesis improvement is yet to become clear. The studies conducted by Fariduddin et al. (2003) in *Brassica juncea*; Khan et al. (2003) in corn and Kumar et al. (2000) in soybean, revealed augmentation of the rate of transpiration, water use efficiency, stomatal conductance, internal CO_2 concentration and the rate of net photosynthesis on exogenous application of salicylic acid.

Sun et al. (2006) found out that salicylic acid pre-treatment in wheat minimised the reduction in intrinsic photochemical efficiency (Fv/Fm), quantum yield of PSII, maximum fluorescence value (Fm) and photochemical quenching (qp) and encouraged increase in non photochemical quenching (NPQ). Besides, salicylic acid has also been observed to diminish and efficiently obstruct the destruction of D1 protein during high temperature and high light stress and sustain D1 protein phosphorylation ETR of the whole chain and PSII, Fv/Fm (the highest photochemical efficiency of PSII) actual photochemical efficiency of PSII, q (p) the photochemical quenching coefficient, and net photosynthetic rate at a greater level (Wang et al., 2010).

The treatment with SA augmented photosynthetic parameters (gs, Ci, WUE, Pn and E) the mentioned parameters considerably (Hayat et al., 2009). In cucumber foliar spraying of 0.5–2.5 mM SA prior to low temperature and light intensity treatment reversed the stress generated reduction of the leaf Pn, gs, transpiration rate and Fv/Fm and increase of the Ci (Liu et al., 2009). Salicylic acid pre-treatment allayed the heat stress generated reduction in photosynthesis chiefly by means of retaining higher RUBISCO activation state, quickened photosynthesis primarily through effect on PSII. These impacts of salicylic acid may be interrelated to improve level of HSP 21 (Wang et al., 2010). SA pre-treatment did not notably affect photosynthesis of grape leaves at optimal growth temperatures. Nevertheless, SA pre-treatment lessened the drop in Pn under heat stress, ostensibly partially via retaining a greater Rubisco activation state and higher PSII efficiency. SA also quickened the enhancement of Pn largely by means of the faster revival of PSII function following heat stress. These SA effects possibly are linked up to enhanced levels of HSP21. Many other means through which SA shields photosynthesis in grape leaves are yet to be identified (Wang et al., 2010). Foliar treatment of 0.3 mM SA has been established to delay the reduction of D1 protein content in wheat plants during heat and high light stress (Zhao et al., 2011). Plenty of two oxygen evolving enhancer (OEE) proteins and the PSII stability/assembly factor HCF136 is up-regulated in stressed seedlings with SA pre-treatment than in stressed wheat seedlings with no SA pre-treatment (Kang et al., 2012b). Treatment with 0.5 mM SA to no-stress or heat-stressed plants improved photosynthetic traits considerably in wheat (Khan et al., 2013). In the current investigation, induced *psbA* transcription by foliar applied SA may perhaps improve the *de novo* synthesis of the new copies of D1 protein, and after that enhance the turnover of it and scatter surplus light energy, which are all favourable for the enhancement of PSII tolerance in heat and high light stressed and recovered wheat plants (Wang et al., 2014). This was recently reported that foliar treatment of 0.3 mM SA was competent to keep the Fv/Fm at constant level as non-stressed control and keep 79 % of Fv/Fo, 86 % of ETR, and 71 % of Pn, which were 29, 92, 23 and 52 % higher compared to their corresponding stressed wheat plants without SA treated (Wang, 2014).

2.3.1 Pigment contents

Karim et al. (1999) reported that prominent impairment of chlorophyll a and b was greater in the developed leaves than in the developing leaves due to heat stress. In addition, Guo et al. (2006) found that active oxygen species are generated due to damage of chlorophyll. Heat shock decreases the quantity of photosynthetic pigments (Todorov et al., 2003), soluble proteins, rubisco binding proteins (RBP) and large-(LS) and small subunits (SS) of rubisco in darkness although it enhances them in light (Kepova et al., 2005). An enhanced proportion of chlorophyll a to chlorophyll b and a reduced proportion of the chlorophyll to carotenoids were found in the tolerant genotypes of tomato in high temperatures (Wahid and Ghazanfar, 2006).

Khodary et al. (2004) reported an obvious improvement in growth characteristics, pigment contents and photosynthetic rate in maize, sprayed with SA. Moreover, salicylic acid when employed exogenously to wheat seedlings amplified the size and mass of plantlets appreciably, than the untreated control (Shakirova, 2007). Carotenoids guard the cellular structures in a range of plant species experiencing abiotic stress (Wahid and Ghazanfar, 2006; Wahid, 2007). The synthesis of xanthophylls and carotenoids get triggered and the de-epoxidation rate, chlorophyll pigments and ratio of chlorophyll a/b gets improved in wheat and moong on application of salicylic acid (Moharekar et al., 2003). Foliar treatment with salicylic acid, safeguarded mustard from the stress created by temperature and considerably enhanced the growth, the level of chlorophyll and photosynthetic parameters. Additionally, salicylic acid inhibits xanthophyll cycle pool in cucumber leaves and enhanced the de-epoxidation level of xanthophyll cycle, as well. A decreased concentration of SA recuperates the photosynthetic net CO_2 assimilation in mustard seedlings. The advantageous influences of low dose of salicylic acid in photosynthesis could possibly be linked to the deterrence of oxidation of AUX by salicylic acid, as high AUX concentrations enhances net photosynthesis (Ahmad et al., 2001). Plants in receipt of SA only acquired the maximum value for SPAD chlorophyll and reflected 11.9% higher as compared to the control. On the other hand, application of high temperature stress to the plants reduced the SPAD by 17.5% less than that of the check. Soaking of seeds or foliar treatment with salicylic acid can also enhance the pigments, which was demonstrated in rapeseed (*Brassica napus* L.) (Ghai et al., 2002) and in wheat (Hayat et al., 2005). But the positive effect of SA upon photosynthetic activity may not be caused by an increased chlorophyll level. In cowpea (*Vigna unguiculata*) plants, SA application enhanced or reduced chlorophyll content, depending on the genotype (Chandra and Bhatt, 1998) or the applied concentration, for example 0.001–10 uM SA increased, whereas 1 mM SA decreased chlorophyll and carotenoid contents together in the cotyledons of sunflower plants (Cag et al., 2009). SA lessens heat stress influences on photosynthesis, SPAD chlorophyll, rubisco activity, and water-use efficiency. Exogenous SA supplementation had a concentration dependent guard on the chlorophyll content. Stressed wheat plants supplied with 0.3 mM SA revealed 32 % higher chlorophyll content as compared to those experiencing heat and high light stress alone (Wang, 2014).

2.4 Stem reserve mobilization, water soluble carbohydrates biosynthesis, enzyme activity and gene expression

2.4.1 Stem reserve mobilization

In wheat, maximum photosynthesis is observed at 22 - 25°C but it gets reduced abruptly over 31°C. A genetic basis for disparity in carbon exchange rates of the leaf and ear subsists during heat stress; however, thermotolerance is a chief motive for persistent yield and grain filling during HT stress (Blum 1988; Alkhatib and Paulsen, 1992). On blockage of the photosynthetic supply asa result of biotic or abiotic stress, the grain filling becomes dependent on transported reserves of the stem (Blum, 1988). In wheat, the stem reserves are an imperative resource for grain filling (Kiniry, 1993). Prior studies proved that the penultimate internodes are the chief sites for the storage of carbohydrates (Scofield et al., 2009).

The leaf and ear photosynthesis at elevated temperatures critically prejudice the grain filling in *Triticum aestivum* L., where it is also determined by the stem reserve mobilisation to the developing grains (Yang and Zhang, 2006). Consequently, the involvement of stored carbohydrates might turn out to be the prime resource of mobilized reserves (Plaut et al., 2004). The 10–20% grain yield in wheat is contributed solely by the reserve materials. These contributions promote enhancement up to 30-50% during drought and heat stress (Davidson and Chevalier, 1992; van Herwaarden et al., 1998b; Shearman et al., 2005). Several research findings indicate that grain yield bears a positive correlation with WSC/NSC under normal and stress environments (Foulkes et al., 2007; Saint Pierre et al., 2010). Significance of WSC to grain yield is realised only after HT decreases the total production of photo-assimilate, while in severe heat stress conditions, the developing grain gets majority of its carbohydrates prevalently through WSC. Greater remobilization due to high WSC storage was achieved during unfavourable influences of terminal drought (van Herwaarden et al., 1998b) and heat (Wang et al., 2012). Consequently, high WSC concentration is deemed as a vital factor contributing to grain yield and weight under diverse stress conditions (Xue et al., 2009; Saint Pierre et al., 2010). Exhaustive study has been done in wheat to elucidate the genotypic divergence in WSC (Ehdaie et al., 2006; Ruuska et al., 2006) and tolerance to high temperature stress (Wardlaw et al., 1989b. Typically, input of penultimate internode WSC towards grain filling accounts to 56 per cent (Shakiba et al., 1996). Virgona and Barlow (1991) informed that WSCs of stem comprise fructans, glucose (Glc), fructose (Fru), sucrose (Suc) and different oligosaccharides. Reserve accumulation and storage capacity of the stem convincingly rely on the growth environment ahead of anthesis. During anthesis, the TNC (Total non-structural carbohydrates) of the stem as observed by Kiniry (1993) varied from 50-350 g kg^{-1} dry mass in distinct experiments. Under normal growing circumstances, carbon assimilation rates are elevated as compared to temperature, water regime (Davidson & Chevalier, 1992) and mineral nutrition (Papakosta & Gagianas, 1991) and a portion of the assimilates are stored. The stored assimilates in the stems decline during stress due to reduced carbon assimilation during stem elongation. For instance, in water-stressed and irrigated wheat, remobilise WSC were measured at 641 mg and 1047 mg, respectively, as the previous one had fewer storage compared to the latter (Davidson and Chevalier, 1992). In dryland conditions, merely one part of the WSC were accessible for remobilisation through grain filling, compared with irrigated conditions. During heat acclimation and SA pre-treatments, an enhancement in total soluble sugars was evidenced amongst all the genotypes and action of soluble neutral invertase also enhanced on heat acclimation and SA pre-treatments (Kaur et al., 2009).

2.4.2 Enzymes involved in water soluble carbohydrates biosynthesis, enzyme activity and gene expression

Goggin and Setter (2004) have observed that fructan is the key storage type of WSC in the stem in wheat. These are fru-based oligo- and polysaccharides resulting from Suc (Valluru and Van den Ende, 2008). These fructans in wheat comprising both β-(2,1) and β-(2,6) linkages are largely of the ‘graminan-type’. Wheat graminan biosynthesis involves three distinct fructosyl transferases (FTs) like sucrose: sucrose 1-fructosyltransferase (1-SST), sucrose: fructan 6-

fructosyltransferase (6-SFT) and fructan: fructan 1-fructosyltransferase (1-FFT) (Yoshida et al., 2007). 1-SST uses two Suc molecules to generate the tri-saccharide 1-kestotriose (1-K) and Glc. Subsequently, 6-SFT utilizes 1-K to produce 1 and 6-kestotetraose (1&6-K). Additional extension takes place by 1-FFT and 6-SFT activity. Thus, 1-SST and 6-SFT are said to be the major enzymes for graminan synthesis (Duchateau et al., 1995).

The gene expression patterns and activities of 1-SST and 6-SFT were studied in barley leaves by Nagaraj et al. (2004) who observed that transcripts and activities of 1-SST were regulated. They anticipated that the flow of carbon into fructan is mainly caused by 1-SST. The hydrolysis of fructans, catalyzed by fructan exohydrolases (FEHs), is responsible for the translocation of reserved carbohydrates. FEHs consist of fructan 1-exohydrolases (1-FEHs) and fructan 6-exohydrolases (6-FEHs) (Van den Ende et al., 2004). The degree of polymerization (DP) and total concentration in wheat blades and sheaths are decided by 1-SST and total FEH activities (Yukawa et al., 1995). The activity of SST seems to be associated to the sucrose concentration that is influenced by the stem's sucrose synthase activity (Wardlaw & Willenbrink, 1994). Dubois et al. (1990) reported that a high sucrose in the penultimate internode of wheat results in accumulation of fructan. The shading experiments conducted by Kiniry in 1993 substantiated that the starch is found in minute quantities in wheat stems but is not mobilised. Opposite to SST activity which decreases under stress condition, however FEH activities greatly enhanced under stress condition and closely related to the fructan remobilization from the stem (Yang et al., 2004).

The expression of genes coding for sucrose: sucrose 1-fructosyltransferase (1-SST) and fructan: fructan 1-fructosyltransferase (1-FFT), both fructan biosynthesizing enzymes, and the determination of the fructo-oligosaccharides (FOS) building up in response to the exogenous treatment of salicylic acid (1mM) had produced a 36-fold accumulation of FOS of considerable amount of polymerization (DP) in stems of *Agave tequilana*. FOS accumulation in the above treatments was strongly coupled to greater expression levels of either the 1-FFT or the 1-SST gene in tissues of both *Agave* species (Edgar et al., 2014). Talukder et al., (2013) reported that water soluble carbohydrates mobilizing efficiency of wheat genotypes under heat stress were higher (67–81%) as compare to control (59–67%) plants.

2.5 Endogenous level of SA and cross-talk with other hormones

Concentrations of SA vary by a large extent in diverse plant species. When the environmental conditions become most favourable, generally very low concentrations (10–100 ng/g FW) can be observed from the leaves of Arabidopsis, tobacco and maize (Meuwly and Me´traux, 1993; Mateo et al. 2006; Szalai and Janda, 2009). Some environmental stimuli such as pathogen infection, high temperature, heavy metal contamination, or the use of direct oxidative agent may evidently, increase it up to the range of µg/g FW which amounts to 10–50-fold increase (Raskin, 1992b; Enyedi, 1999; Pal et al., 2005). Still, in some species, for instance rice, the basic SA level in the leaves is normally as high as 30–40 µg/g FW (Pal et al., 2014). Wheat seedling basal level of SA varies from 500 to 8000 ng/g FW (Rakhmankulova et al., 2010).

Exogenous treatment with SA has been demonstrated to endow protection against a number of stressors. Hostile environment have been proven to improve the endogenous SA levels in plants (Szalai and Janda, 2009; Pal et al., 2013). The function of SA in plant tolerance to a range of biotic stresses has been exhaustively investigated; still, contemporary reports have confirmed that SA additionally modulates the plant response to many abiotic stresses, together with salt, drought, chilling, heat, ultraviolet radiation, and heavy metals. The result of exogenous SA is based on several factors, like genotype and developmental stage of the plant, the method of treatment, and the level of SA and its endogenous level in the specified plant (Horvath et al., 2007; Ashraf et al., 2010). Hormones and their signalling carries out a significant function in high temperature stress.

Salicylic acid has been observed to be concerned with basal as well as upward acquired thermo tolerance in plants (Dat et al., 1998b; Larkindale and Knight, 2002). Salicylic acid application averts the reduction in IAA and cytokinin content absolutely which lowers the stress induced inhibition of plant growth and higher ABA level are retained in salicylic applied wheat seedling (Sakhabutdinova et al., 2003). In *Arabidopsis*, giberrelic acids influence the salicylic acid biosynthtic pathway. Priming of seeds for 24 h with GA_3, and the over-expression of a GA-induced gene in *Arabidopsis*, doubles the SA concentrations as compared to the seeds primed in water (Alonso-Ramírez et al., 2009). Complementary evidence corroborating for ABA and SA signalling is the enhanced synthesis proteins under

ABA regulation, for instance dehydrins, LEA proteins, and heat shock proteins, expressed due to the application of 0.5 mM salicylic acid (Rajjou et al., 2006). In wheat SA (0.05mM) application limited the ethylene development in heat-stressed plants to normal range by restraining activity of 1-aminocyclopropane carboxylic acid (ACC) synthase (Khan et al., 2013). The enhancing influence of SA was investigated on endogenous ABA and IAA contents. Stress notably enhanced the ABA/IAA ratio than the untreated control, but SA extensively reduced the ABA/IAA ratio during stress although the value was observed to be greater than that of untreated control. The lower ratio of ABA/IAA is because of the balance between the two phytohormones equally produced by SA and is advantageous for plant development under stress (Fahad et al., 2012).

High temperature augmented total endogenous SA quickly, while SA treatment and heat acclimation stimulated similar series of variation in the ascorbate and glutathione pools and antioxidant enzymes. Thus, enhancement in endogenous SA and variation in antioxidants possibly be concerned in heat acclimation in mustard (Wang et al., 2006). It was observed that variation in endogenous SA and antioxidants is associated with induced thermotolerance. Thirty minutes to one hour heat acclimation treatment enhanced glucosylated SA 5.5-fold and then dropped in the period of subsequent six hours. Rise in free SA were less (2-fold) but significant (Dat, 1998b). Experiments on grapevine (*Vitis vinifera* L.) (Wang and Li, 2006) also demonstrated an abrupt rise in the concentration of salicylic acid at the commencement of high temperature tolerance, while application of SA also stimulated thermotolerance. Following the exogenous SA treatment the leaf SA concentration of greenhouse raised tomato attained a peak two days after the application start-up (DAAS) and then progressively reduced to a concentration statistically equal to that of the control plants 10 days after application (Guzmán-Tllez et al., 2014).

It was also observed that the molecular mechanisms underlying the influence of ABA in increasing the susceptibility of rice to *M. grisea,* concentrating on the antagonistic interaction of ABA with the SA signalling pathway. ABA noticeably suppressed the transcriptional upregulation of *WRKY45* and *OsNPR1*, the two main components of the SA signalling pathway in rice, stimulated by SA or benzothiadiazole or by blast infection. Overexpression of *OsNPR1* or *WRKY45* greatly annulled the development of blast susceptibility by ABA, signifying that ABA performs upstream of WRKY45 and OsNPR1 in the rice SA pathway (Jiang et al., 2010).

2.6 Oxidative stress and antioxidants defence

In the biochemical perspective, elevated temperatures instigate denaturation of a large number of heat-labile proteins and increase the level of detrimental reactive oxygen species (ROS) in plant cells (Mittler et al., 2012; Grover et al., 2013). High temperature stress hastens the production of reactive oxygen species (ROS) like singlet oxygen, superoxide radical, hydrogen peroxide (H_2O_2) and hydroxyl radical (OH•), thus generating oxidative stress (Mittler, 2002; Yin et al., 2008). In heat and intense light stress situation, surplus energy that has not been utilized for photosynthesis may possibly generate substantial amounts of ROS, which may bring about oxidative injury to chloroplasts and other cell structures (Singh and Singhal, 2001). ROS trigger the peroxidation of pigments and membrane lipids thereby altering the semi-permeability of the membrane as well as its function (Hasanuzzaman et al., 2013).

Pre-treatment by a foliar spray of SA might have a signalling role that functions to induce heat tolerance in the wheat plants as revealed by the reduction in O^{2-} generation rate, and H_2O_2 concentration (Wang et al., 2014). Depressed concentrations of ROS, particularly H_2O_2, are recognized to function as signal molecules commencing numerous defensive resistance mechanisms against pathogens, chilling, and heat stress (Horva´th et al., 2007). Conversely, if ROS accumulation stimulated by SA were immense, acute oxidative stress can take place along with irreparable membrane impairment (Rao et al., 1997). During high temperature stress, plants accumulate large quantities of non-enzymatic antioxidant and up-regulate the performance of antioxidant enzymes. Nevertheless, in susceptible genotypes, these increased activities are ineffective for generation of stress tolerance (Almeselmani et al., 2009). They observed that in response to heat stress, the SOD, APX, CAT, GR and POX activities were enhanced dramatically during active growth in heat-tolerant cultivars (C 306) while, the susceptible cultivar (PBW 343) demonstrated a considerable decline in activities of CAT, GR and POX. Wang and Li, (2006) showed that heat and cold together changed the antioxidant defence system in grape plants. However, exogenous salicylic acid (SA) pre-treatment facilitated the grape leaves to retain somewhat elevated activities of APX, GR, MDHAR, and redox ratio in the AsA–GSH pool equally in normal temperature and heat or cold stress. They also advocated that Ca^{2+} homeostasis and antioxidant systems implicate SA-induced heat or cold tolerance. Hayat et al. (2008) observed that exogenous application of SA increased the performance of antioxidant enzymes, CAT, peroxidise and superoxide dismutase, in the drought experiencing plants of *Lycopersicon esculentum*. Similar results were also obtained by Krantev et al. (2008) in maize who accounted that the exogenous application of salicylic acid geared up the activities of antioxidant enzymes APX and SOD while regressed the activity of CAT. Foliar application of low concentrations of salicylic acid bestowed HT tolerance in mustard. The SA application together with hardening at 45°C for one hour enhanced the H_2O_2 concentration and decreased the activity of CAT, thus improving the capability of mustard plants to endure the HT stress (Dat et al., 1998). Similarly, *in vitro* raised potato plantlets, complemented with low concentrations of acetyl salicylic acid showed an analogous response (Lopez-Delgado et al., 1998).

Larkindale et al., (2004) observed that salicylic acid protected the *Agrostis stolonifera* plants from oxidative injury and thus improved their high temperature tolerance. Salicylic acid pre-treatment had consequence on POX activity while, the activity of CAT decreased. In contrary, application of salicylic acid witnessed an increased CAT and SOD activity in HT stressed *Poa pratensis* (He et al., 2005). Chakraborty and Tongden, (2005) communicated that the HT stress caused membrane damage in chickpea was appreciably decreased by the treatment of SA. The application also increased the proline as well as protein contents considerably with a synchronized expression of different stress enzymes viz. POX and APX but the CAT activity declined. Foliar supplementation of SA have been shown to retain or promote the activities of antioxidative enzymes, like superoxide dismutase, ascorbate peroxidase, and catalase, that impart protection against oxidative stress for wheat crops (Wang et al., 2014).

Chen et al. (1993) anticipated that the SA functions by inhibiting catalase which scavenges H_2O_2, thereby raising its concentrations within the cell. It in turn works as a second messenger stimulating genes for plant defence. Wang et al. (2006) deliberated that 0.1mM SA was able to induce thermotolerance in grape leaves. Hardening of plants together with exogenous SA treatment, elevated the levels of

AsA, GSH, and hydrogen peroxide, enhanced the activities of the antioxidant enzymes superoxide dismutase, peroxidase, glutathione reductase, and ascorbic peroxidase and reduced the catalase activity in grapes. SA enhances the activities of antioxidant enzymes and the contents of ASA and GSH in of abiotic stress conditions (Syeed et al., 2011; Miura and Tada, 2014). Salicylic acid has been found capable to induce prolonged thermotolerance, by engaging the antioxidant systems and the calcium ion homeostasis (Wang and Li, 2006).

2.7 Heat shock protein and heat shock factors

2.7.1 Heat shock proteins

Heat shock proteins (HSPs) have been established to operate as chaperones to shield cellular proteins against irreparable heat-induced denaturation and to promote refolding of heat injured proteins (Boston et al., 1996). The key HSPs discovered in eukaryotes can be categorized into six structurally discrete classes: Small HSPs (sHSPs), Hsp60, Hsp70, Hsp90, Hsp100 and ubiquitin (Efeoğlu, 2009). Every organism expresses HSPs from the above cited classes (Park and Bong, 2002).

The small heat shock proteins are low-molecular-mass HSPs ranging from 12 to 40 kDa. Plants generate over 20 sHSPs (counting unique 17-30 kDa in higher plants), which are generally copious and stress responsive set of HSPs in plants (Heckathorn et al., 1999). Plants are the exclusive eukaryotes wherein organelle confined sHSP have been reported. Plant sHSPs are nuclear encoded and are classified into 6 classes; three of them are restricted within the cytosol or in the nucleus whereas other three are localized in the plastids, endoplasmic reticulum and mitochondria (Waters et al., 1996). It has been observed that sHSPs exhibit extremely low sequence similarity among divergent species and classes of plants (Sun and Montagu, 2002). The abundance and heterogeneity of sHSPs is exceptional to plants which propose their distinctive physiological role in plant-acquired stress tolerance (Sun and Montagu, 2002). Although, there is no substantiation for their prerequisite during normal cellular functioning, the small HSPs are synthesized ubiquitously in stressed plants (Ledesma and Kawabata, 2004). The electron transport system in mitochondria and chloroplast are reported to be guarded by the sHSPs situated within these organelles (Sun and Montagu, 2002). They have a great competence to attach to non-native proteins via hydrophobic interaction and impede non-native aggregation, in this manner assist their consequent refolding by ATP-dependent chaperones (Lee and Vierling, 2000). Under stress situation, sHSPs may encompass one percent of the cellular proteins (Agarwal et al., 2003). Function of chloroplast-confined sHSPs in high-temperature tolerance has been described in tomato (Preczewski et al., 2000). Yeh et al. (2002) illustrated that overexpression of *OsHSP16.9* in *E. coli* bestows thermotolerance to the bacterial cells. Transgenic rice plants over-expressing *OsHSP17.7* gene evidenced improved thermotolerance and enhanced resistance to UV-B irradiation (Murkami et al., 2004). HSP16.9, HSP17.1, HSP17.8 and HSP18.1 have been known to check the aggregation or denaturation of proteins in heat stress in different crops (Low et al., 2000). Members of this Hsp 70 family are largely conserved in the course of evolution. In plants, numerous HSP70 proteins have been documented (Boston et al., 1996). HSP70 accumulates in nucleus (Hendrick and Hartl, 1993) and functions in imparting thermal tolerance and enhanced resistance concerning different environmental stresses (Sung and Guy, 2003). Frydman (2001) observed that HSP70 chaperones promote protein folding in virtually all cellular compartments by avoiding aggregation non-native proteins and their refolding during normal as well as in stress conditions. An apparent attribute of Hsp90 is that its substrates are signal transduction proteins like steroid hormone receptors and signalling kinases (Young et al., 2001). Plant cytosolic, ER and plastidic HSP90 genes displayed 63–71% resemblance with that of yeast and animals (Krishna and Gloor, 2001). The main responsibility of HSP90 is to supervise protein folding; besides, they perform an important function in signal transduction networks, cell cycle control, protein degradation, protein trafficking, morphological development and stress adaptation, by the use of ATP (Queitsch et al., 2002). In *Drosophila* and *Arabidopsis* developmental anomalies and phenotypic alterations are caused by genetic mutation or by the treatment with geldanamycin, an HSP90 inhibitor (Queitsch et al., 2002). Hsp 101 members are a part of the larger ATPase superfamily (Agarwal et al., 2001). Proteins of this category commonly carry out protein denaturation and degradation (Wang et al., 2004) and eliminate non-functional and detrimental polypeptides developing owing to misfolding, denaturation or aggregation to sustain cellular homeostasis. Similar to other HSPs, these too are constitutively expressed in plants, although their expression is developmentally regulated and is induced by various environmental stresses (Adam and Clarke, 2002). HSP100 have been described in a number of plant species, together with wheat (Agarwal et al., 2001). Innumerable research has suggested the function of these proteins in deliberating thermotolerance in heat stress (Lee et al., 2007). Loss-of-function mutants of HSP101 in *Arabidopsis* hot1 (Hong and Vierling, 2001) and maize (Nieto-Sotelo et al., 1999) were not capable to gain thermotolerance at distinct growth stages and exhibited an acute heat sensitive phenotype. In wheat HSP101 cDNA was studied by Wells et al. (1998), that was soon designated as *TaHSP101a*. Transformants expressing *HSP101* demonstrated increased tolerance to high temperature (Katiyar- Agarwal et al., 2003). *A. thaliana* plants under-expressing their own HSP100 proteins were detected to lack both basal and induced thermotolerance (Nieto-Sotelo et al., 2002), therefore, establishing their impact on plant endurance.

2.7.2 Salicylic acid mediated HSPs and HSFs gene expression

Stress responses are intricate processes concerning transcription factors, enzymes and effectors (Agarwal et al., 2006). Plants express over 20 different HSF genes (*Arabidopsis*) in contrast to four HSFs in human and animal cells (Nover et al., 2001). Over expression of transcription factor interceding transcription of mRNAs encoding HSPs, triggered an improvement in thermotolerance of *Arabidopsis* plants (Prandl et al., 1998). Charng et al. (2007) have revealed that under expression of HsfA2 (heat-inducible trans-activator protein, that prolongs the expression of HSP genes and protracts the attained thermotolerance in *A. thaliana*) produces an improved sensitivity of the mutant plants to stress. Stimulation of several genes that are induced by high temperatures is ascribed to the conserved regions of the heat-shock element (HSE) within the promoter (Scharf, et al., 1998). AtHsfA2, *A. thaliana* TF *A* mutants exhibited decreased basal and acquired thermotolerance in addition to oxidative stress tolerance, even as the over-expression lines demonstrated enhanced tolerance (Li et al., 2005).

Heat shock transcription-factor-regulated expression of antioxidant enzyme APX in *Arabidopsis* (Sharkey, 2005) proposed that HSFs are occupied in HSP synthesis as well as in oxidative stress mediated antioxidant gene expression. Wheat breeders to date have exploited a restricted quantity of germplasms to achieve superior yield potential in congenial environmental circumstances, as a result tapering down genetic diversity of stress resistance traits, involving heat stress tolerance (Holden et al., 1993). Consequently, there exists a intense requirement to reveal the molecular and genetic base of traits concerning heat shock transcription factors and heat shock proteins, those capable of employing towards development of temperature tolerant wheat genotypes with superior yield. *Arabidopsis* is known to have 15 Hsfs (Nover et al., 2001) and tomato possesses 21 Hsfs all of which play vital roles in HT stress (Scharf et al., 1998). HsfA2 and HsfB1 are heat inducible Hsfs in tomato (Scharf et al., 1990), whose expression is regulated by HsfA1 (Mishra et al., 2002), considered as chief modulator of the HSR. Actually, the HsfA2 and HsfA1 interaction is crucial for their nuclear co-localization (Scharf et al., 1998). HsfA2 is presumed to be the central Hsf throughout HT stress condition (Mishra et al., 2002). As soon as the cells are subjected to a variety of stress like the high temperature stress, the cytosolic Hsfs, get detached from Hsp70, get stimulated, and go through trimming. These Hsf trimmers move to the nucleus after phosphorylation where they combine with the HSEs, that is positioned in the promoter of the *Hsp* (Pelham, 1982). This results in enhanced levels of Hsps in the cytosol. *Per se*, they perform as chaperones which support the movement and destruction of impaired proteins (Bukau and Horwich, 1998). In wheat Hsf family total 56 TaHaf members are recognized (Xue et al, 2014) which are categorized into A, B, and C classes. Many TaHsfs are constitutively expressed. Upon heat stress, the transcript levels of A2 and A6 members turn out to be dominant Hsfs, implicating a significant regulatory role throughout heat stress.

Wahid et al. (2007a) observed that salicylic acid facilitates the heat shock transcription factors to attach the HSE to the promoter of the genes related heat shock. HSP expression is regulated by means of heat shock factors (Hsfs). In heat shock-stressed (40°C) tomato seedlings, SA treatment improved the binding of Hsf to DNA, HSP70 transcription, and enhanced the expression of hsfA1, hsfA2, and hsfB1, entailing SA-imposed potentiation of HSP70 may be because of inflection of these Hsfs by SA (Snyman and Cronje, 2008). SA encourages thermotolerance all over the heat shock and stimulates the heat shock protein Hsp17.6, despite the fact that NahG has a little thermotolerance and a minimal level of Hsp17.6 (Clarke et al., 2004). HSP70 is concerned with the assembly and disassembly of multimeric structures and in the denaturation/dysfunction of defensive proteins in stress situations (Kim et al., 2007). HSP21 is located within the chloroplast alone, and a 21 kDa peptide was present in the grape leaves in SA-pre-treated and control leaves together. SA did not significantly ($P < 0.05$) vary the immune signal of HSP21 prior to heat stress. Once SA-pretreated and control leaves were stressed, they confirmed elevated levels of the immune signal together. On the other hand, in the course of recuperation, HSP21 levels in the SA-pre-treatment continued to be elevated till the termination of the experiment although their levels in the control diminished below pre-stress levels. In addition, SA geared up the increase of Pn chiefly owing to the fast recovery of PSII function after heat stress. These SA effects may possibly be associated to higher levels of HSP21 (Wang, 2010).

2.8 Salicylic acid biosynthesis and gene expression of biosynthesis pathways

The isochorismate (IC) pathway and the phenylalanine ammonia-lyase (PAL) pathway are responsible for the biosynthesis of SA. These pathways commence with chorismic acid, which is is synthesized in the plastid via shikimate pathway. In *Arabidopsis*, tobacco and tomato IC is the predominant pathway for SA synthesis (Catinot et al., 2008). Isochorismate synthase (ICS) transforms the chorismic acid to IC. A large number of plants, together with pepper, tobacco, rice, tomato, soybean, grape vine and poplar are known to have *ICS* homologs (Dewdney et al., 2000). In Arabidopsis, *ICS1/SID2* is up- regulated by biotic as well as abiotic stresses (Ogawa et al., 2005; Kilian et al., 2007; Wan et al., 2012). PAL pathway is the alternative pathway for SA biosynthesis where PAL catalyses the production of *trans*-cinnamic acid by deamination of phenylalanine. Varied phenolic compounds are synthesized through *trans*-cinnamic acid (Dempsey et al., 2011). *Ortho-coumaric* acid and benzoic acid are the two intermediates which convert *trans*-cinnamic acid to SA (Ylpani et al., 1993). SA accumulation in *Nicotiana benthamiana* depends on ICS activity: total SA levels enhanced more in wild-type plants than in plants with silenced ICS expression after UV irradiation treatment (Catinot et al., 2008). Alike other stressors, low temperature can also bring about endogenous SA accumulation in cucumber leaves, and this SA build up can be prevented by spraying with an inhibitor of SA biosynthesis, paclobutrazol. In addition, exogenous SA may possibly augment the plant’s own *de novo* SA synthesis, as it was exemplified by the alteration in free and bound SA levels in pea plants growing from seeds pre-treated with SA prior to sowing (Szalai et al., 2011).

2.9 ABA biosynthesis, catabolism and gene expression

Studies report abscisic acid (ABA) accumulation during abiotic stresses (Thomashow, 1999; Chinnusamy et al., 2004). ABA is also known to be engaged with the response to heat stress (Toh et al., 2008). In contrast to cold-sensitive lines, the cold-tolerant rice accumulated less ABA in response to cold treatment, which may be attributed to lower ABA biosynthesis as well as faster turnover of ABA (Oliver et al., 2007). ABA concentrations in plants are the consequence of equilibrium amid biosynthesis and catabolism (Nambara and Marion-Poll, 2005). The first committed step in ABA synthesis is catalyzed by 9-cis-epoxycarotenoid dioxygenase (NCED; Schwartz et al., 1997) that plays a regulatory function in several plant species (Qin and Zeevaart, 1999). Moreover, the gene encoding zeaxanthin epoxidase (ZEP) is also known to control the ABA levels (Frey et al., 1999). Out of a number of pathways for ABA catabolism, the 8'-hydroxylation of ABA to form phaseic acid seem to be the major pathway for ABA inactivation, which is catalyzed by the cytochrome P450 8'-hydroxylase (CYP707A; Nambara and Marion-Poll, 2005). The precise equilibrium concerning ABA synthesis and catabolism ascertains the ABA levels in plant tissues (Nambara and Marion-Poll, 2005). Ji et al. (2011) accounted that drought stress stimulates abscisic acid (ABA) biosynthesis genes in anthers and causes ABA accumulation in spikes of drought-sensitive wheat varieties. On the contrary, low ABA levels accumulate in drought-tolerant wheat, which in associated with lower ABA biosynthesis and higher ABA catabolic gene expression (ABA 8'-hydroxylase). Wheat TaABA8'-OH1 deletion lines were reported to be more drought sensitive and accumulate higher spike ABA concentrations. Initially, the rate of ABA accumulation was comparable in cold-sensitive and cold-tolerant lines for the first 8 h of cold treatment in cold stressed rice. However, in the tolerant rice lines, ABA catabolism decreased ABA levels between 8 and 16 h of cold treatment. Ji et al. (2011) reported that ABA and ABA 8'-hydroxylase play significant role in controlling anther ABA homeostasis and reproductive stage abiotic stress tolerance in cereals.

2.10 Yield response

The optimum temperatures for anthesis and grain filling stages in wheat varies from 12 - 22°C and the grain yield reduces drastically when wheat is exposed to temperatures beyond the optimal during these stages (Tewolde et al., 2006). Wardlaw and Wrigley (1994) observed that the high temperature stress at the time of anthesis results in increased abortion of florets. Heat stress during reproductive stage can bring about pollen sterility, dehydration of tissues, lesser assimilation of CO_2 and enhanced photorespiration (Kase and Catsky, 1984) which is not offset by the improved growth rate (Zahedi and Jenner, 2003). Nevertheless, Saini and Aspinal (1982) observed that temperatures greater than 30°C, during floret development, can instigate complete sterility. Consequently, the grain yield is affected due to the reduction in time to acquire resources when the temperatures rise during the anthesis up to the grain maturity. Devasirvatham et al. (2012) reported that elevated temperature stress influences the source and sink equally for assimilates and thus diminish crop yield.

Exposure to high temperatures considerably reduces grain weight and accelerates physiological maturity, i.e., curbing the grain filling phase in bread and durum wheat (Dias et al., 2000). Delayed sowing in wheat decreased the grain filling in several genotypes owing to the exposure to elevated temperature at the time of grain filling stage (Pandey et al., 2009). In a contemporary study, Prasad et al. (2011) reported that spring wheat plants raised in high temperature evinced a considerable decrease in number of grains per spike, total dry weight, grain yield and harvest index. Heat vulnerability has also been reported to be accountable for yield loss in *Arachis hypogea* and *Phaseolus vulgaris* (Vara Prasad et al., 1999; Rainey and Griffiths, 2005). Under high night temperature, reduction in the mean endosperm cell area caused shrinkage in grain length and width (Morita et al., 2005). Additionally, crops normally counter the high temperature stress by means of a proliferation in the rate of kernel growth, which causes a reduction in the extent of dry matter accumulation (Devasirvatham et al., 2012). In B-73 inbred line of maize, dry weight of kernels decreased from 79 to 95% in heat stress (Commuri and Jones, 2001). Shah and Paulsen, 2003 illustrated that grain biomass and sugar contents of kernels dropped off fast during high temperature stress. High temperatures have an effect on the endosperm development in maize and decreased the grain yield at the time of endosperm cell division (Monjardino et al., 2005). A decline in seed yield of sorghum by 10 and 99% due to the elevation in temperature from 32/22°C to 36/26°C and 40/30°C, correspondingly (Prasad et al., 2006). Djanaguiraman et al. (2010) and Mohammed and Tarpley, (2010) found noteworthy variations in yield attributes and yield of sorghum plants grown at HT (40/30°C). Johkan et al. (2011) reported that elevated temperature increased dark respiration which produced more immature grains and reduced yields in wheat. SA (0.05 mM) encouraged the growth of young wheat seedlings. This outcome was also evident in field experiments when elements of yield structure were evaluated. Plants pre-treated with SA were attributed by larger ear sizes, higher mass of 100 seeds and higher grain yield representing a supportive effect of the presowing treatment of the seeds, which generates a invigorative result on the productivity of wheat (Shakirova et al., 2003).

3. MATERIALS AND METHODS

The present study was performed in the Division of Plant Physiology, Indian Agricultural Research Institute, New Delhi (latitude 28°N and longitude 77°E, and about 250m above mean sea level), to understand the role of salicylic acid in terminal heat stress tolerance of wheat. In this respect three independent experiments were conducted. Two small experiments were done in-vitro/ in laboratory conditions. The first experiment was performed on wheat seedlings (10days old) in laboratory condition, to identify the optimum salicylic acid concentration based on plant growth and development of wheat genotypes under study. The second experiment were performed in 30 days old wheat plant, with single wheat genotype to see the response of optimum dose (identified in first experiment) of SA to heat stress during vegetative stage. The basic idea of second experiment was to confirm whether optimum dose of SA, which was identified in first experiment, was capable of making some positive changes in physiological, biochemical and molecular parameters or not under heat stress. In the third experiment, plants were grown in pot culture, Division of Plant Physiology, in two *rabi* seasons during 2013-14 and 2014-15, during November (normal sowing) and January (late sowing) both the year, following the recommended cultural practices. Optimum salicylic acid doses (0.1mM) (identified in seedling experiment) were exogenously applied during reproductive phase and all parameters (physiological, biochemical, molecular and yield etc.) were analyzed 2 and 10 days after foliar spray (DAFS) of SA, to confirm whether positive effects are consistent or transient in nature. All recommended agronomic package and practices were followed to raise a healthy wheat crop.

3.1 Experiment-1

Wheat (*Triticum aestivum* L.) seeds of equal size were selected and surface sterilized using 1 % sodium hypochlorite solution for 10 minutes and followed by washing five times with distilled water. Two sets of experiments were performed. The seeds were sown in petri plates lined with three layers of filter paper that had been moistened with distilled water for germination and allowed to grow in incubator for 6 days. On the sixth day after the germination, the plants were foliar pre-treated with 0.1, 0.25, 0.5, 1.0, 2.0, 3.0 mM of salicylic acid (SA) with water as the control. After one day, the pre-treated plants were subjected to heat stress (temperature, 39 ±

2°C) for 12 hrs and then recovered under non-stressed conditions (temperature, 25 ± 2°C) for 5 days in the incubator/growth chamber. In second set of experiment the plants were only treated with different concentrations of SA (0.1, 0.25, 0.5, 1.0, 2.0, 3.0 mM) with water as control and grown under ambient/ unstressed conditions for 5 days after SA treatments. And then shoot length, root length and total seedling length were measured in different sets of plants. The germinated seeds were raised in to seedlings with one-fourth strength Hoagland nutrient medium (Hoagland and Arnon, 1950).

3.2 Experiment-2

About 6 cm long seedlings of genotype HD3086 were transplanted in pots filled with soil and four to six plants were maintained in each pot. Pots were supplied with only double distilled water. After twenty five days of transplantation, the plants were foliar pre-treated with 0.1 mM of salicylic acid (SA). One days later, the pre-treated plants were subjected to heat stress (temperature, 39 ± 2°C) for 36 h and then recovered under non-stressed conditions (temperature, 25 ± 2°C) for 24 h. Uppermost leaves from all the treated and control plants were sampled after heat shock and analysed for biochemical, physiological and molecular parameters.

3.3 Experiments-3

A pot culture experiment was conducted in completely randomized design (CRD) with three replications using four wheat varieties (two relatively tolerant and two susceptible, as suggested by wheat breeder). The experiments were performed at pot culture facility of Division of Plant Physiology, IARI, in the year of 2013-14 and 2014-15. The staggered sowing strategy was used for terminal heat stress treatment. The sowing was done at two dates during both the cropping season: normal (20th November, 2013), late (9th January, 2014) and normal (20th November, 2014), late (9th January, 2015) to induce varied and prolonged high temperature stress conditions during reproductive stage. All recommended package and practices were followed to raise a healthy wheat crop. When anthesis started, foliar application of salicylic acid (SA) (0.1mM) was done and distilled water was sprayed on control plant.

3.4 Plant Material

Four wheat genotypes were used in the study, viz., HD3086, HD2985, HD3043, and HD3076 as suggested by the wheat breeder. Among these, HD3086 and HD 2985 were relatively heat stress tolerant while and HD3043 and HD3076 were heat susceptible. The wheat genotypes were obtained from the wheat breeder, Division of Genetics, IARI, New Delhi.

3.5 Weather data

The data of maximum and minimum temperature during the cropping season from November (2013) to April (2014) and November (2014) to April (2015) is presented in Table 3.1 and Table 3.2.

3.6 Sample collection

Flag leaves were collected from control and SA treated wheat genotypes at 2 days and ten days after foliar spray (DAFS) of SA for biochemical estimations. Physiological parameters were also measured in flag leaf at 2 DAFS and 10 DAFS of SA in all samples while, for gene expression study only 2 DAFS samples were used. For estimation of remobilization efficiency, stem reserve mobilization and water soluble carbohydrates (WSC) in stem (including leaf sheath); five main stems were harvested 10 days after anthesis (in November sown crop) and just after anthesis in January (late sown crop) and stems at physiological maturity. The main stem were harvested and instantly dried in an oven at 80°C for 28 h to minimise respiration and weight losses. The penultimate internodes were used for estimation of non-structural carbohydrates (NSC) analysis and remobilization efficiency (RE) was also calculated.

3.7 Observation recorded

3.7.1 Membrane stability Index (MSI)

Membrane stability index (MSI) was estimated according to the procedure illustrated by Premachandra et al. (1990). For evaluation of membrane stability index 100 mg of the leaf material was taken in two sets in test tubes containing 10 ml of double distilled water. One set was heated at 40ºC for 30 min in a metabolic water bath, and the electrical conductivity of the solution was recorded on a conductivity bridge (C1). Second set was boiled at 100ºC on a boiling water bath for 10 min, and its conductivity was measured on a conductivity bridge (C2). Membrane stability index (MSI) was calculated as:

$MSI = [1 - (C1/C2)] * 100$

Table 3.1: Temperature regimes (°C) during anthesis (started), 2DAFS and 10 DAFS (from November 2013-April 2014).

		Temperature regimes (°C) of normal sowing (21-11-2013)				Temperature regimes (°C) of late sowing (9-01-2014)			
		HD2985	**HD3086**	**HD3076**	**HD3043**	**HD2985**	**HD3086**	**HD3076**	**HD3043**
Anthesis started	Date	02-03-2014	26-02-2014	02-03-2014	04-03-2014	27-03-2014	27-03-2014	27-03-2014	30-03-2014
	Max.	19.6	24.0	19.6	24.0	31.0	31.0	31.0	31.5
	Min.	7.4	10.4	7.4	10.6	18.8	18.8	18.8	15.8
	Mean	13.5	17.2	13.5	17.3	24.9	24.9	24.9	23.7
1st Sampling	Date	04-03-2014	28-02-2014	04-04-2014	06-03-2014	29-03-2014	29-03-2014	29-03-2014	01-04-2014
	Max.	24.0	24.0	24.0	25.4	32.0	32.0	32.0	32.8
	Min.	10.6	11.4	10.6	9.7	16.6	16.6	16.6	14.2
	Mean	17.3	17.7	17.3	17.6	24.3	24.3	24.3	23.5
2nd Sampling	Date	12-03-2014	08-03-2014	12-03-2014	14-03-2014	06-04-2014	06-04-2014	06-04-2014	07-04-2014
	Max.	25.6	24.6	25.6	25.0	35.0	35.0	35.0	37.6
	Min.	12.9	10.6	12.9	10.0	17.4	17.4	17.4	20.4
	Mean	19.3	17.6	19.3	17.5	26.2	26.2	26.2	29.0

Table 3.2: Temperature regimes (°C) during anthesis (started), 2DAFS and 10 DAFS (from November 2014-April 2015).

		Temperature regimes (°C) of normal sowing (21-11-2014)				**Temperature regimes (°C) of late sowing (9-01-2015)**			
		HD2985	**HD3086**	**HD3076**	**HD3043**	**HD2985**	**HD3086**	**HD3076**	**HD3043**
Anthesis started	Date	03-03-2015	27-02-2015	03-03-2015	05-03-2015	25-03-2015	25-03-2015	25-03-2015	30-03-2015
	Max.	21.0	26.0	21.0	22.4	33.2	33.2	33.2	34.2
	Min.	10.5	8.9	10.5	7.6	15.9	15.9	15.9	18.9
	Mean	15.8	17.5	15.8	15.0	24.6	24.6	24.6	26.6
1st Sampling	Date	05-03-2015	01-03-2015	05-03-2015	07-03-2015	27-03-2015	27-03-2015	27-03-2015	03-04-2015
	Max.	22.4	27.2	22.4	26.0	35.2	35.2	35.2	33.4
	Min.	7.6	14.4	7.6	10.2	20.2	20.2	20.2	18.4
	Mean	15.0	20.8	15.0	18.1	27.7	27.7	27.7	25.9
2nd Sampling	Date	13-03-2015	08-03-2015	13-03-2015	14-03-2015	03-04-2015	03-04-2015	03-04-2015	11-04-2015
	Max.	27.0	26.0	27.0	27.4	33.4	33.4	33.4	34.4
	Min.	12.2	12.4	12.2	13.2	18.4	18.4	18.4	17.2
	Mean	19.6	19.2	19.6	20.3	25.9	25.9	25.9	25.8

3.7.2 SPAD chlorophyll meter reading

Soil and plant analyser development (SPAD) values were determined in the centre of flag leaves by means of portable Minolta SPAD-502 chlorophyll meter (Minolta camera Co. Ltd., Osaka, Japan) from control plants and SA treated leaves. The average readings of 10 leaves per pot was recorded and used in analysis.

3.7.3 Chlorophyll and carotenoid content

Chlorophyll and carotenoid content were measured by extracting 0.05 g of the leaf material in 10 ml dimethylsulfoxide (DMSO) (Hiscox and Israelstam, 1979). The method for determination of chlorophyll content in plants is footed on the captivation of light by chlorophyll extracts made by incubating the leaf tissues in DMSO (dimethyl sulfoxide). DMSO confers permeability to the plasmalemma and consequently triggers the leaching of the pigments (Hiscox and Israelstam, 1979). The absorbance of the known volume of solution comprising known amount of leaf tissue at two individual wavelengths (663 nm and 645 nm) was ascertained for chlorophyll content as well as at 470 nm for total carotenoid contents (Lichtenthaler and Welburn, 1983). Chlorophyll content was measured employing the formula specified by Arnon, (1949) and expressed as mg g^{-1} DW.

3.7.4 Photosynthesis rate

Leaves were classified into green and yellow/dead leaves, and the rate of photosynthesis (Pn) was estimated by means of Infrared Gas Analyser (IRGA), LI-6400XT Model (Li-COR Ltd., Lincoln, Nebraska, USA) by commissioning the IRGA in the closed mode amid 10.00-11.00 a.m. when parameters like, relative humidity (50-60%,) temperature (30 to 35^{o}C), photosynthetic photon flux density (1200 µmol $m^{-2}s^{-1}$) and CO_2 concentration (350 to 360 µmol mol^{-1}) were in required range reading from IRGA were taken. Fifteen flag leaves per treatment were chosen arbitrarily for Pn estimation and expressed in µmol CO_2 m^{-2} s^{-1}.

3.7.5 Hydrogen peroxide content

Hydrogen peroxide content was determined as per the procedure explained by Rao et al., 1997.

Reagents:

1. Titanium reagent: One-gram titanium dioxide and 10 g potassium sulphate were digested in 150 ml concentrated sulphuric acid on a hot plate for 4 h. The digested mixture was diluted to 500-600 ml and stirred with a magnetic stirrer cum heater at 70-80^{o}C till the solution became clear transparent. It was further diluted to 1.5 liter and stored in dark brown bottle.
2. Acetone: Analytical grade reagent
3. Liquid ammonia: Analytical grade reagent

Estimation

One gram of the leaf material was crushed to fine powder using liquid nitrogen, and subsequent addition of 10 ml chilled acetone. The resultant mixture was filtered with Whatman No. 1 filter paper following the addition of 4 ml titanium reagent and 5 ml liquid ammonia solution to precipitate the titanium-hydro peroxide complex. Reaction mixture was centrifuged at 10000 g for 10 min at 4^{o}C. The precipitate was suspended in 10 ml 2M H_2SO_4 and then centrifuged again. Supernatant was read at 415 nm against reagent blank in UV-visible spectrophotometer. Concentration of hydrogen peroxide was calculated by referring to a standard curve made from known concentrations of hydrogen peroxide ($y = 0.124\,x$) and was expressed as µmolg^{-1}DW.

3.7.6 Lipid peroxidation (TBARS content)

Lipid peroxidation was estimated as the thiobarbituric acid reactive substances (TBARS), adhering to the method given by Heath and Packer (1968).

Reagents:

1. Trichloroacetic acid solution (TCA, 0.1%)
2. Thiobarbituric acid reagent (TBA, 0.5%)

Estimation:

0.5 g leaf sample were homogenized in 10 ml TCA, then centrifugation were done at 15,000 g for 15 min. Finally 1.0 ml aliquot of the supernatant and 4.0 ml of 0.5% TBA was added. The mixture was kept in water bath at 95ºC for 30 min and then cooled to room temperature. Again centrifuged at 10,000 g for 10 min and then the absorbance of the supernatant were taken at 532 nm and 600 nm in spectrophotometer.

3.8.1 Antioxidant enzyme activity

3.8.1.1Enzymatic antioxidant activity

3.8.1.2 Estimation of superoxide dismutase (SOD) activity

The SOD activity was estimated based on formation of blue coloured formazone, adhering to the method given by (Dhindsa et al., 1981).

Grinding media: Phosphate buffer (0.1 M), pH 7.5 and also added 0.5 mM EDTA for extraction of SOD, CAT, GR and POX

Extraction of enzyme

Grinding of 1.0 g leaf sample was done in extraction buffer, the homogenates were filtered and after filtration centrifuged for 20 min at 15000 g and the finally supernatant was used as enzyme.

Enzyme assay

Three ml of the reaction mixture was prepared by addition of the following reagents in a given sequence

1. 13.33 mM methionine (0.2 ml of 200mM)
2. 75 µM nitroblue tetrazolium chloride (NBT) (0.1 ml of 2.25 mM)
3. 0.1 mM EDTA (0.1 ml of 3mM)
4. 50 mM phosphate buffer (pH 7.8) (1.5 ml of 100 mM)
5. 50 mM sodium carbonate (0.1 ml of 1.5M)
6. 0.1 ml enzyme
7. 0.9 to 0.95 ml of water (to make a final volume of 3.0 ml)

Two micro molar riboflavin (0.1 ml) were added in a reaction mixture and exposed to 85 W fluorescent lamps for 15 min, and then kept the tube in dark to stop the reaction. For control we added entire reaction mixture without enzyme that gave maximal colour. The absorbance was recorded at 560 nm. The enzyme activity was expressed as units per mg protein per hour.

3.8.1.3 Estimation of Catalase (CAT) Activity

The CAT activity was estimated, adhering to the method given by Aebi et al., (1984).

Preparation of Enzyme Extract

Homogenigetion of leaf samples (1g) were done with 10 ml extraction buffer containing. Homogenate was filtered and filtrate was centrifuged at 15000g for 20 minutes and finally the supernatant was used for estimation of enzyme.

Enzyme Assay

Reaction mixture was prepared with the following reagents

1. Potassium phosphate buffer 50 mM
2. Enzyme (50 µL)
3. Water (950 µL)

After addition of freshly prepared hydrogen peroxide (0.5 ml) reaction start and absorbance was recorded for 1 minute at 240 nm.

3.8.1.4 Estimation of glutathione reductase (GR) activity

The total Glutathione reductase activity was estimated following the method described by Smith et al., (1988).

Preparation of enzyme extract

Grinding of 1.0 g leaf sample was done in extraction buffer, the homogenates were filtered and after filtration centrifuged for 20 min at 15000 g and the finally supernatant was used as enzyme.

Enzyme assay

The reaction mixture was prepared with the following reagents:

1. 0.5 mM DTNB
2. 66.67 µM NADPH
3. 666.67µM GSSG
4. 0.1 ml enzyme extract

Distilled water to make up a final volume of 3.0 ml

Finally 0.1 ml of 20.0 mM GSSG (oxidized glutathione) added and then increase in absorbance at 412 nm was recorded spectro-photometrically.

3.8.1.5 Estimation of Peroxidase (POX) activity

The total peroxidase activity was estimated following the method described by Castillo et al. (1984).

Preparation of Enzyme Extract

Grinding of 1.0 g leaf sample was done in extraction buffer, the homogenates were filtered and after filtration centrifuged for 20 min at 15000 g and the finally supernatant was used as enzyme.

Enzyme Assay

The reaction mixture was prepared with the following reagents

1. Potassium phosphate buffer 50 mM
2. Guaiacol 16 mM (0.5 ml of 96 mM)
3. Hydrogen peroxide 2mM (0.5 ml of 12 mM)
4. Enzyme (0.1 ml)
5. Water (0.4 ml) and the total volume were made up to 3 ml.

Finally absorbance was recorded at 470nm (owing to the formation of tetra-guaiacol).

3.8.1.6 Estimation of Ascorbate peroxidase (APOX) activity

The total Ascorbate peroxidase activity was determined following the procedure described by Nakano and Asada, (1981).

Enzyme assay

Ascorbate peroxidase was assayed by documenting the decrease in optical density due to ascorbic acid at 290 nm. The 3 ml reaction mixture included 50 mM potassium phosphate buffer (pH 7.0) (1.5 ml of 100 mM), 0.5 mM ascorbic acid (0.5 ml of 3.0 mM), 0.1 mM EDTA (0.1 ml of 3.0 mM), 0.1 mM H_2O_2 (0.2/0.6 ml of 1.5 mM), 0.1 ml enzyme and water 0.6 ml (to make a final volume of 3.0 ml). To start the reaction 0.2 ml of hydrogen peroxide added. Decline in absorbance for a period of 30 seconds was recorded at 290 nm in an UV-visible spectrophotometer (model Specord Bio-200, Analytik Jena, Germany).

3.9.1 Non enzymatic antioxidant Metabolites

3.9.1.2 Reduced Ascorbic Acid

Dipyridyl-ferric chloride reagents methods

Extraction

Leaf samples were prepared for ascorbic acid (AsA) by homogenising 1g fresh leaf material in 10 ml of cold 5% metaphosphoric acid. The homogenate was centrifuged at 22000 g for 15 min at 4^{o}C, and the supernatant was collected for the assay of AsA. AsA were analyzed as per Law et al. (1983).

Assay

Plant extracts (aliquot)	0.3 ml
Phosphate buffer 150 mM, pH 7.4 (5 mM EDTA)	0.75 ml
Water	0.3 ml

Colour was developed in the reaction mixtures by addition of the following:

TCA 10%	0.6ml
Ortho-phosphoric acid 44%	0.6 ml
Dipyridyl 4% in 70% ethanol	0.6 ml
$FeCl_3$ 0.3%	0.3 ml

Before recording the absorbency at 525nm the reaction mixture vortexed and then incubated at 40^{o}C for 40 minutes. The concentration was estimated by referring to a standard curve of reduced ascorbic acid in the range of 0-100 µg ml^{-1}.

3.9.1.3 Oxidized Glutathione

Extraction

Leaf samples were prepared for oxidized glutathione (GSSG) by homogenising 1g fresh leaf material in 10 ml of cold 5% metaphosphoric acid. The homogenate was centrifuged at 22000 g for 15 min at 4^{o}C, and the supernatant was collected for assay of glutathione.

Assay

GSSG were assayed according to the methods given by Smith, (1985).

The 3 ml reaction mixture for glutathione content included the following:

0.2 mM NADPH	0.1 ml of 6.0 mM
100 mM phosphate buffer, pH 7.5	0.6 ml of 0.5 mM
5mM EDTA	0.1 ml of 150 mM
0.6 mM DTNB	0.1 ml of 18 mM
3 units of GR	1 ml
Water	1 ml

Reaction was initiated by adding 0.1 ml of extract samples as explained above. The reaction rate was examined by recording the difference in absorbance at 412 nm for 1 min.

The concentration of glutathione is quantified by referring to a standard curve based on GSSH in the range of 0 to 50 µm ml^{-1}.

3.10.1 Estimation of non-structural carbohydrates

Stems were ground to obtain fine powder. Non-structural carbohydrates (NSC) analysis was conducted on two fractions, ethanol soluble carbohydrate (ESC) comprising primarily mono and disaccharides and water-soluble carbohydrate (WSC) including mostly fructans and oligosaccharides, in consistent with the technique described by Virgona and Barlow, (1991). The total NSC was estimated by addition of ESC and WSC fractions and estimated by the anthrone method (Yemm and Willis, 1954).

Estimation of total NSC

To 1.0 ml of sugar sample, 4 ml of anthrone regent was added and the mixture was heated on a boiling water bath for 8 minutes with subsequent cooling. The optical density of green to dark green colour was read at 630 nm in UV-visible spectrophotometer. A blank and two freshly prepared fructose standards were also incorporated with each set of samples.

3.10.2 Mobilised non-structural carbohydrates and remobilisation efficiency (RE)

Mobilization efficiency was estimated as described by Ehdaie et al. (2006)

Mobilised NSC (MNSC) = Maximum NSC – Minimum NSC

Remobilization efficiency = (MNSC/ Maximum NSC) × 100.

Where, Maximum NSC = NSC present in anthesis + 10 sample (penultimate internodes part of stem)

Minimum NSC= NSC present in harvested sample of stem (penultimate internodes)

3.11.1 Estimation of endogenous level of abscisic acid (ABA) and Salicylic acid (SA) by HPLC

3.11.1.1 Estimation of abscisic acid (ABA)

ABA in the leaf sample was estimated by using HPLC, pursuing the procedure of Zeevart, 1980.

Preparation of sample

Frozen leaf samples (2g) were extracted three times with 10 ml of 80 % v/v acetone (80 ml acetone, 1 ml glacial acetic acid and 100 mg of 2, 6 di-tert-butyl 4-methyl phenol in a total volume of 100 ml) and collected in a 100 ml volumetric flask. The tissue residue was thereafter homogenized with pestle and mortar with acetone (80 % v/v). The homogenate was filtered through Whatman No. 1 filter. The filtrate together with the extracts prepared earlier was shifted to the boiling flask of rotary flash vacuum evaporator for eliminating the acetone. As the acetone evaporated, the lipid soluble material formed sediments on the walls of the round bottomed boiling flask. This was dissolved in 1% acetic acid solution and the amber coloured aqueous solution was poured into small vials.

Conditions of assay

Detector: A variable wavelength 150 UV-visible detector.

Injector: Rheodyne injector (20 µl loop).

Column/stationary phase: The column used is µBondapak TM/C18 P/N 841/6 S/N. The stationary phase consisted of a Lichrosorb C-18.

Mobile phase: 1 % acetic acid in 95 % methanol solution attached to the pump.

Flow rate: 2.5 ml/min

Wavelength: Wavelength was set at 265 nm before injecting the sample.

The concentration of ABA present in the sample was computed by calculating the area under the peak using the formula as described in the principle of the instrument ($A = ½ b \times h$), and comparing it with the standard curve prepared with known concentrations of ABA. Standard curve was prepared by plotting concentrations on X-axis and respective areas (of the peak) of the standard ABA on Y-axis.

3.11.1.2 Estimation of free and total salicylic acid

Salicylic acid was extracted using the procedure specified by O’Donnell et al. (2001) with modifications. Six gram tissue of flag leaves was crushed in liquid N_2 and extracted in 15ml of 90% methanol followed by 10 ml of 100% methanol in a centrifuge at 12000 rpm for 15 minutes. The pooled extracts were separated into two and dried independently by rotary evaporator. For free SA determination, residues were re-suspended in 1.5ml of 100% methanol and subjected to HPLC analysis. For conjugated SA, residue were hydrolysed in 2M HCl for 60 min at 65^0C and the ensuing fractions were subsequently extracted twice in diethyl ether, dried down and re-suspended in methanol. Salicylic acid was identified and quantified by reverse-phase HPLC (Varian, Prostar) equipped with quaternary pump, UV detector and connected with Rheodyne injection system using Lichrospher C-18 stainless steel column (4×250 mm i.d), acetonitrile: 0.1% aqueous o-phosphoric acid (40:60) as a mobile phase at a flow rate of 1.0 min^{-1} and at a wavelength of 230 nm. The recovery of SA estimated by extracting tissue to which a known amount of SA has been added. The results were assessed from the graph prepared by running the standards (Sigma) of different concentrations.

3.12.1 Gene expression study for selected enzymes/proteins by sqRT-PCR

Nucleotide sequences for candidate genes were acquired from National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). The Basic Local Alignment Search Tool (http://www.ncbi.nlm.nih.gov/BLAST/) was used to recognize the homologs of candidate genes. For sqRT-PCR expression analysis, the oligonucleotide primers were designed (Table 3.3) manually, and oligo quality (to avoid primer dimmer, self-dimer etc.), GC % and Tm were examined by using Oligoanalyzer 3.0 tool (http://www.idtdna.com/analyzer/Applications/OligoAnalyzer/, Intergrated DNA Technologies, Coralville, IA 52241, USA).

3.12.1.1 Isolation of total RNA from leaf sample

Total RNA was extracted from leaf tissues by using TRIzol® reagent (Invitrogen, USA). Approximately 100 mg sample was used for isolating the RNA. The sample was finely ground using liquid nitrogen in a pre-chilled pestle and mortar. The tissue powder along with a little liquid nitrogen was decanted into 2ml eppendorf tube followed by immediate addition of 1000 µL of TRIzol to the tubes. Every tube was capped and the cells were lysed by vortexing briskly for 3 minutes. The samples were incubated for 10 minutes at room temperature after which 200 µL of chloroform was added to each tube and vortexed vigorously for 1 min. Samples were again incubated for 1 minute at room temperature. The samples were centrifuged at 13,000 rpm for 20 minutes at 4°C for phase separation. Three phases were observed viz., lower brown, middle 1-2 mm chloroform-phenol having DNA and upper aqueous phase having RNA. The upper aqueous phase alone was transferred to a fresh tube. RNA was precipitated by adding 600 µL of chilled isopropyl alcohol. Tubes were inverted up and down to mix and incubated in -20°C for 30 min. After incubation, the samples were centrifuged for 10 minutes at 12000 g at 4°C. Finally, the RNA formed a pellet. The supernatant was discarded and the pellet was washed with 1000µL of 80 % ethanol twice, before centrifugation at 12000 g for 1 minute at 4°C. The pellet was air dried for 5-10 minutes; however, complete drying of pellet was avoided. The pellet was dissolved in 50 to 80 µl RNase free water or autoclaved double distilled water and incubated for 3 minutes at 36°C. RNase-free DNase I (Promega, USA) was applied to remove contaminating genomic DNA at 37°C for 1 h. Quality and integrity of total RNA were then determined by running appropriate amount in a formamide denaturing gel, and quantity of total RNA was determined using a NanoDrop™ 1000 spectrophotometer (Thermo Fisher Scientific, USA). The first-strand cDNA was synthesized according to the instructions of the cDNA Synthesis Superscript® III First- Strand Synthesis System (Invitrogen, USA). Resulting cDNA was stored at -20°C and employed as template for two-step RT-PCR reactions following recommended conditions provided in user's manual. Sequences of gene specific forward and reverse primers employed are

Table 3.3 Primer sequence and amplicon size of genes

Gene	Sequence	Amplicon size (bp)
TaActin	F>>AATACAGTGTCTGGATCGGTG R>>GGTACACATCTTCTACAGAACAC	215
TahsfA1a	F>>GCTTTCATCCTCTTCCTCTG R>>GGGTTCTTGGAGGAGTAGAG	200
TahsfA2b	F>>ACATCAGGCGTCGTAAAC R>>CTATCCTCCATGGCTTTCAG	191
Tahsp101	F>>CTCACACAGGCGAACATTAG R>>CCACCACAAGAATCACTTC	200
Tahsp70	F>>CAA GTC TCT TGG AAG CTT CCG ACTG R>>CTC ACC ACG ATC CAT TGA CCA TC	611
Tahsp16.9	F>>AGGAGATCGACGAGAACTG R>>CATGACCCGCATCTTCTTC	200
Tahsp17.8	F>>AGATGATGCGCAAGTTCG R>>GCTACTCTCTGCTTCGATTC	195
TaICS	F>>ATCCTCTCCTTCACCTTCAG R>>ATGAACGTGTTGGGCTTG	191
TaNCED	F>>GCACGGAGAACGTGAAGAAC R>>CGCTCTGCTCTAGTTAGACTC	200
Ta6-FEH	F>>TCTTGGAGGGAAGCTCATAC R>>GGTTGTCGTTGACAGAGTTG	190
TapsbA	F>>GAGAGACGCGAAAGTACAAG R>>AGAAACAGGCTCACGAATAC	189
TapsbO	F>>AGAAGACCATCACCGACTAC R>>TAGTACTGCTTACCGTCCAC	178

described in Table 3.2. Every RT-PCR measurement was performed at least thrice. Expression of *TaActin* was used as an internal standard for normalization.

3.12.1.2 Agarose Gel Electrophoresis

Material and Reagents

1. Agarose

2. 5X TBE Buffer (pH 8.0)

Tris base	54 g
Boric acid	27.5 g
EDTA 0.5 M (pH 8.0)	20 ml
Final volume	1000 ml with distilled water

The buffer was autoclaved and stored at room temperature.

3 Gel loading dye (6X): 10 mM Tris-HCI (pH 7.6), 0.03 % bromophenol blue, 0.03 % xylene cyanol FF, 60 % glycerol and 60 mM EDTA

4. Ethidium bromide stock solution: 10 mg per ml in water

5 Mini gel apparatus and power supply

6 Gel documentation system: Alpha Imager

Procedure:

Agarose gel was prepared by heat dissolution of agarose in 1X TBE buffer. Ethidium bromide from stock solution (10 mg ml^{-1}) was added to a final concentration of 0.5 mg $ml^{-1}$of gel. Once the gel was ready, it was placed in the electrophoresis tank after removing the comb and tape. The electrophoresis tank was filled with 1X TBE buffer till the gel was completely submerged. The total RNA samples were mixed with appropriate volume of 6X loading dye before loading. The samples were loaded and run at 5 V/cm, with the help of an electric supply. After one hour of electrophoresis, the agarose gel was viewed using gel documentation system.

3.12.1.3 sqRT-PCR expression analysis of target genes

Semi quantitative Reverse transcriptase-polymerase chain reaction (sqRT-PCR) mixture using Qiagen One Step RT PCR Kit with gene specific and degenerate forward and reverse primers was prepared as per the protocol given below:

Reagents	**Quantity for 50 µl of reaction mixture**
Nuclease free water	21.0 µl
5X RT buffer (Tris Cl, KCI, $(NH_4)_2SO_4$, 13.5 mM $MgCI_2$, DTT; pH 8.7 at 20 °C)	10.0 µl
dNTP Mix (10 mM each)	2.0 µl
Forward + Reverse primers (10 µM)	5.0 µl
RNA template (1 µg)	10.0 µl
RT Enzyme mix	2.0 µl
Total volume	50.0 µl

The above reaction mixture was prepared in 0.2 ml PCR tubes and reactions were conducted using QB 96 Thermal cycler (Quanta Biotech, England), under the following conditions. Number of cycles was standardized as 25-27 cycles by conducting semi quantitative RT-PCR, amplified products were not visible in susceptible cultivars when the number of cycles was less than 25.

Reverse Transcription 30 min 50 ºC

Initial PCR activation step 15 min 95 ºC

3-step cycling

Denaturation 1 min 94 ºC

Annealing 1 min 57 ºC

Extension 2 min 72 ºC

Number of cycles 27

Final extension 10 min 72 ºC

Linear amplification for semi-quantitative RT-PCR was obtained with 27 cycles. To check the amplification, an aliquot of 5 µl of the reaction mixture was run on a 0.8 % (w/v) agarose gel stained with 0.5 µg ml^{-1} ethidium bromide. 100bp DNA ladder was included as a marker for size comparison of the amplified products. 1X TAE buffer was used to prepare the gel as well as for running buffer and electrophoresis was carried out at 5 V/cm (Sambrook et al., 1989). 6X loading dye were used to load the samples. The stained DNA products were photographed using Gel Documentation system.

3.14.1 Yield and yield related parameters

The yield associated parameters like, 1000-grain weight, grain weight per plant and total biomass per plant were recorded after harvest, from which harvest index (HI) were computed using the formula specified below:

Harvest Index (HI): HI = [Economic yield / Biological Yield] x 100

3.15.2 Statistical analysis

Experiments were conducted in completely randomized design. Statistical analysis of the data was done following the methods of analysis of variance (ANOVA) (Panse and Sukhatme, 1967). The critical difference was calculated at 5% probability level.

4. RESULTS

Present study was done to understand the role of salicylic acid in terminal heat stress tolerance of wheat. In this regard extensive studies were done at seedling, vegetative and reproductive stages and three independent experiments were formulated. Two experiments were done *in-vitro*/ in laboratory conditions. The first experiment was performed on wheat seedlings (10 days old) in laboratory condition, to identify the optimum salicylic acid concentration based on plant growth and development of wheat genotypes under study. The second experiment was performed in 30 days old wheat plant, with single wheat genotype (HD3086) to see the response of optimum dose (identified in first experiment) of SA to heat stress during vegetative stage. It was observed that 0.1mM SA concentration effectively/positively modulated the physiological as well as biochemical traits and gene expression in wheat plants. The third experiment was conducted to see the response of optimum dose of SA during reproductive stage of four wheat genotypes under study which were grown in two rabi seasons *viz.* 2013-14 and 2014-15, during normal (control) and late sown conditions (to induce high temperature stress during reproductive phase). All the parameters were analyzed at 2 and 10 DAFS of SA, to confirm whether positive effects are consistent or transient in nature. Results of various experiments conducted during this study are reported below:

4.1 Experiment 1: Identification of optimum exogenous salicylic acid concentrations for the genotypes under study based on phenotypic changes (shoot length, root length and plant height) in control and heat-stressed wheat seedlings.

4.1.1 Effect of different concentrations of SA on shoot length, root length and plant height

Different concentration of SA (0.1, 0.25, 0.5, 1.0, 2.0 and 3.0 mM) were foliar applied on wheat seedlings 24 h before exposure to heat stress (39 ± 2°C, in incubator) as well as on control plant (not exposed to heat stress). The result of the effect of foliar spray of different concentrations of SA on growth (shoot length, root length and plant height) of wheat seedling of two wheat genotypes (HD3086 and HD3076) were presented in Fig 4.1.1. High temperature stress (39 ±2 ^{0}C) significantly retarded the growth of shoot length, root length and plant height of both the genotypes and maximum retardation occurs in HD3076 compared to HD3086. However, pre-treatment with lower concentration (0.1mM and 0.25mM SA) helped in maintenance of higher growth which was reflected in terms of higher shoot, root and total length of seedlings compared to respective control (heat stressed without SA treated plants), however, it was still lower than plant grown under ambient conditions (control plant). Pre-treatment with higher concentrations (beyond 0.5mM) of SA severely retarded the growth of seedlings (shoot length, root length and plant height) of heat stressed plants. In control plant pre-treatment with lower concentration of SA (0.1mM and 0.25mM) did not make any significant phenotypic change in any genotypes, however, concentrations 0.5mM and above retarded the growth (shoot length, root length and plant height) in both the genotypes.

4.2 Experiment 2: Effects of SA on biochemical, physiological and molecular traits in wheat genotype subjected to heat stress during vegetative stage

4.2.1 Effect of SA on H_2O_2 and TBARS contents

Lipid peroxidation (TBARS content) and H_2O_2 contents are important indicators of oxidative stress. The result of the effect of foliar application of SA (0.1mM) on TBARS and H_2O_2 contents in upper most leaf of 30 days old wheat genotypes (HD3086) was recorded in control, heat stressed without SA treated plant and heat stressed with SA treated plant (Fig 4.2.1). The TBARS and H_2O_2 contents increased significantly under heat stress without SA treated plant as compared to control plant (without heat stress). The application of SA minimised the content of H_2O_2 (37.5%) and TBARS (22.9%) compared with heat stressed without SA treated, however, it was still 74% and 10% higher than non-stressed control plant, respectively.

4.2.2 Effect of SA on antoxidant enzymes activity

The result of the effect of foliar application of SA (0.1mM) on superoxide dismutase (SOD), ascorbate peroxidase (APOX) and catalase (CAT) activity in upper most leaf of 30 days old wheat genotypes (HD3086) recorded in control, heat stressed without SA treated plant and heat stressed with SA treated plant are presented in Fig 4.2.2. The SOD, APOX and CAT activity enhanced significantly under heat stress without SA treated plant as compared to control plant (without heat stress). The application of SA further enhanced the activity of SOD (33.3%), APOX

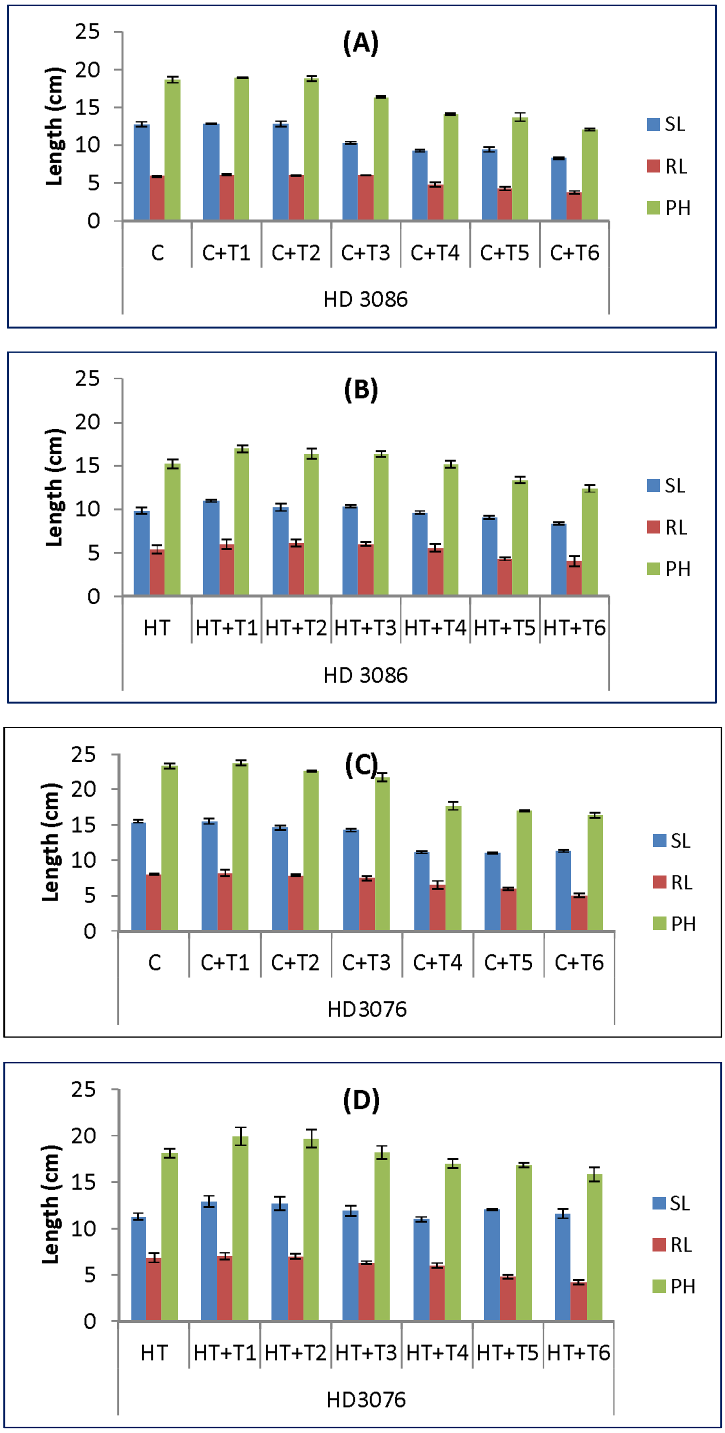

Fig 4.1.1: Effect of different concentrations (T1=0.1, T2=0.25, T3=0.5, T4=1.0, T5=2.0, and T6=3.0 mM) of salicylic acid on growth [shoot length (SL), root length (RL) and plant height (PH)] of 10 days old control (C) (grown in ambient condition) and heat-stressed (HT) seedlings of wheat genotypes HD3086 (A,B) and HD3076 (C, D).

(32.6%) significantly and reduced the catalase (19%) activity compared to heat stressed without SA treated plant.

4.2.3 Effect of SA on total chlorophyll, photosynthesis rate (Pn), photochemical efficiency (Fv/Fm)

The result of the effect of foliar application of SA (0.1mM) on total chlorophyll, Pn and Fv/Fm in upper most leaf of 30 days old wheat genotypes (HD3086) recorded in control, heat stressed without SA treated plant and heat stressed with SA treated plant are presented in Fig 4.2.3. The total chlorophyll, Pn and Fv/Fm decreased significantly under heat stress without SA treated plant as compared to control plant (without heat stress). The application of SA increased the total chlorophyll (71%), Pn (82%) and Fv/Fm (33%) compared to heat stressed without SA treated plant. However, these parameters were still lower than control plant (without heat stress).

4.2.4 RT-PCR expression analysis of photosynthesis genes and *HSP70* in wheat leaves at vegetative stage

The results of the effect of exogenous foliar application of salicylic acid on *TapsbA* gene expression in the leaf (vegetative stage) of wheat genotypes grown under high temperature stress are presented in plate 1. The *TapsbA* expression study was done in one wheat genotype (HD3086) in vegetative stage sample. In case of *TapsbA* gene, sqRT-PCR was performed with gene specific primers and expected amplicons of size 189 bp were obtained in treated as well as in its respective control. The expression of *TapsbA* in control sample (without heat stress treatment) was higher as compared to heat stress and salicylic acid treated plant. The expression was more in salicylic acid + heat stress treated plant as compared to heat stressed plant alone.

In case of *TapsbO* gene, sqRT-PCR was performed with gene specific primers and expected amplicons of size 180 bp were obtained in treated and respective control (plate 1). The expression of *TapsbO* in control sample (without heat stress treatment) was higher as compared to heat stress and salicylic acid + heat stress treated leaf sample. In salicylic acid + heat stress treated plant, expression was more but not so obvious compared to heat stressed plant alone, although it was less compared to control plants.

In case of *TaHSP70* gene, sqRT-PCR was performed with gene specific primers and expected amplicons of size 611 bp were obtained in both treated and its respective control (plate 1). The expression of *TaHSP70* in control sample (without heat stress treatment) was less compared to heat stressed and salicylic acid + heat stress treated leaf sample. The expression of *Tahsp70* was induced under high temperature stress compared to control plant (without heat stress treated). In salicylic acid + heat stress treated plant expression was enhanced compared to both heat stressed and control plants.

4.3.1 Experiment 3: Effects of SA (0.1mM) on biochemical and physiological traits in four wheat genotypes grown in pot culture at 2 and 10 DAFS (days after foliar spray) under normal and high temperature stress (induced by late sowing)

4.3.1.1 TBARS contents (Lipid peroxidation)

Result of the effect of SA on lipid peroxidation in terms of TBARS contents in flag leaf of four wheat genotypes (details of temperature regimes during SA treatment and sampling are mentioned in table 3.1) recorded at 2 and 10 days after foliar spray (DAFS) under control (normal sown) and temperature stress (induced by late sowing) conditions during flowering stage (reproductive stage) are presented in Table 4.3.1.1 and Fig 4.3.1.1. The TBARS contents increased significantly in all genotypes grown under late sown (high temperature stress) condition as compared to the normal sown condition. Foliar spray of SA decreased TBARS contents significantly in late sown crop with the maximum decrease validated in HD3086 (16%) and HD2985 (14%) and minimum in HD3076 (9%), however, 10 DAFS the decreased content started to increase in all the genotypes. Almost similar trends were observed in both the years regarding response to SA and lipid peroxidation in wheat genotypes. The TBARS contents which were reduced upon SA spray started increasing 10 DAFS in late sown conditions.

4.3.1.2 Membrane stability index (MSI)

Result of the effect of SA on membrane stability index (MSI) of flag leaf of four wheat genotypes recorded at 2 and 10 DAFS under control (normal sown) and temperature stress (induced by late sowing) conditions during flowering stage are presented in Table 4.3.1.2 and Fig 4.3.1.2. The MSI reduced significantly in all

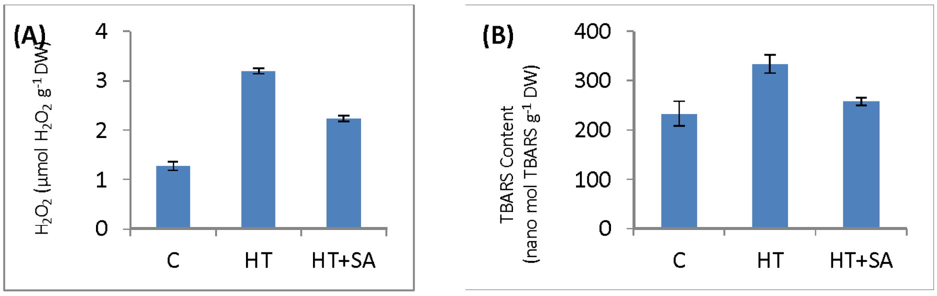

Fig 4.2.1: Effect of foliar spray of salicylic acid on (A) hydrogen peroxide contents (µmol H_2O_2 g^{-1} DW) and (B) lipid peroxidation (nmol TBARS g^{-1} DW) in leaves of 30 days old wheat plant grown under heat stress. (C=control, HT=high temperature, HT+SA=high temperature + salicylic acid)

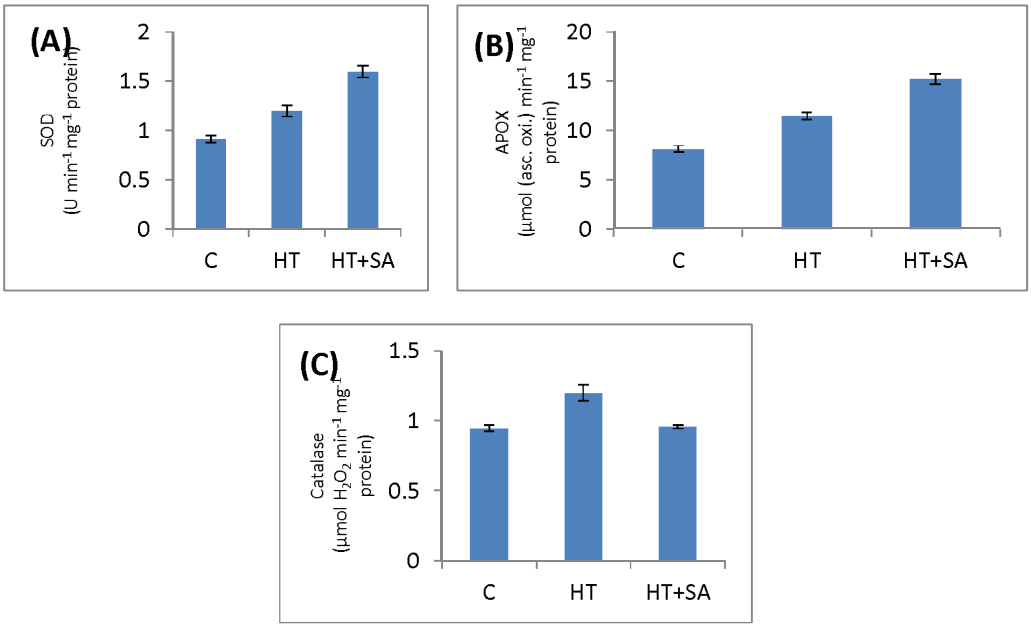

Fig 4.2.2: Effect of foliar spray of salicylic acid on activity of (A) superoxide dismutase (units mg^{-1} protein min^{-1}) (B) ascorbate peroxidise (µmol H_2O_2 reduced mg^{-1} protein min^{-1}) and (C) catalase (µmol H_2O_2 reduced mg^{-1} protein min^{-1}) in leaves of 30 days old wheat plant grown under heat stress. (C=control, HT=high temperature, HT+SA=high temperature + salicylic acid)

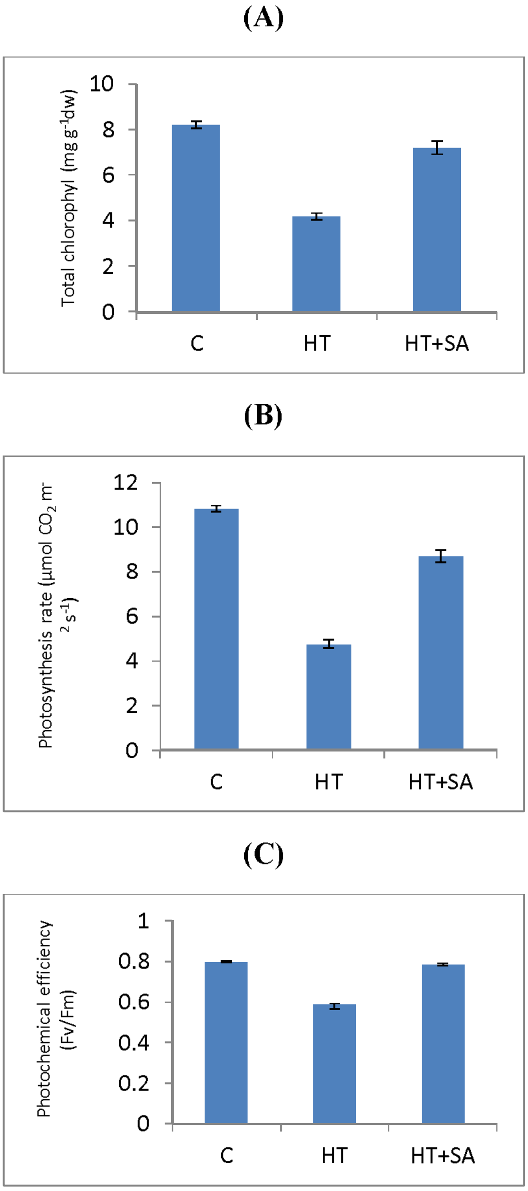

Fig 4.2.3: Effect of foliar spray of salicylic acid on (A) total chlorophyll (mg g^{-1} DW) (B) photosynthesis (µmolCO_2 m^{-2} s^{-1}) and (C) photochemical efficiency of PSII (Fv/Fm) in leaves of 30 days old wheat plant grown under heat stress. (C=control, HT=high temperature, HT+SA=high temperature + salicylic acid)

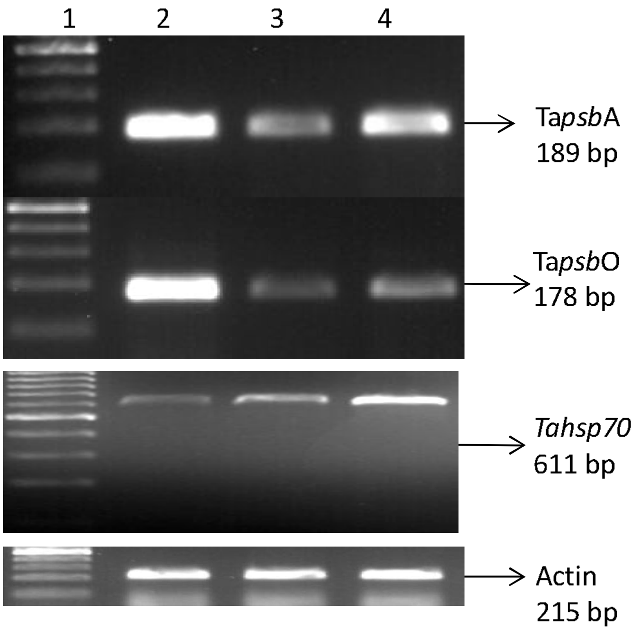

Plate 1: sqRT-PCR expression analysis of *TapsbA, TapsbO and Tahsp70* genes in wheat leaves of 30 days old plant. Lane -1 100 bp DNA marker, Lane -2 control (non stress condition (25 ± 2 °C) without salicylic acid) , Lane - 3 heat stress (39 ± 2 °C, for 36 h) without salicylic acid, lane- 4 heat stress + foliar spray of salicylic acid (24 h before exposer to stress).

Table 4.3.1.1: Effect of SA on the lipid peroxidation (nmol TBARS g^{-1}DW) in wheat genotypes at 2 and 10 DAFS (Days after foliar spray) under normal and high temperature stress (induced by late sowing) conditions during reproductive stage.

Time (Days)	Genotypes	Lipid peroxidation (nmol TBARS g^{-1}DW)							
		Normal sown				Late sown			
		C	C+SA	Mean	%change	HT	HT+SA	Mean	%change
2DAFS	HD2985	729.05	712.3	720.675	-2.30	983.75	841.9	912.825	-14.42
	HD3086	748.25	723.3	735.775	-3.33	1025.4	861.4	943.4	-15.99
	HD3076	740.45	741.2	740.825	0.10	1160.75	1052.55	1106.65	-9.32
	HD3043	762.15	735.55	748.85	-3.49	1091.45	921.25	1006.35	-15.59
10DAFS	HD2985	732.9	721.8	727.35	-1.51	1025.45	964.25	994.85	-5.97
	HD3086	752.25	739.75	746.00	-1.66	1082.8	1012.1	1047.45	-6.53
	HD3076	788.3	790.65	789.475	0.30	1239.8	1239.80	1239.8	0.00
	HD3043	751.3	748.15	749.725	-0.42	1162.45	1091.95	1127.2	-6.06

	Factors	Sowing (S)	Treatment (T)	S×T	Variety (V)	S×V	T×V	S×T×V
2DAFS	C.D.	28.925	28.925	40.907	40.907	57.851	N/A	N/A
10DAFS	C.D.	21.788	21.788	30.813	30.813	43.577	N/A	N/A

C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid

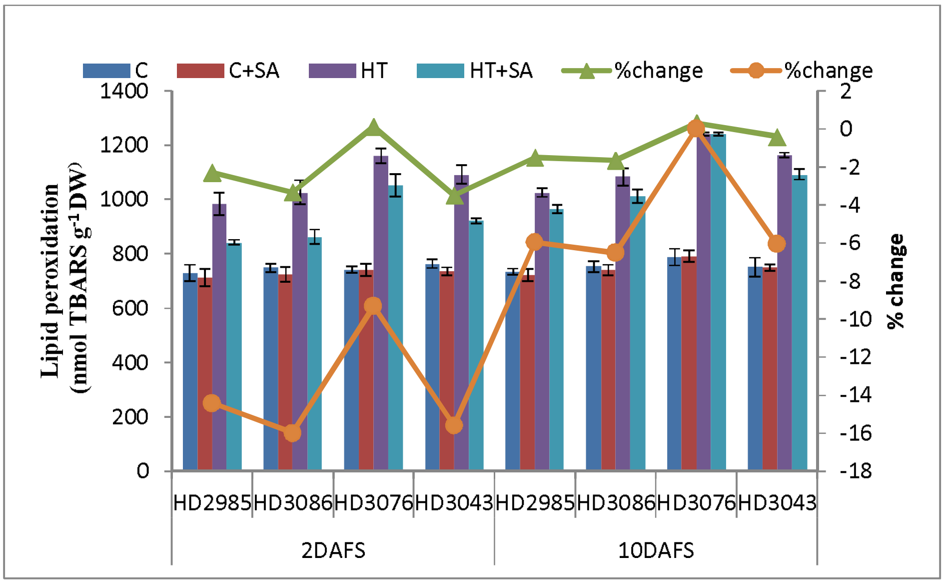

Fig. 4.3.1.1: Effect of SA on the lipid peroxidation (nmol TBARS g^{-1}DW) in wheat genotypes at 2 and 10 DAFS (Days after foliar spray) under normal and high temperature stress (induced by late sowing) conditions during reproductive stage. (C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid)

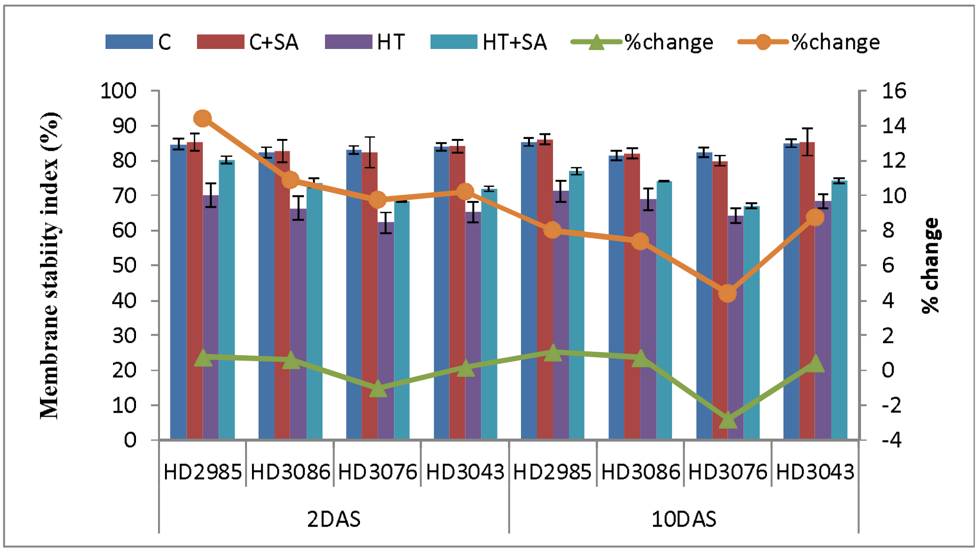

Fig. 4.3.1.2: Effect of SA on the membrane stability index (%) in wheat genotypes at 2 and 10 DAFS (Days after foliar spray) under normal and high temperature stress (induced by late sowing) conditions during reproductive stage. (C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid)

genotypes grown under late sown (high temperature stress) condition in contrast to the normal sown condition, with maximum decrease measured in HD3076 (28%) and minimum in HD2985 (20%). Foliar spray of SA maintained significantly higher MSI in late sown condition. 2 DAFS of SA, the MSI enhanced significantly in the late sown crop, with maximum increase evinced equally by HD 2985 (15%) and HD3086 (15%) and minimum by HD3076 (7%). Even after 10 days of foliar spray under late sown conditions MSI was maintained higher from its respective control. The MSI which was improved after SA spray was still maintained higher in tolerant genotypes than its respective control. The effect of SA on MSI was almost similar in both the cropping seasons in wheat genotypes.

4.3.1.3 Peroxidase (POX) activity

Result of the effect of SA on peroxidise activity, recorded at 2 and 10 days after foliar spray (DAFS), in flag leaf of four wheat genotypes grown under control (normal sown) and temperature stress (induced by late sowing) conditions during flowering stage (reproductive stage) are presented in Table 4.3.1.3 and Fig 4.3.1.3. As compared to normal sown condition, peroxidase activity increased significantly in all genotypes grown under late sown (high temperature stress) conditions with the greatest increase evinced by HD2985 (21%). Foliar spray of SA also enhanced peroxidase activity significantly in normal sown crop with maximum increase observed in HD2985 (9.36%) and minimum 4.82% recorded in HD3043, however, 10 DAFS the increased activity declined in all the genotypes apart from HD3043 which still retained the activity 6.2% excess of the untreated plants. The peroxidase activity significantly increased under late sown condition, 2 days after foliar spray (DAFS) of SA, with highest activity corroborated by HD 2985 (29.69%) and HD3086 (29.04) and least activity by HD3076 (15.36%), however, 10 days after foliar spray under late sown conditions peroxidise activity declined, albeit it was greater than its respective control. The POX activity that improved upon SA spray was retained at 15.93% higher in HD2985 (tolerant genotypes) but in susceptible genotypes it was barely 4.18% higher than the respective control.

4.3.1.4 Catalase (CAT) activity

Result of the effect of SA on catalase activity in flag leaf of four wheat genotypes recorded at 2 and 10 DAFS under control and stress are presented in Table 4.3.1.4 and Fig 4.3.1.4. In contrast to normal sown condition, catalase activity augmented significantly under late sown (high temperature stress) condition in all genotypes with the highest increase measured in HD2985 (12%). Foliar spray of SA reduced the catalase activity significantly in normal sown as well in late sown crops. Under late sown condition, 2 DAFS of SA, a significant drop in the catalase activity in all genotypes was measured with most of the decrease substantiated in HD3076 (18%) and least decrease in HD2985 (15%), however, 10 days after foliar spray the catalase activity enhanced, even though it was reasonably lower (10-11%) than its respective control.

4.3.1.5 Superoxide dismutase (SOD) activity

The findings of the consequence of SA on SOD activity in flag leaf of four wheat genotypes, recorded at 2 and 10 DAFS grown under control and stress are presented in Table 4.3.1.5 and Fig 4.3.1.5. The SOD activity relatively increased evidently under late sown (high temperature stress) conditions as compared to the normal sown condition in all genotypes with the maximum increase evinced in HD2985 (26%) and minimum in HD3076 (15%). Foliar spray of SA further enhanced the SOD activity significantly in normal sown and in late sown crops, together, however, 10 DAFS the increased activity dropped in all the genotypes. Under late sown condition, 2 DAFS of SA, the SOD activity significantly increased in HD 2985 (19%) and minimum in HD3076 (16%), although, 10 days after foliar spray, under late sown conditions SOD activity decreased, even though it was still higher than respective control. The SOD activity that improved after SA spray was sustained at 12% higher in HD2985 (tolerant genotypes) and 6.2% higher in susceptible genotypes from their respective controls subsequent to 10DAFS.

4.3.1.6 Glutathione reductase (GR) activity

Result of the effect of SA on GR activity in flag leaf of four wheat genotypes recorded at 2 and 10 DAFS grown under control and stress are presented in Table 4.3.1.6 and Fig 4.3.1.6. Distinct from the normal sown condition, GR activity increased significantly under late sown (high temperature stress) condition, in all genotypes with maximum increase observed in HD2985 (26%) followed by HD3086 (25%) and minimum increase in HD3076 (13%). In addition, the foliar spray of SA enhanced GR activity significantly, in normal sown crop with maximum increase measured in HD2985 (9%) and minimum in HD3076 (5%), but, 10 DAFS the increased activity decreased in all the genotypes. Under late sown condition, 2 days

Table 4.3.1.2: Effect of SA on the membrane stability index (%) in wheat genotypes at 2 and 10 DAFS (Days after foliar spray) under normal and high temperature stress (induced by late sowing) conditions during reproductive stage.

		Membrane stability index (%)							
		Normal sown				Late sown			
Time (Days)	**Genotypes**	**C**	**C+SA**	**Mean**	**%change**	**HT**	**HT+SA**	**Mean**	**%change**
2DAFS	HD2985	84.64	85.29	84.965	0.77	70.12	80.23	75.175	14.42
	HD3086	82.27	82.78	82.525	0.62	66.38	73.61	69.995	10.89
	HD3076	83.15	82.3	82.725	-1.02	62.28	68.35	65.315	9.75
	HD3043	83.98	84.12	84.05	0.17	65.32	72	68.66	10.23
10DAFS	HD2985	85.31	86.2	85.755	1.04	71.2	76.9	74.05	8.01
	HD3086	81.41	82	81.705	0.72	69	74.1	71.55	7.39
	HD3076	82.3	80	81.15	-2.79	64.28	67.12	65.7	4.42
	HD3043	84.95	85.3	85.125	0.41	68.31	74.3	71.305	8.77

	Factors	**Sowing (S)**	**Treatment (T)**	**S×T**	**Variety (V)**	**S×V**	**T×V**	**S×T×V**
2DAFS	C.D.	0.297	0.297	0.421	0.421	0.595	0.595	0.841
10DAFS	C.D.	0.255	0.255	0.361	0.361	0.51	0.51	0.722

C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid

Table 4.3.1.3: Effect of SA on the peroxidase activity (µmol TG mg^{-1} protein min^{-1}) in wheat genotypes at 2 and 10 DAFS (Days after foliar spray) under normal and high temperature stress (induced by late sowing) conditions during reproductive stage.

Time (Days)	Genotypes	Peroxidase activity (µmol TG mg^{-1} protein min^{-1})							
		Normal sown				Late sown			
		C	C+SA	Mean	%change	HT	HT+SA	Mean	%change
2DAFS	HD2985	122.4	133.8	128.1	9.36	148.2	192.2	170.2	29.69
	HD3086	116.9	125.1	121.0	7.02	137.1	176.9	157.0	29.04
	HD3076	103.0	111.1	107.1	7.86	122.3	141.8	132.0	15.90
	HD3043	118.4	124.1	121.2	4.82	133.2	165.4	149.3	24.14
10DAFS	HD2985	124.1	127.4	125.7	2.70	140.0	162.3	151.2	15.93
	HD3086	115.8	119.3	117.5	3.02	125.5	143.3	134.4	14.14
	HD3076	98.7	100.9	99.8	2.23	107.7	112.2	110.0	4.18
	HD3043	116.1	123.4	119.7	6.24	132.6	143.8	138.2	8.45

	Factors	Sowing (S)	Treatment (T)	S×T	Variety (V)	S×V	T×V	S×T×V
2DAFS	C.D.	4.826	4.826	6.826	6.826	N/A	N/A	N/A
10DAFS	C.D.	4.254	4.254	6.016	6.016	N/A	N/A	N/A

C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid

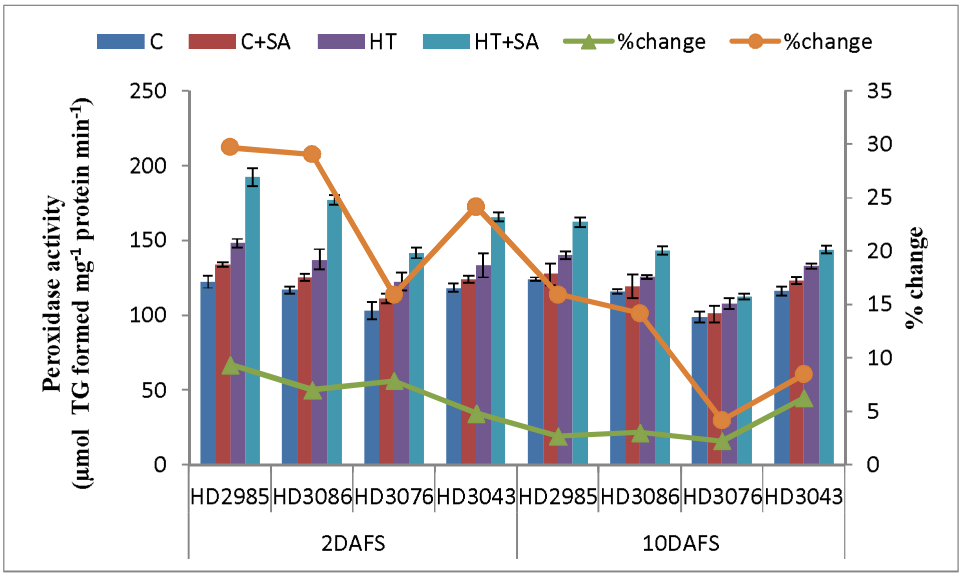

Fig. 4.3.1.3: Effect of SA on the peroxidase activity (µmol TG mg^{-1} protein min^{-1}) in wheat genotypes at 2 and 10 DAFS (Days after foliar spray) under normal and high temperature stress (induced by late sowing) conditions during reproductive stage. (C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid)

Table 4.3.1.4: **Effect of SA on the catalase activity (µmol H_2O_2 reduced mg^{-1} protein min^{-1}) in wheat genotypes at 2 and 10 DAFS (Days after foliar spray) under normal and high temperature stress (induced by late sowing) conditions during reproductive stage.**

		Catalase activity (µmol H_2O_2 reduced mg^{-1} protein min^{-1})							
		Normal sown				Late sown			
Time (Days)	**Genotypes**	**C**	**C+SA**	**Mean**	**%change**	**HT**	**HT+SA**	**Mean**	**%change**
2DAFS	HD2985	5.48	5.00	5.24	-8.76	6.12	5.22	5.67	-14.71
	HD3086	4.98	4.61	4.80	-7.43	5.59	4.70	5.15	-15.92
	HD3076	3.51	3.31	3.41	-5.70	3.89	3.21	3.55	-17.48
	HD3043	4.98	4.51	4.75	-9.44	5.38	4.55	4.97	-15.43
10DAFS	HD2985	5.98	5.77	5.88	-3.51	6.43	5.75	6.09	-10.58
	HD3086	5.58	5.28	5.43	-5.38	6.11	5.50	5.81	-9.98
	HD3076	4.09	3.89	3.99	-4.89	3.98	3.51	3.75	-11.81
	HD3043	5.41	5.11	5.26	-5.55	5.69	5.31	5.50	-6.68

	Factors	**Sowing (S)**	**Treatment (T)**	**S×T**	**Variety (V)**	**S×V**	**T×V**	**S×T×V**
2DAFS	C.D.	0.211	0.211	0.298	0.298	N/A	N/A	N/A
10DAFS	C.D.	N/A	0.216	N/A	0.306	N/A	N/A	N/A

C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid

Table 4.3.1.5: Effect of SA on the superoxide dismutase activity (units mg^{-1} protein min^{-1}) in wheat genotypes at 2 and 10 DAFS (Days after foliar spray) under normal and high temperature stress (induced by late sowing) conditions during reproductive stage.

Time (Days)	Genotypes	Superoxide dismutase activity (units mg^{-1} protein min^{-1})							
		Normal sown				Late sown			
		C	C+SA	Mean	%change	HT	HT+SA	Mean	%change
2DAFS	HD2985	4.96	5.31	5.14	7.06	6.28	7.48	6.88	19.11
	HD3086	4.48	4.59	4.54	2.46	5.23	6.11	5.67	16.83
	HD3076	3.17	3.25	3.21	2.52	3.58	3.89	3.74	8.66
	HD3043	4.61	4.91	4.76	6.51	5.25	5.87	5.56	11.81
10DAFS	HD2985	5.03	5.17	5.10	2.78	5.85	6.51	6.18	11.28
	HD3086	4.39	4.41	4.40	0.46	5.07	5.59	5.33	10.26
	HD3076	3.44	3.49	3.47	1.45	3.84	4.08	3.96	6.25
	HD3043	4.62	4.67	4.65	1.08	5.10	5.58	5.34	9.41

	Factors	Sowing (S)	Treatment (T)	S×T	Variety (V)	S×V	T×V	S×T×V
2DAFS	C.D.	0.286	0.286	N/A	0.404	0.571	N/A	N/A
10DAFS	C.D.	0.266	0.266	N/A	0.376	N/A	N/A	N/A

C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid

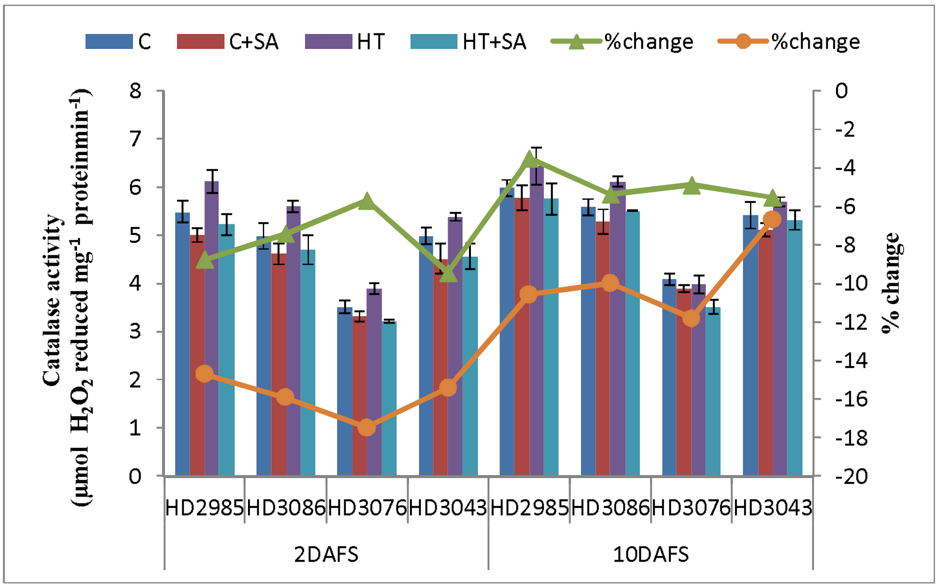

Fig. 4.3.1.4: Effect of SA on the catalase activity (µmol H_2O_2 reduced mg^{-1} protein min^{-1}) in wheat genotypes at 2 and 10 DAFS (Days after foliar spray) under normal and high temperature stress (induced by late sowing) conditions during reproductive stage. (C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid)

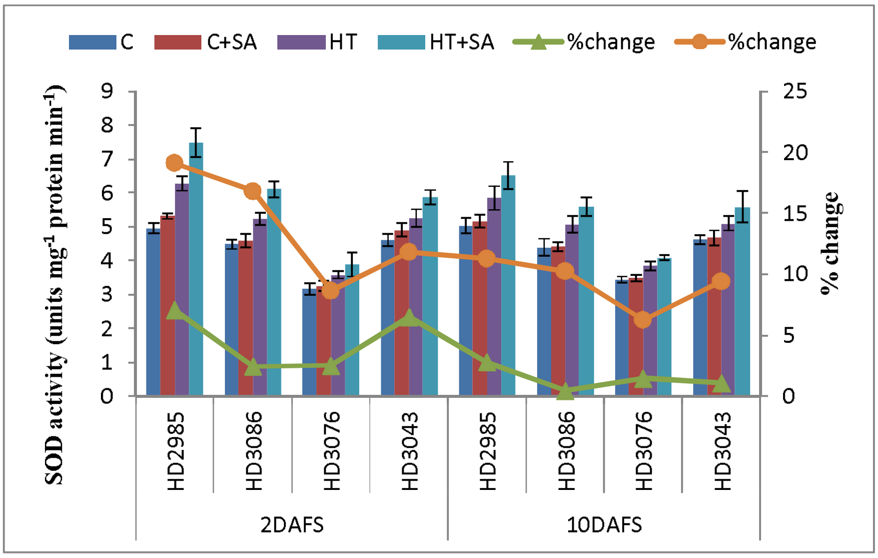

Fig 4.3.1.5: Effect of SA on the superoxide dismutase activity (units mg^{-1} protein min^{-1}) in wheat genotypes at 2 and 10 DAFS (Days after foliar spray) under normal and high temperature stress (induced by late sowing) conditions during reproductive stage. (C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid)

after foliar spray (DAFS) of SA, the GR activity enhanced significantly with maximum activity observed in HD 2985 (22%) followed by HD3086 (29.04 %) and minimum in HD3076 (11%), however, 10 days after foliar spray under late sown conditions GR activity decreased, even though it was still higher than its respective control. The GR activity that enhanced upon SA spray was sustained 11 % higher in HD2985 (tolerant genotypes) while, in susceptible genotypes it was only 7 % higher than its respective control.

4.3.1.7 Ascorbate peroxidise (APOX) activity

Result of the effect of SA on APOX activity in flag leaf of four wheat genotypes recorded at 2 and 10 DAFS raised under control and stress are presented in Table 4.3.1.7 and Fig 4.3.1.7. In comparison to normal sown condition, APOX activity increased significantly under late sown (high temperature stress) condition in all the genotypes with highest increase quantified in HD2985 (49%) followed by HD3086 (41%) and minimum in HD3043 (28%). Besides, the foliar spray of SA enhanced the APOX activity considerably in the normal sown crop with maximum increase determined in HD2985 (7%) and minimum in HD3076 (5%), though, 10 DAFS the increased activity diminished in all the genotypes. Under late sown condition, 2 DAFS of SA, the APOX activity enhanced significantly with maximum activity being observed in HD 2985 (25%) followed by HD3086 (25.28%) and minimum in HD3076 (18%), conversely, 10 days after foliar spray under late sown conditions, the APOX activity decreased but was still higher than its respective control. The APOX activity that increased upon SA spray was maintained 12% higher in HD3086, but in susceptible genotypes it was simply 6.4% higher than its respective control.

4.3.1.8 Oxidised glutathione (GSSG) contents

Result of the effect of SA on GSSH contents in flag leaf of four wheat genotypes recorded at 2 and 10 DAFS grown under control and stress are presented in Table 4.3.1.8 and Fig 4.3.1.8. In comparison to normal sown condition, the GSSG contents decreased significantly under late sown (high temperature stress) condition in all genotypes with maximum decrease of 22% recorded in HD2985 and 20% in HD3086 and minimum of 10% decrease in HD3076. Foliar spray of SA also decreased the GSSG contents significantly in normal sown crop along with a greatest increase measured in HD2985 (9%) and minimum in HD3076 (7%), however, 10

DAFS the decreased GSSG content began to increase in all the genotypes. Under late sown condition, 2 DAFS of SA the GSSG contents significantly decreased with a maximum decrease observed in HD2985 (20%) and minimum in HD3076 (11%), however, 10 days after foliar spray under late sown conditions GSSG contents started increasing but it was still lower than its respective control. The GSSG contents which was lowered upon SA spray started increasing 10DAFS.

4.3.1.9 Reduced ascorbate (AsA) contents

Result of the effect of SA on AsA contents in flag leaf of four wheat genotypes recorded at 2 and 10 DAFS grown under control and stress are presented in Table 4.3.1.9 and Fig 4.3.1.9. In comparison to the normal sown condition, the genotypes grown under late sown (high temperature stress) condition illustrated significant decrease in AsA contents in all genotypes with maximum decrease evinced by HD2985 and minimum by HD3073. Foliar spray of SA also decreased AsA contents significantly in normal sown crop and maximum decrease was substantiated by HD2985 (9%) and minimum by HD3076 (7%), however, 10 DAFS the decreased content started to increase in all the genotypes. Under late sown condition, 2 DAFS of SA, the AsA activity decreased significantly with maximum decrease was corroborated by HD 2985 (17%) and minimum by HD3076 (9%), however, 10 days after foliar spray under late sown conditions AsA contents increased abruptly in tolerant genotypes HD2985 but it was still lower than its respective control. The AsA contents which decreased upon SA spray started increasing 10DAFS in late sown conditions.

4.3.1.10 Photochemical efficiency of PSII (Fv/Fm)

Result of the effect of SA on photochemical efficiency of PSII presented in terms of ratio of maximum to variable fluorescence (Fv/Fm), in flag leaf of four wheat genotypes recorded at 2 and 10 DAFS grown under control and stress are presented in Table 4.3.1.10 and Fig 4.3.1.10. The Fv/Fm reduced significantly under late sown (high temperature stress) condition as compared to normal sown condition, in all genotypes with maximum decrease corroborated in HD3043 (22%) and HD3076 (24%) and minimum in HD2985 (11%). Foliar spray of SA maintained higher photochemical efficiency of PSII significantly in late sown condition. 2 DAFS of SA the efficiency of PSII ratio enhanced significantly in late sown crop, with maximum increase measured in HD 2985 (8.3%) followed by HD3086 (7.81%) and

Table 4.3.1.6: **Effect of SA on the glutathione reductase activity (µmol glutathione reduced mg^{-1} protein min^{-1}) in wheat genotypes at 2 and 10 DAFS (Days after foliar spray) under normal and high temperature stress (induced by late sowing) conditions during reproductive stage.**

Time (Days)	Genotypes	Glutathione reductase activity (µmol glutathione reduced mg^{-1} protein min^{-1})							
		Normal sown				Late sown			
		C	C+SA	Mean	%change	HT	HT+SA	Mean	%change
2DAFS	HD2985	625.20	681.00	653.10	8.93	788.21	958.20	873.21	21.57
	HD3086	615.50	670.12	642.81	8.87	772.51	925.68	849.10	19.83
	HD3076	517.80	545.30	531.55	5.31	584.20	650.10	617.15	11.28
	HD3043	611.80	657.12	634.46	7.41	698.21	798.60	748.41	14.38
10DAFS	HD2985	637.70	652.28	644.99	2.29	825.31	914.20	869.76	10.77
	HD3086	625.37	631.29	628.33	0.95	792.34	854.10	823.22	7.79
	HD3076	519.32	521.98	520.65	0.51	591.20	631.20	611.20	6.77
	HD3043	623.51	627.95	625.73	0.71	711.25	775.30	743.28	9.01

	Factors	Sowing (S)	Treatment (T)	S×T	Variety (V)	S×V	T×V	S×T×V
2DAFS	C.D.	21.103	21.103	29.845	29.845	42.206	N/A	N/A
10DAFS	C.D.	0.214	0.214	0.303	0.303	0.428	N/A	N/A

C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid

Table 4.3.1.7: Effect of SA on the ascorbate peroxidase activity (µmol H_2O_2 reduced mg^{-1} protein min^{-1}) in different wheat genotypes at 2 and 10 DAFS (Days after foliar spray) under normal and high temperature stress (induced by late sowing) conditions during reproductive stage.

Time (Days)	Genotypes	Ascorbate peroxidase activity (µmol H_2O_2 reduced mg^{-1} protein min^{-1})							
		Normal sown				Late sown			
		C	C+SA	Mean	%change	HT	HT+SA	Mean	%change
2DAFS	HD2985	6.11	6.57	6.34	7.53	9.12	11.35	10.24	24.45
	HD3086	5.80	6.25	6.03	7.76	8.15	10.21	9.18	25.28
	HD3076	5.10	5.25	5.18	2.94	7.32	8.65	7.99	18.17
	HD3043	6.10	6.40	6.25	4.92	7.81	9.51	8.66	21.77
10DAFS	HD2985	6.51	6.71	6.61	3.07	9.00	10.00	9.50	11.11
	HD3086	5.81	5.95	5.88	2.41	6.51	7.31	6.91	12.29
	HD3076	4.81	4.86	4.84	1.04	5.00	5.32	5.16	6.40
	HD3043	6.13	6.25	6.19	1.96	6.21	6.82	6.52	9.82

	Factors	Sowing (S)	Treatment (T)	S×T	Variety (V)	S×V	T×V	S×T×V
2DAFS	C.D.	0.302	0.302	0.427	0.427	0.604	N/A	N/A
10DAFS	C.D.	0.214	0.214	0.303	0.303	0.428	N/A	N/A

C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid

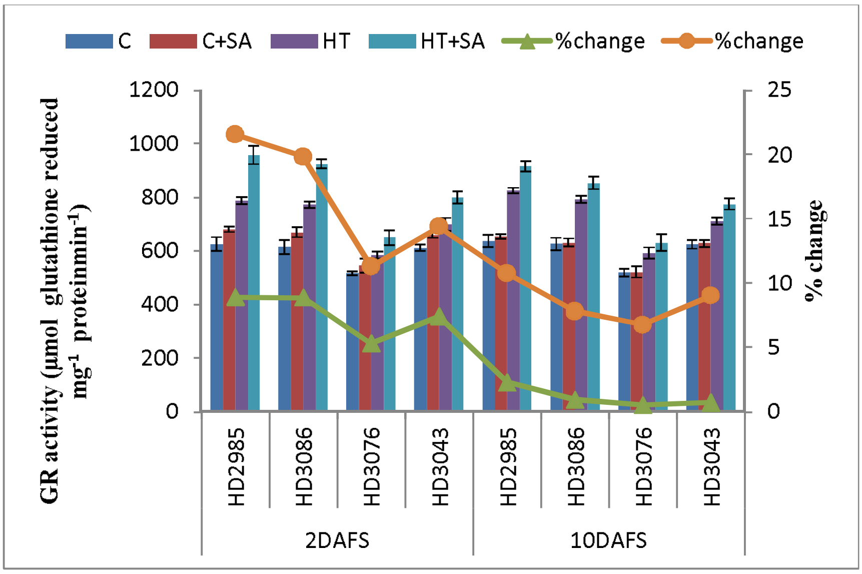

Fig. 4.3.1.6: Effect of SA on the glutathione reductase activity (µmol glutathione reduced mg^{-1} protein min^{-1}) in wheat genotypes at 2 and 10 DAFS (Days after foliar spray) under normal and high temperature stress (induced by late sowing) conditions during reproductive stage. (C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid)

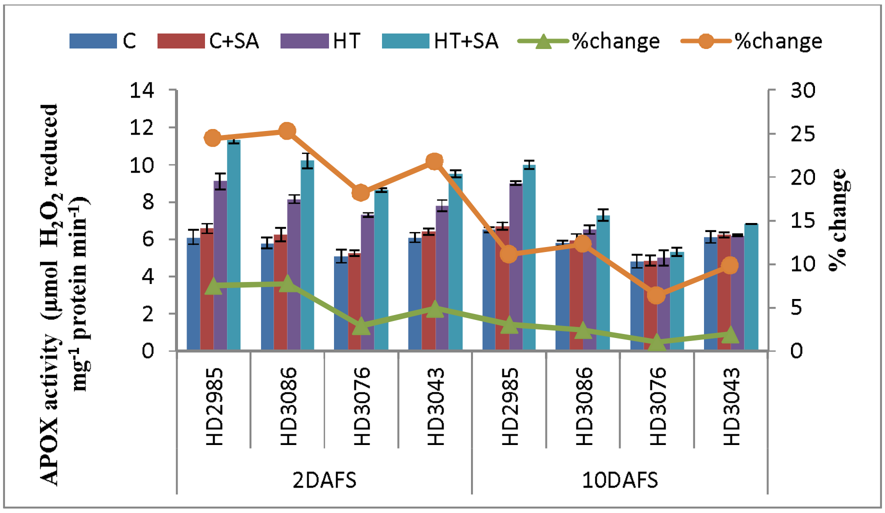

Fig. 4.3.1.7: Effect of SA on the ascorbate peroxidase activity (µmol H_2O_2 reduced mg^{-1} protein min^{-1}) in different wheat genotypes at 2 and 10 DAFS (Days after foliar spray) under normal and high temperature stress (induced by late sowing) conditions during reproductive stage. (C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid)

Table 4.3.1.8: **Effect of SA on the oxidised glutathione content (mmol g^{-1} DW) in wheat genotypes at 2 and 10 DAFS (Days after foliar spray) under normal and high temperature stress (induced by late sowing) conditions during reproductive stage.**

		Oxidised glutathione content (mmol g^{-1} DW)							
		Normal sown				**Late sown**			
Time (Days)	**Genotypes**	**C**	**C+SA**	**Mean**	**%change**	**HT**	**HT+SA**	**Mean**	**%change**
2DAFS	HD2985	8.98	8.14	8.56	-9.35	7.00	5.59	6.30	-20.14
	HD3086	8.98	8.32	8.65	-7.35	7.12	6.00	6.56	-15.73
	HD3076	7.55	7.00	7.28	-7.28	6.75	6.00	6.38	-11.11
	HD3043	8.24	7.56	7.90	-8.25	6.85	5.69	6.27	-16.93
10DAFS	HD2985	8.91	8.46	8.69	-5.05	6.73	6.00	6.37	-10.85
	HD3086	7.58	7.48	7.53	-1.32	6.11	5.59	5.85	-8.51
	HD3076	6.41	6.38	6.40	-0.47	5.21	5.00	5.11	-4.03
	HD3043	7.69	7.55	7.62	-1.82	6.38	5.98	6.18	-6.27

	Factors	**Sowing (S)**	**Treatment (T)**	**S×T**	**Variety (V)**	**S×V**	**T×V**	**S×T×V**
2DAFS	C.D.	0.249	0.249	N/A	0.352	0.498	N/A	N/A
10DAFS	C.D.	0.183	0.183	N/A	0.259	0.367	N/A	N/A

C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid

Table 4.3.1.9: Effect of SA on the reduced ascorbate content (µmol g^{-1} DW) in wheat genotypes at 2 and 10 DAFS (Days after foliar spray) under normal and high temperature stress (induced by late sowing) conditions during reproductive stage.

Time (Days)	Genotypes	Reduced ascorbate content (µmol g^{-1} DW)							
		Normal sown				Late sown			
		C	C+SA	Mean	%change	HT	HT+SA	Mean	%change
2DAFS	HD2985	16.25	14.81	15.53	-8.86	11.21	9.22	10.22	-17.75
	HD3086	14.65	13.86	14.26	-5.39	10.94	9.12	10.03	-16.64
	HD3076	14.12	13.65	13.89	-3.33	10.54	8.89	9.72	-15.65
	HD3043	14.85	14.12	14.49	-4.92	10.80	9.51	10.16	-11.94
10DAFS	HD2985	15.23	14.85	15.04	-2.50	9.87	7.98	8.93	-19.15
	HD3086	15.12	14.55	14.84	-3.77	13.21	11.00	12.11	-16.73
	HD3076	13.89	13.75	13.82	-1.01	13.21	11.89	12.55	-9.99
	HD3043	14.95	14.68	14.82	-1.81	14.68	13.42	14.05	-8.58

	Factors	Sowing (S)	Treatment (T)	S×T	Variety (V)	S×V	T×V	S×T×V
2DAFS	C.D.	0.407	0.407	N/A	0.576	0.815	N/A	N/A
10DAFS	C.D.	0.351	0.351	0.496	0.496	0.702	N/A	N/A

C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid

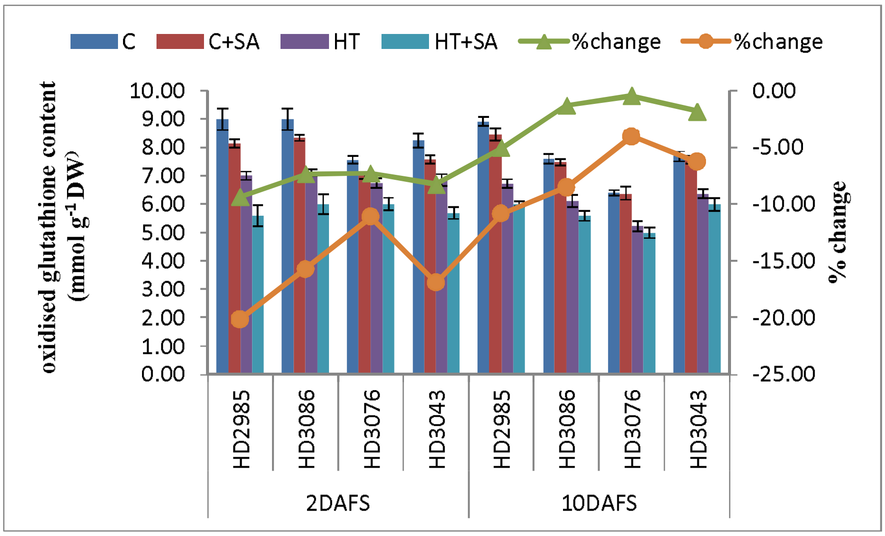

Fig 4.3.1.8: Effect of SA on the oxidised glutathione content (mmol g^{-1} DW) in wheat genotypes at 2 and 10 DAFS (Days after foliar spray) under normal and high temperature stress (induced by late sowing) conditions during reproductive stage. (C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid)

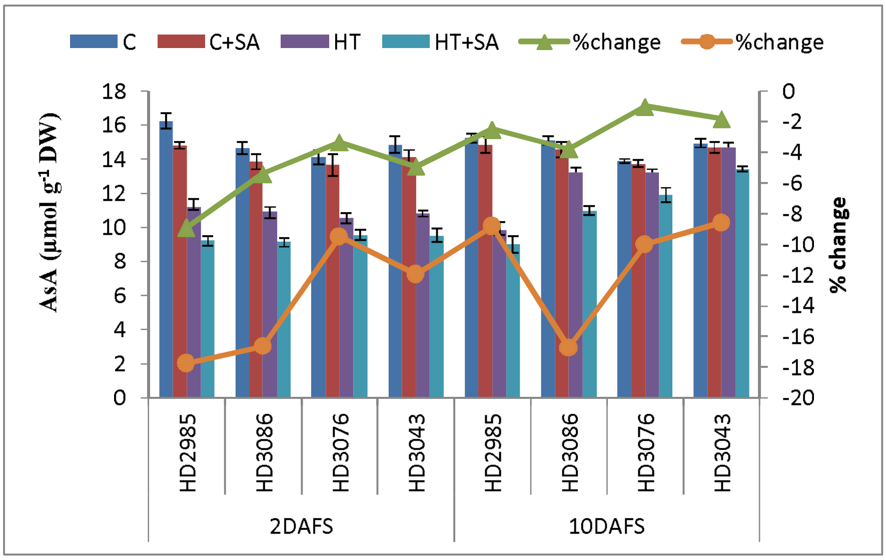

Fig 4.3.1.9: Effect of SA on the reduced ascorbate content (µmol g^{-1} DW) in wheat genotypes at 2 and 10 DAFS (Days after foliar spray) under normal and high temperature stress (induced by late sowing) conditions during reproductive stage. (C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid)

minimum in HD3076 (5%), however, 10 days after foliar spray under late sown conditions Fv/Fm was maintained higher from its respective control. The efficiency of PSII that was increased upon SA spray was still maintained 12% higher in HD2985 (tolerant genotypes) but in susceptible genotypes it was only 5% higher than its respective control.

4.3.1.11 Photosynthesis (Pn) rate

Result of the effect of SA on Pn in flag leaf of four wheat genotypes recorded at 2 and 10 DAFS grown under control and stress are presented in Table 4.3.1.11 and Fig 4.3.1.11. The Pn decreased significantly in all genotypes grown under late sown (high temperature stress) condition as compared to normal sown condition, with maximum decrease evinced in HD3043 (28%) followed by HD3076 (27%) and HD 3086 (27%) and minimum in HD2985 (17%). Foliar spray of SA enhanced Pn significantly in late sown condition. 2 DAFS of SA, the Pn enhanced significantly in late sown crop, with maximum increase observed in HD 2985 (13%) followed by HD3086 (12%) and minimum in HD3076 (9%), however, 10 days after foliar spray under late sown conditions Pn decreased, even though it was still higher than their respective control. The Pn which increased upon SA spray was still maintained 10.53% higher in HD2985 (tolerant genotypes) however, in susceptible genotypes it was only 5.5% higher than their respective control.

4.3.1.12 Chlorophyll a content

Result of the effect of SA on chlorophyll a contents measured in flag leaf of four wheat genotypes recorded at 2 and 10 DAFS grown under control and stress are presented in Table 4.3.1.12 and Fig 4.3.1.12. The chlorophyll a contents decreased significantly in all genotypes grown under late sown (high temperature stress) condition as compared to the normal sown condition, and maximum decrease was recorded in HD3076 (27%). Foliar spray of SA increased significantly (7 to 10%) chlorophyll a contents in tolerant genotypes under late sown condition in both the cropping seasons. 10 DAFS of SA, the chlorophyll a value was still 10-11% higher in late sown crop in tolerant genotypes. Similar response to SA was observed in terms of chlorophyll a contents in wheat genotypes in both the cropping seasons.

4.3.1.13 Chlorophyll b content

Result of the effect of SA on chlorophyll b contents measured in flag leaf of four wheat genotypes recorded at 2 and 10 DAFS grown under control and stress are presented in Table 4.3.1.13 and Fig 4.3.1.13. The chlorophyll b contents decreased significantly in all genotypes grown under late sown (high temperature stress) condition as compared to the normal sown condition. Foliar spray of SA maintained higher (7 to 8%) chlorophyll b contents in tolerant genotypes under late sown condition in both the cropping seasons but it was non-significant. 10 DAFS of SA, the chlorophyll b value was still 8% higher in late sown crop in tolerant genotypes. However in cropping season 2014-15, we could not get any significant change in chlorophyll b response to SA in any wheat genotypes.

4.3.1.14 Carotenoid contents

Result of the effect of SA on carotenoid contents measured in flag leaf of four wheat genotypes recorded at 2 and 10 DAFS grown under control and stress are presented in Table 4.3.1.14 and Fig 4.3.1.14. The carotenoid contents decreased significantly in all genotypes grown under late sown (high temperature stress) condition as compared to the normal sown condition. Foliar spray of SA maintained higher (2 to 3%) carotenoid contents in tolerant genotypes under late sown condition in both the cropping seasons but it was non-significant. 10 DAFS of SA, the carotenoid contents was still 1-2% higher in late sown crop in tolerant genotypes but it was non-significant.

4.3.1.15 SPAD value

Result of the effect of SA on SPAD measured in flag leaf of four wheat genotypes recorded at 2 and 10 DAFS grown under control and stress are presented in Table 4.3.1.15 and Fig 4.3.1.15 the SPAD value decreased significantly in all genotypes grown under late sown (high temperature stress) condition as compared to the normal sown condition, and maximum decrease was recorded in HD3076 (23%). Foliar spray of SA maintained significantly higher SPAD value in late sown condition and maximum value recorded in HD2985 (9%) than respective control. 10 DAFS of SA, the SPAD value maintained higher in late sown crop, with maximum increase observed in HD 2985 (11%). Almost similar trends were observed in both the cropping seasons.

Table 4.3.1.10: **Effect of SA on the photochemical efficiency (F_V/F_M) in wheat genotypes at 2 and 10 DAFS (Days after foliar spray) under normal and high temperature stress (induced by late sowing) conditions during reproductive stage.**

Time (Days)	Genotypes	Photochemical efficiency (F_V/F_M)							
		Normal sown				Late sown			
		C	**C+SA**	**Mean**	**%change**	**HT**	**HT+SA**	**Mean**	**%change**
2DAFS	HD2985	0.801	0.803	0.802	0.25	0.711	0.77	0.7405	8.30
	HD3086	0.803	0.805	0.804	0.25	0.704	0.759	0.7315	7.81
	HD3076	0.801	0.802	0.8015	0.12	0.615	0.651	0.633	5.85
	HD3043	0.81	0.815	0.8125	0.62	0.634	0.691	0.6625	8.99
10DAFS	HD2985	0.802	0.805	0.8035	0.37	0.685	0.76	0.7225	10.95
	HD3086	0.784	0.789	0.7865	0.64	0.681	0.75	0.7155	10.13
	HD3076	0.762	0.766	0.764	0.52	0.612	0.643	0.6275	5.07
	HD3043	0.802	0.804	0.803	0.25	0.617	0.657	0.637	6.48

	Factors	**Sowing (S)**	**Treatment (T)**	**SXT**	**Variety (V)**	**S×V**	**T×V**	**S×T×V**
2DAFS	C.D.	0.017	0.017	0.025	0.025	0.035	N/A	N/A
10DAFS	C.D.	0.017	0.017	0.024	0.024	0.035	N/A	N/A

C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid

Table 4.3.1.11: Effect of SA on the photosynthesis rate (µmolCO_2 m^{-2} s^{-1}) in wheat genotypes at 2 and 10 DAFS (Days after foliar spray) under normal and high temperature stress (induced by late sowing) conditions during reproductive stage.

Time (Days)	Genotypes	Photosynthesis rate (µmolCO_2 m^{-2} s^{-1})							
		Normal sown				Late sown			
		C	C+SA	Mean	%change	HT	HT+SA	Mean	%change
2DAFS	HD2985	22.6	23	22.8	1.77	18.6	21.1	19.85	13.44
	HD3086	23.9	24.1	24	0.84	17.5	19.7	18.6	12.57
	HD3076	19.3	19.5	19.4	1.04	14.1	15.3	14.7	8.51
	HD3043	21.4	21.4	21.4	0.00	15.3	17	16.15	11.11
10DAFS	HD2985	22	22.1	22.05	0.45	17.1	18.9	18	10.53
	HD3086	24.5	24.6	24.55	0.41	14.3	15.9	15.1	11.19
	HD3076	20	20.1	20.05	0.50	9.1	9.6	9.35	5.49
	HD3043	21.4	21.1	21.25	-1.40	10.9	11.8	11.35	8.26

	Factors	Sowing (S)	Treatment (T)	S×T	Variety (V)	S×V	T×V	S×T×V
2DAFS	C.D.	0.614	0.614	0.868	0.868	1.227	N/A	N/A
10DAFS	C.D.	0.691	N/A	N/A	0.977	1.381	N/A	N/A

C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid

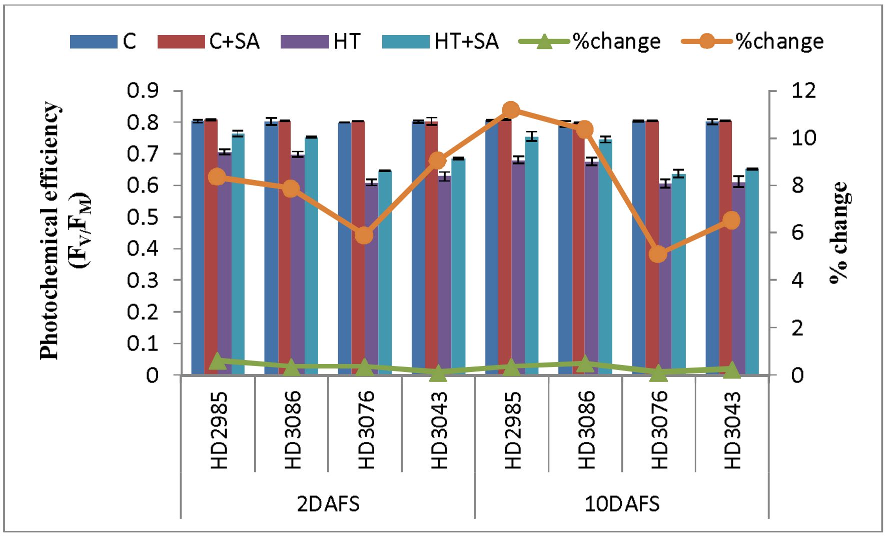

Fig. 4.3.1.10: Effect of SA on the photochemical efficiency (F_V/F_M) in wheat genotypes at 2 and 10 DAFS (Days after foliar spray) under normal and high temperature stress (induced by late sowing) conditions during reproductive stage. (C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid)

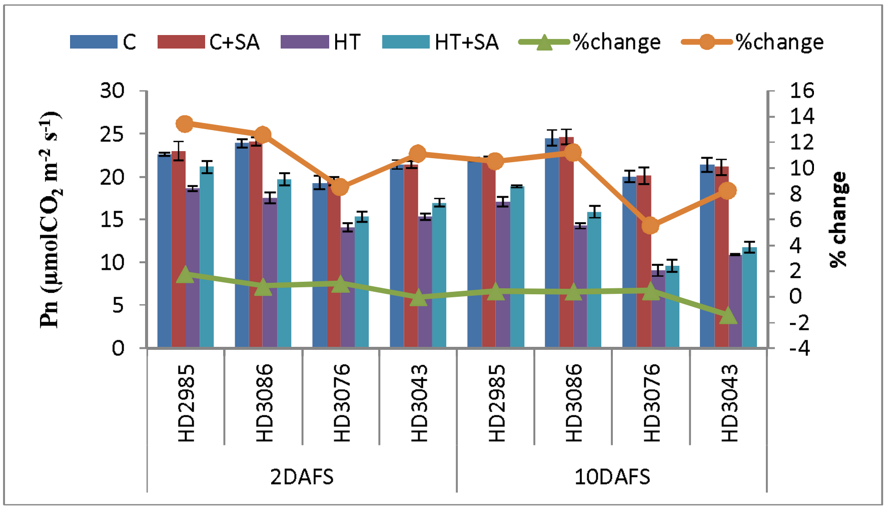

Fig 4.3.1.11: Effect of SA on the photosynthesis rate (µmolCO$_2$ m^{-2} s^{-1}) in wheat genotypes at 2 and 10 DAFS (Days after foliar spray) under normal and high temperature stress (induced by late sowing) conditions during reproductive stage. (C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid)

Table 4.3.1.12: Effect of SA on the Chlorophyll a (mg g^{-1} DW) content in wheat genotypes at 2 and 10 DAFS (Days after foliar spray) under normal and high temperature stress (induced by late sowing) conditions during reproductive stage.

Time (Days)	Genotypes	Chlorophyll a (mg g^{-1} DW)							
		Normal sown				Late sown			
		C	C+SA	Mean	%change	HT	HT+SA	Mean	%change
2DAFS	HD2985	9.81	10.12	9.96	3.11	7.88	8.55	8.22	8.50
	HD3086	9.64	9.85	9.74	2.23	7.37	8.11	7.74	10.04
	HD3076	8.39	8.41	8.40	0.24	6.11	6.51	6.31	6.55
	HD3043	9.12	9.15	9.14	0.33	7.24	7.67	7.46	5.94
10DAFS	HD2985	9.85	10.00	9.93	1.52	7.32	8.12	7.72	10.93
	HD3086	9.62	9.65	9.64	0.31	6.35	7.10	6.73	11.81
	HD3076	8.36	8.45	8.41	1.08	5.09	5.51	5.30	8.25
	HD3043	9.17	9.20	9.19	0.33	6.11	6.70	6.41	9.66

	Factors	Sowing (S)	Treatment (T)	S×T	Variety (V)	S×V	T×V	S×T×V
2DAFS	C.D.	0.273	0.273	N/A	0.386	N/A	N/A	N/A
10DAFS	C.D.	0.285	0.285	N/A	0.403	N/A	N/A	N/A

C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid

Table 4.3.1.13: Effect of SA on the Chlorophyll b (mg g^{-1} DW) content in wheat genotypes at 2 and 10 DAFS (Days after foliar spray) under normal and high temperature stress (induced by late sowing) conditions during reproductive stage.

Time (Days)	Genotypes	Chlorophyll b (mg g^{-1} DW)							
		Normal sown				Late sown			
		C	C+SA	Mean	%change	HT	HT+SA	Mean	%change
2DAFS	HD2985	2.11	2.13	2.12	0.95	1.30	1.40	1.35	7.69
	HD3086	2.13	2.15	2.14	0.94	1.45	1.57	1.51	8.28
	HD3076	2.00	2.01	2.005	0.50	1.11	1.19	1.15	7.21
	HD3043	2.06	2.08	2.07	0.97	1.32	1.39	1.355	5.30
10DAFS	HD2985	2.04	2.05	2.045	0.49	1.27	1.38	1.325	8.66
	HD3086	2.10	2.12	2.11	0.95	1.21	1.31	1.26	8.26
	HD3076	1.97	1.97	1.97	0.00	0.89	0.94	0.915	5.62
	HD3043	2.06	2.06	2.06	0.00	1.22	1.28	1.25	4.92

	Factors	Sowing (S)	Treatment (T)	S×T	Variety (V)	S×V	T×V	S×T×V
2DAFS	C.D.	0.07	N/A	N/A	0.099	N/A	N/A	N/A
10DAFS	C.D.	0.05	0.05	N/A	0.071	0.1	N/A	N/A

C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid

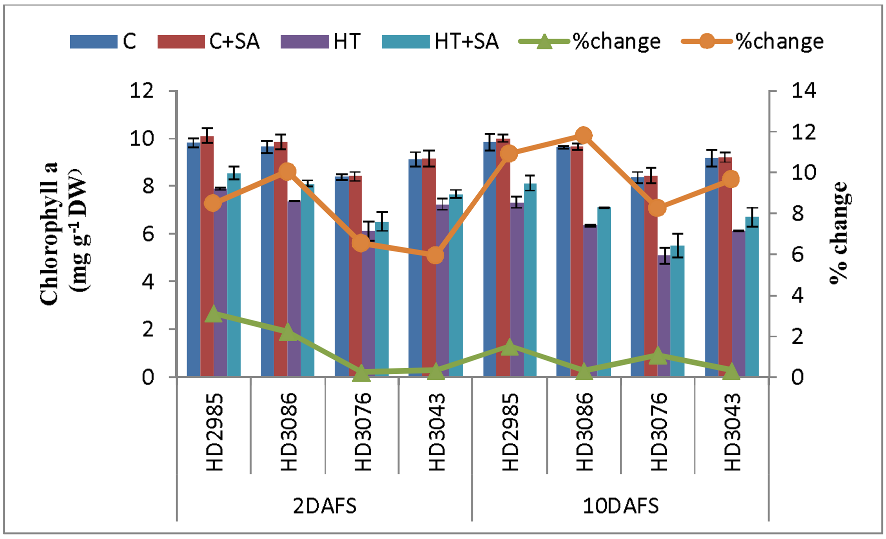

Fig 4.3.1.12: Effect of SA on the Chlorophyll a (mg g^{-1} DW) content in wheat genotypes at 2 and 10 DAFS (Days after foliar spray) under normal and high temperature stress (induced by late sowing) conditions during reproductive stage. (C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid)

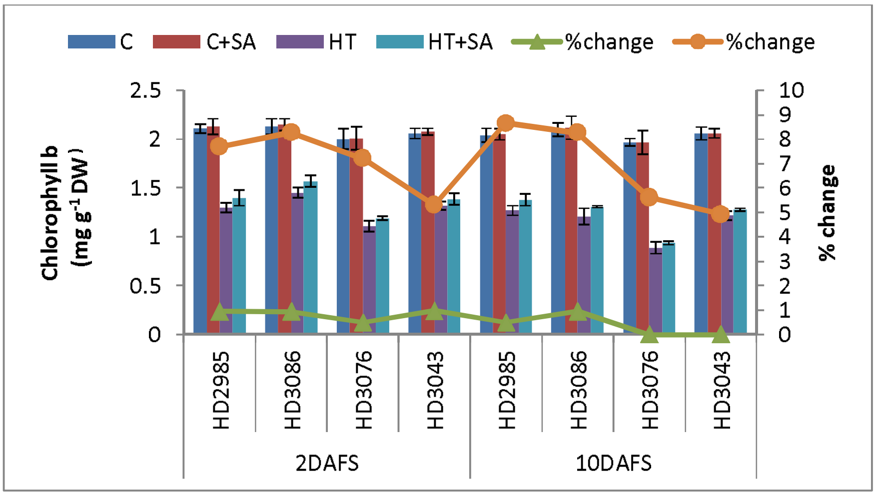

Fig 4.3.1.13: Effect of SA on the Chlorophyll b (mg g^{-1} DW) content in wheat genotypes at 2 and 10 DAFS (Days after foliar spray) under normal and high temperature stress (induced by late sowing) conditions during reproductive stage. (C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid)

Table 4.3.1.14: Effect of SA on the Carotenoid (mg g^{-1} DW) content in wheat genotypes at 2 and 10 DAFS (Days after foliar spray) under normal and high temperature stress (induced by late sowing) conditions during reproductive stage.

		Carotenoid (mg g^{-1} DW)							
		Normal sown				Late sown			
Time (Days)	**Genotypes**	**C**	**C+SA**	**Mean**	**%change**	**HT**	**HT+SA**	**Mean**	**%change**
2DAFS	HD2985	0.83	0.83	0.83	0.00	0.67	0.69	0.68	2.99
	HD3086	0.81	0.81	0.81	0.62	0.60	0.61	0.61	1.67
	HD3076	0.83	0.83	0.83	0.61	0.58	0.59	0.59	1.72
	HD3043	0.98	0.98	0.98	0.00	0.64	0.65	0.65	1.56
10DAFS	HD2985	0.87	0.87	0.87	0.00	0.64	0.65	0.65	1.56
	HD3086	0.83	0.83	0.83	0.00	0.57	0.58	0.58	1.75
	HD3076	0.84	0.84	0.84	0.60	0.51	0.52	0.52	1.96
	HD3043	0.93	0.93	0.93	0.54	0.59	0.60	0.60	1.69

	Factors	**Sowing (S)**	**Treatment (T)**	**S×T**	**Variety (V)**	**S×V**	**T×V**	**S×T×V**
2DAFS	C.D.	0.054	N/A	N/A	0.076	N/A	N/A	N/A
10DAFS	C.D.	0.045	N/A	N/A	0.064	N/A	N/A	N/A

C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid

Table 4.3.1.15: Effect of SA on SPAD value in wheat genotypes at 2 and 10 DAFS (Days after foliar spray) under normal and high temperature stress (induced by late sowing) conditions during reproductive stage.

Time (Days)	Genotypes	SPAD value							
		Normal sown				Late sown			
		C	C+SA	Mean	%change	HT	HT+SA	Mean	%change
2DAFS	HD2985	49.2	49.3	49.3	0.20	41.1	44.8	43.0	9.00
	HD3086	46.7	46.9	46.8	0.43	38.2	41.2	39.7	7.85
	HD3076	46.0	46.2	46.1	0.43	35.2	38.0	36.6	8.11
	HD3043	46.4	47.0	46.7	1.40	38.1	41.1	39.6	7.87
10DAFS	HD2985	50.2	50.2	50.2	0.00	39.5	43.8	41.7	10.89
	HD3086	46.1	46.5	46.3	0.98	36.5	39.7	38.1	8.77
	HD3076	44.4	44.5	44.5	0.23	32.2	34.1	33.1	6.07
	HD3043	46.4	46.7	46.6	0.65	34.4	37.5	36.0	9.01

	Factors	Sowing (S)	Treatment (T)	S×T	Variety (V)	S×V	T×V	S×T×V
2DAFS	C.D.	1.498	1.498	N/A	2.119	N/A	N/A	N/A
10DAFS	C.D.	1.399	1.399	N/A	1.978	N/A	N/A	N/A

C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid

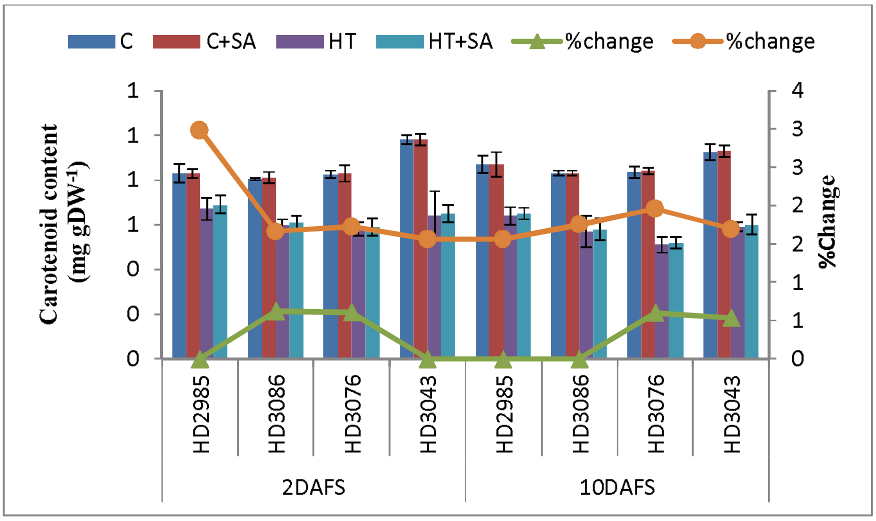

Fig 4.3.1.14: Effect of SA on the Carotenoid (mg g^{-1} DW) content in wheat genotypes at 2 and 10 DAFS (Days after foliar spray) under normal and high temperature stress (induced by late sowing) conditions during reproductive stage. (C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid)

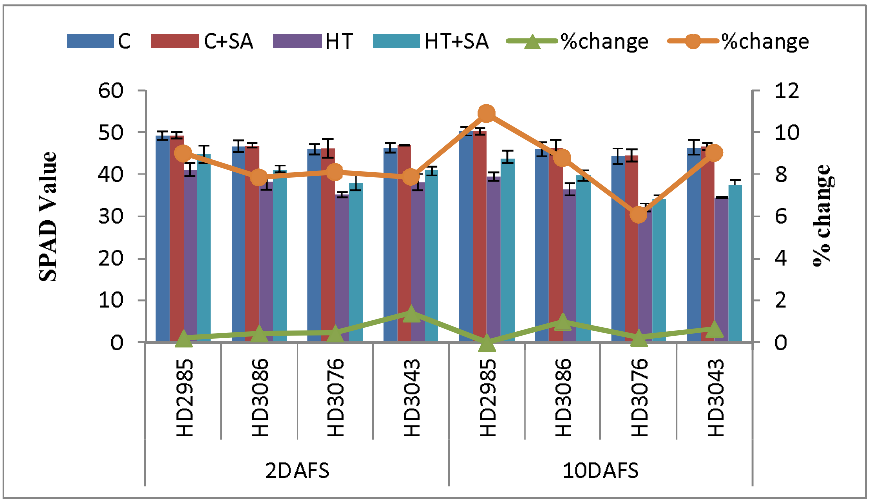

Fig 4.3.1.15: Effect of SA on SPAD value in wheat genotypes at 2 and 10 DAFS (Days after foliar spray) under normal and high temperature stress (induced by late sowing) conditions during reproductive stage. (C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid)

4.3.1.16 Total Chlorophyll

Result of the effect of SA on total chlorophyll contents measured in flag leaf of four wheat genotypes recorded at 2 and 10 DAFS grown under control and stress are presented in Table 4.3.1.16 and Fig 4.3.1.16. The total chlorophyll contents decreased significantly in all genotypes grown under late sown (high temperature stress) condition as compared to the normal sown condition, and maximum decrease was recorded in HD3076 (30%). Foliar spray of SA maintained significantly higher (8 to 9%) chlorophyll contents in tolerant genotypes under late sown condition in both the cropping seasons. 10 DAFS of SA, the chlorophyll a value was still 10-11% higher in late sown crop in tolerant genotypes. Similar response to SA were observed in terms of chlorophyll contents in wheat genotypes in both the cropping seasons.

4.3.2 Effects of SA (0.1mM) on non-structural carbohydrates in four wheat genotypes at anthesis +10 days and at harvest in penultimate internodes under normal and high temperature stress (induced by late sowing)

4.3.2.1 Non-structural carbohydrates (NSC)

Result of the effect of exogenous foliar spray of SA on maximum non-structural carbohydrates (NSC) (anthesis+10 days in normal sown and at anthesis in late sown plant) in measured in penultimate internodes of four wheat genotypes grown under control and stress condition (cropping season 2013-14) are presented in Table 4.3.2.1 (A) and Fig 4.3.2.1 (A). In comparison to the normal sown condition, maximum NSC was decreased significantly in all wheat genotypes grown under late sown (high temperature stress) condition. High temperature stress reduced maximum NSC content by 26 to 30 per cent in different wheat genotypes. Maximum NSC contents recorded higher in HD3086 and HD 2985 in comparison to the susceptible genotypes. Under normal condition the maximum NSC contents was higher in HD3086 and HD2985 and minimum in HD3076. The foliar spray of SA did not significantly alter the maximum NSC contents in any genotypes in late sown as well in normal sown crops.

The result of the effect of exogenous foliar spray of SA on minimum non-structural carbohydrates (NSC) measured in penultimate internodes of four wheat genotypes in harvested sample grown under control and stress condition (cropping season 2013-14) are presented in Table 4.3.2.1(B) and Fig 4.3.2.1 (B). In comparison to normal sown condition, under late sown (high temperature stress) condition minimum NSC was reduced significantly in all genotypes with maximum decrease observed in HD3086 and HD2985 and minimum in HD3076 and HD3043. Under normal condition the minimum NSC contents was higher in HD3086 and HD 2985. The foliar spray of SA did not significantly change the maximum NSC contents in any genotype in late sown as well as in normal sown crops. Almost similar results were obtained in both cropping seasons. The results of maximum and minimum NSC cropping season 2014-15 presented in Table 4.3.2.3 (A), Fig 4.3.2.3 (A) and Table 4.3.2.3 (B), Fig 4.3.2.3 (B), respectively, and it clearly indicates that maximum and minimum NSC were not significantly affected by application of SA.

4.3.2.2 Mobilised non-structural carbohydrates (MNSC) and remobilisation efficiency (RE)

Result of the effect of exogenous foliar spray of SA on mobilised non-structural carbohydrates (MNSC) measured in penultimate internodes of four wheat genotypes grown under control and stress conditions (cropping season 2013-14) are presented in Table 4.3.2.2 (A) and Fig 4.3.2.2 (A). In comparison to normal sown condition, under late sown (high temperature stress) condition MNSC was reduced significantly in all genotypes, however, it was higher in HD3086 and HD2985 as compared to HD3076. MNSC content decreased by 12 to18 per cent across all genotypes. Under late sown condition MNSC content was higher in HD3086 and HD 2985 in comparison to susceptible genotypes. In normal condition the MNSC contents was also higher in HD3086 and HD 2985 and minimum in HD 3076. The foliar spray of SA did not significantly enhance the MNSC contents in any genotype in late sown as well as in normal sown crops. The results of MNSC of cropping season 2014-15 are presented in Table 4.3.2.4 (A) and Fig 4.3.2.4 (A), respectively, and it clearly indicates that MNSC were not significantly affected by application of SA.

Result of the effect of exogenous foliar spray of SA on remobilisation efficiency (RE) of non-structural carbohydrates were measured in penultimate internodes of four wheat genotypes grown under control and stress conditions, of cropping season 2013-14, are presented in Table 4.3.2.2 (B) and Fig 4.3.2.2 (B). In comparison to normal sown condition, RE was increased significantly in all genotypes grown under late sown (high temperature stress) condition, however, it

Table 4.3.1.16: Effect of SA on the total chlorophyll (mg g^{-1} DW) in wheat genotypes at 2 and 10 DAFS (days after foliar spray) under normal and high temperature stress (induced by late sowing) conditions during reproductive stage.

		Total chlorophyll (mg g^{-1} DW)							
		Normal sown				Late sown			
Time (Days)	**Genotypes**	**C**	**C+SA**	**Mean**	**%change**	**HT**	**HT+SA**	**Mean**	**%change**
2DAFS	HD2985	11.91	12.24	12.075	2.77	9.175	9.95	9.5625	8.45
	HD3086	11.76	12	11.88	2.04	8.815	9.67	9.2425	9.70
	HD3076	10.385	10.42	10.4025	0.34	7.21	7.695	7.4525	6.73
	HD3043	11.175	11.225	11.2	0.45	8.555	9.055	8.805	5.84
10DAFS	HD2985	11.89	12.05	11.97	1.35	8.58	9.495	9.0375	10.66
	HD3086	11.715	11.765	11.74	0.43	7.55	8.4	7.975	11.26
	HD3076	10.325	10.415	10.37	0.87	5.975	6.45	6.2125	7.95
	HD3043	11.22	11.25	11.235	0.27	7.225	7.975	7.6	10.38

	Factors	**Sowing (S)**	**Treatment (T)**	**S×T**	**Variety (V)**	**S×V**	**T×V**	**S×T×V**
2DAFS	C.D.	0.276	0.276	N/A	0.391	N/A	N/A	N/A
10DAFS	C.D.	0.302	0.302	0.427	0.427	N/A	N/A	N/A

C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid

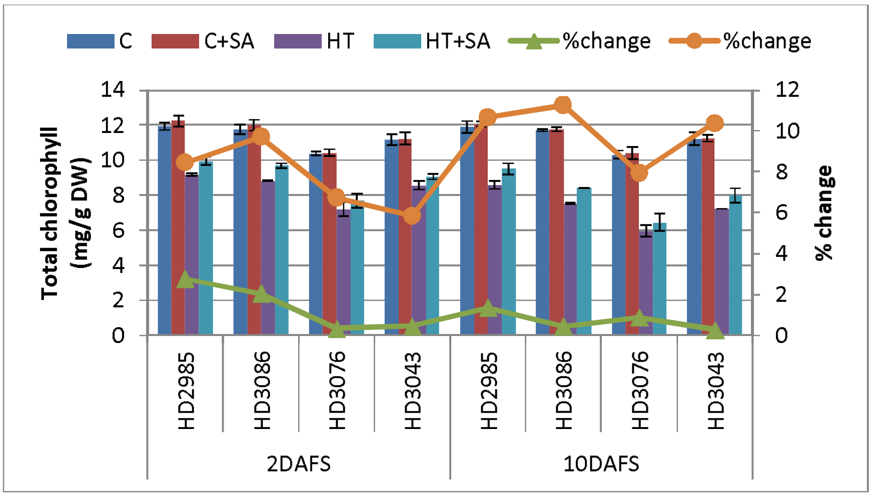

Fig. 4.3.1.16: Effect of SA on the total chlorophyll (mg g^{-1} DW) in wheat genotypes at 2 and 10 DAFS (Days after foliar spray) under normal and high temperature stress (induced by late sowing) conditions during reproductive stage. (C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid)

Table 4.3.2.1: Effect of SA on NSC (mg g^{-1} DW) of penultimate internode in wheat genotypes at (A) anthesis +10 days sample (maximum NSC) (B) harvested sample (minimum NSC) of normal and late sown (high temperature stress) crop (2013-14).

(A)

	Maximum NSC (mg g^{-1} DW)							
Genotypes	**C**	**C+SA**	**Mean**	**% change**	**HT**	**HT+SA**	**Mean**	**% change**
HD2985	135.2	137.6	136.4	1.76	98.1	98.1	98.1	0
HD3086	143.4	147.0	145.2	2.49	99.0	102.7	100.8	3.68
HD3076	98.0	98.0	98.0	-0.01	71.9	73.0	72.4	1.57
HD3043	134.2	137.3	135.8	2.27	98.0	102.5	100.3	4.59
Factors	**Sowing (S)**	**Treatment (T)**	**S×T**	**Variety (V)**	**S×V**	**T×V**	**S×T×V**	
C.D.	4.352	N/A	N/A	6.154	8.704	N/A	N/A	

C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid

(B)

	Minimum NSC (mg g^{-1} DW)							
Genotypes	**C**	**C+SA**	**Mean**	**% change**	**HT**	**HT+SA**	**Mean**	**% change**
HD2985	59.1	57.0	58.1	-3.55	31.4	29.1	30.2	-7.41
HD3086	56.6	56.7	56.7	0.04	28.0	26.8	27.4	-4.46
HD3076	49.3	49.0	49.2	-0.62	32.1	32.4	32.2	0.77
HD3043	56.3	57.3	56.8	1.83	32.1	33.0	32.6	2.80
Factors	**Sowing (S)**	**Treatment (T)**	**S×T**	**Variety (V)**	**S×V**	**T×V**	**S×T×V**	
C.D.	3.194	N/A	N/A	N/A	6.387	N/A	N/A	

C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid

Table 4.3.2.2: Effect of SA on (A) mobilised NSC (mg) (B) and remobilisation efficiency (%) of normal and late sown(high temperature stress) wheat genotypes (2013-14).

(A)

Genotypes	Mobilised NSC (mg)							
	C	**C+SA**	**Mean**	**% change**	**HT**	**HT+SA**	**Mean**	**% change**
HD2985	76.2	80.6	78.4	5.84	66.8	69.1	67.9	3.44
HD3086	86.8	90.4	88.6	4.09	71.0	75.9	73.5	6.90
HD3076	48.7	49.0	48.9	0.62	39.8	40.7	40.2	2.13
HD3043	78.0	80.0	79.0	2.63	65.9	69.5	67.7	5.46
Factors	**Sowing (S)**	**Treatment (T)**	**S×T**	**Variety (V)**	**S×V**	**T×V**	**S×T×V**	
C.D.	3.194	N/A	N/A	N/A	6.387	N/A	N/A	

C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid

(B)

Genotypes	Remobilisation efficiency (%)							
	C	**C+SA**	**Mean**	**% change**	**HT**	**HT+SA**	**Mean**	**% change**
HD2985	56.3	58.6	57.4	4.19	67.7	70.4	69.1	3.99
HD3086	60.5	61.4	61.0	1.50	71.8	73.9	72.8	2.89
HD3076	49.7	49.8	49.7	0.13	55.5	55.5	55.5	0.12
HD3043	58.1	58.2	58.1	0.17	67.3	67.6	67.4	0.50
Factors	**Sowing (S)**	**Treatment (T)**	**S×T**	**Variety (V)**	**S×V**	**T×V**	**S×T×V**	
C.D.	3.389	N/A	N/A	4.792	N/A	N/A	N/A	

C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid

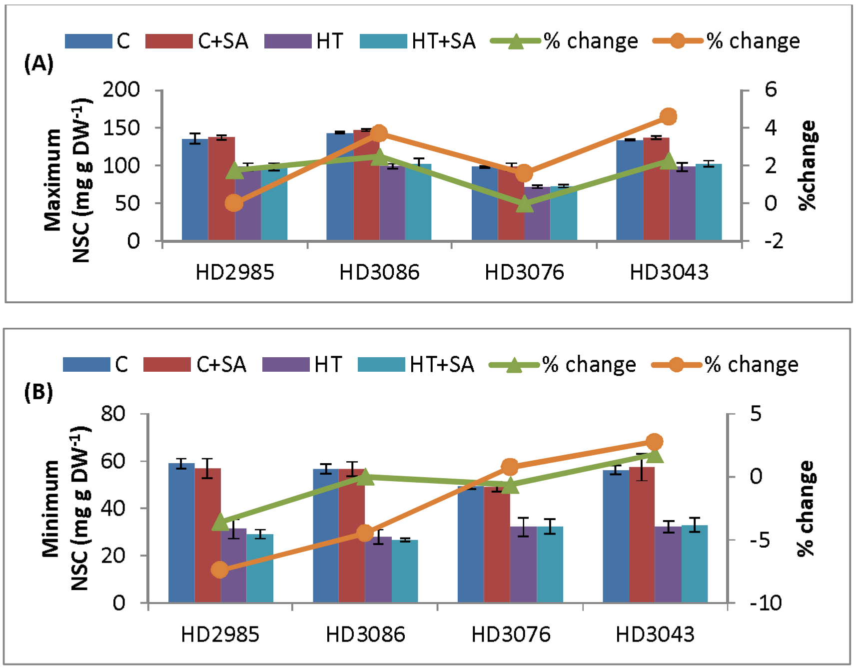

Fig 4.3.2.1: Effect of SA on NSC (mg g^{-1} DW) of penultimate internode in wheat genotypes at (A) anthesis +10 days sample (maximum NSC) (B) harvested sample (minimum NSC) of normal and late sown (high temperature stress) crop (2013-14). (C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid)

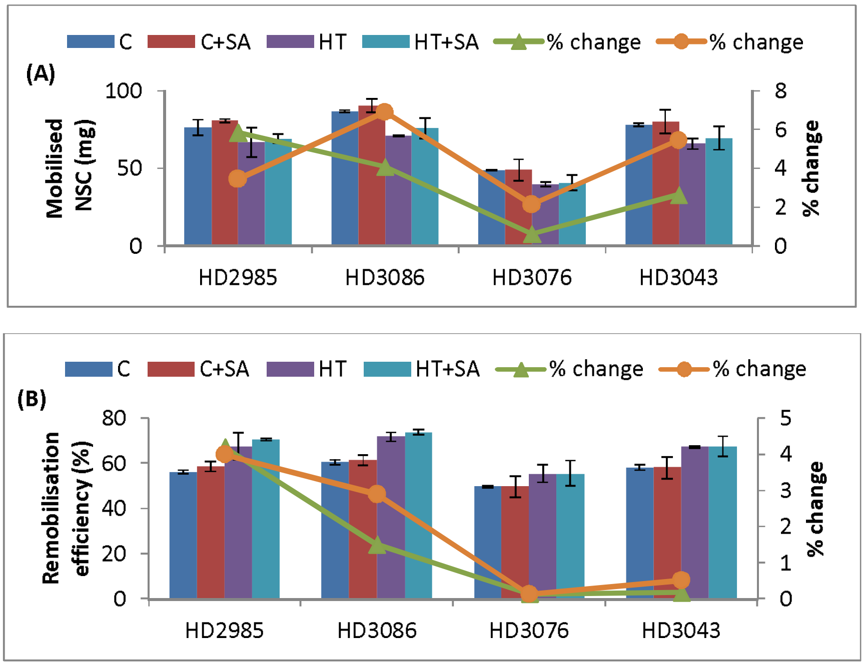

Fig. 4.3.2.2: Effect of SA on (A) Mobilised NSC (mg) (B) and remobilisation efficiency (%) of normal and late sown (high temperature stress) wheat genotypes (2013-14). (C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid)

Table 4.3.2.3: Effect of SA on NSC (mg g^{-1} DW) of penultimate internode in wheat genotypes at (A) anthesis +10 days sample (maximum NSC) (B) harvested sample (minimum NSC) of normal and late sown (high temperature stress) crop (2014-15).

(A)

	Maximum NSC (mg g^{-1} DW)							
Genotypes	**C**	**C+SA**	**Mean**	**% change**	**HT**	**HT+SA**	**Mean**	**% change**
HD2985	154.8	160.3	157.5	3.52	114.4	122.0	118.2	6.64
HD3086	164.4	167.9	166.1	2.10	128.9	129.1	129.0	0.11
HD3076	112.2	116.8	114.5	4.10	103.4	103.7	103.5	0.24
HD3043	134.2	137.3	135.8	2.27	112.1	109.2	110.7	-2.58
Factors	**Sowing (S)**	**Treatment (T)**	**S×T**	**Variety (V)**	**S×V**	**T×V**	**S×T×V**	
C.D.	4.748	N/A	N/A	6.715	9.497	N/A	N/A	

C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid

(B)

	Minimum NSC (mg g^{-1} DW)							
Genotypes	**C**	**C+SA**	**Mean**	**% change**	**HT**	**HT+SA**	**Mean**	**% change**
HD2985	61.2	62.3	61.7	1.80	31.4	31.1	31.2	-0.87
HD3086	56.6	56.7	56.7	0.04	32.2	28.3	30.2	-12.1
HD3076	49.3	49.0	49.2	-0.62	43.2	42.1	42.6	-2.43
HD3043	56.3	57.3	56.8	1.83	42.2	38.2	40.2	-9.48
Factors	**Sowing (S)**	**Treatment (T)**	**S×T**	**Variety (V)**	**S×V**	**T×V**	**S×T×V**	
C.D.	2.985	N/A	N/A	N/A	5.971	N/A	N/A	

C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid

Table 4.3.2.4: Effect of SA on (A) mobilised NSC (mg) (B) and remobilisation efficiency (%) of normal and late sown (high temperature stress) wheat genotypes (2014-15).

(A)

	Mobilised NSC (mg)							
Genotypes	**C**	**C+SA**	**Mean**	**% change**	**HT**	**HT+SA**	**Mean**	**% change**
HD2985	93.7	98.0	95.8	4.64	83.0	90.9	86.9	9.45
HD3086	107.8	111.2	109.5	3.16	96.8	100.8	98.8	4.18
HD3076	62.9	67.8	65.4	7.79	60.3	61.6	60.9	2.15
HD3043	78.0	80.0	79.0	2.63	70.0	71.1	70.5	1.57
Factors	**Sowing (S)**	**Treatment (T)**	**S×T**	**Variety (V)**	**S×V**	**T×V**	**S×T×V**	
C.D.	5.324	N/A	N/A	7.529	N/A	N/A	N/A	

C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid

(B)

	Remobilisation efficiency (%)							
Genotypes	**C**	**C+SA**	**Mean**	**% change**	**HT**	**HT+SA**	**Mean**	**% change**
HD2985	60.4	61.2	60.8	1.27	72.6	74.5	73.6	2.58
HD3086	65.6	66.2	65.9	0.97	75.0	78.1	76.5	4.10
HD3076	55.9	58.1	57.0	3.86	58.3	59.1	58.7	1.37
HD3043	58.1	58.2	58.1	0.17	62.4	65.1	63.7	4.38
Factors	**Sowing (S)**	**Treatment (T)**	**S×T**	**Variety (V)**	**S×V**	**T×V**	**S×T×V**	
C.D.	2.608	N/A	N/A	3.689	5.217	N/A	N/A	

C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid

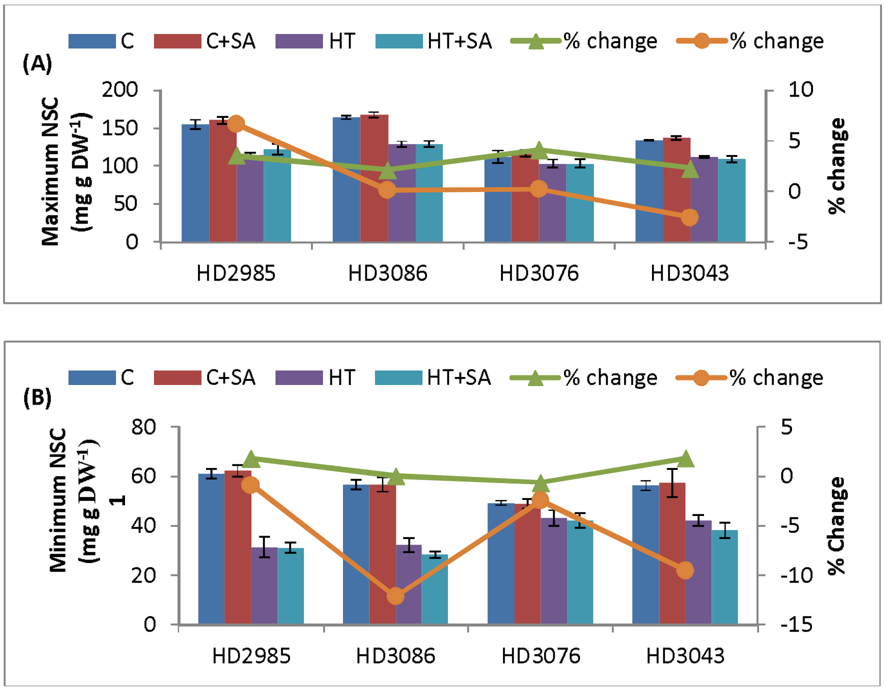

Fig 4.3.2.3: Effect of SA on NSC (mg g^{-1} DW) of penultimate internode in wheat genotypes at (A) anthesis +10 days sample (maximum NSC) (B) harvested sample (minimum NSC) of normal and late sown (high temperature stress) crop (2014-15). (C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid)

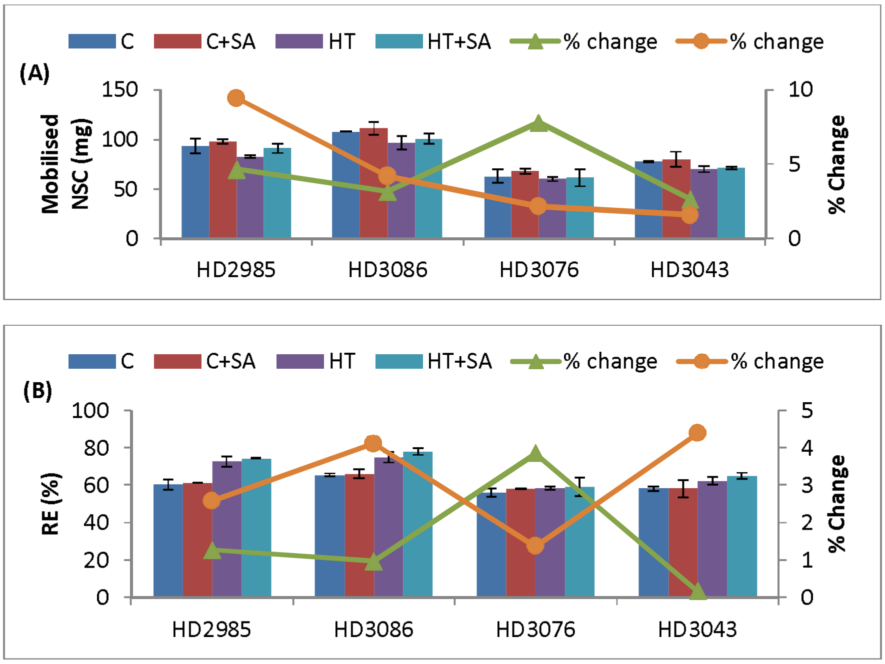

Fig. 4.3.2.4: Effect of SA on (A) Mobilised NSC (mg) (B) and remobilisation efficiency (%) of normal and late sown (high temperature stress) wheat genotypes (2014-15). (C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid)

was higher in HD2985 (20%) followed by HD3086 (18%) as compared to HD3076 (11%). In normal condition the RE was higher in HD3086 and HD 2985 and minimum in HD 3076. The foliar spray of SA did not significantly influence the RE in any genotype in late sown as well as in normal sown crops. The results of RE of cropping season 2014-15 are presented in Table 4.3.2.4 (B) and Fig 4.3.2.4 (B) respectively, and it clearly indicates that RE were not significantly affected by application of SA.

4.3.3 Effects of SA (0.1mM) on endogenous level of salicylic acid and ABA in different wheat genotypes at 2 and 10 DAFS (Days after foliar spray) under normal and high temperature stress (induced by late sowing)

4.3.3.1 Endogenous level of ABA

Result of the effect of exogenous SA on endogenous level of ABA in flag leaf of four wheat genotypes recorded at 2 and 10 DAFS grown under control (normal sown) and temperature stress (induced by late sowing) conditions during flowering stage are presented in Table 4.3.3.1 and Fig 4.3.3.1 With respect to the normal sown condition, the endogenous level of ABA increased significantly in all genotypes grown under late sown (high temperature stress) condition with maximum increase recorded in HD3076 and HD3043 and minimum observed in HD2985 and HD 3086. In normal as well as late sown conditions the foliar spray of SA did not increase the endogenous level of ABA significantly in any genotype. Amongst the genotypes grown under normal sown conditions the highest endogenous level of ABA was found in HD3076.

4.3.3.2 Endogenous level of total salicylic acid

Result of the effect of exogenous SA on endogenous level of total SA in flag leaf of four wheat genotypes recorded at 2 and 10 DAFS grown under control and stress conditions during flowering stage are presented in Table 4.3.3.2 and Fig 4.3.3.2. Foliar spray of SA improved the endogenous level of total SA significantly in all genotypes in normal sown, however with maximum increase evinced in HD2985 (19%) followed by HD3086 (11%) and minimum observed in HD3043 (7) and HD3076 (8%). The basal level of total SA was significantly higher in all genotypes in late sown crop compared to normal sown crop. Under late sown crop (high temperature stress) the exogenous application of SA enhanced the endogenous level of total SA with maximum increase observed in HD2985 (17%).

4.3.3.3 Endogenous levels of free salicylic acid

Result of the effect of exogenous SA on endogenous level of free SA in flag leaf of four wheat genotypes recorded at 2 and 10 DAFS grown under control and stress conditions during flowering stage are presented in Table 4.3.3.3 and Fig 4.3.3.3. In comparison to the normal sown condition, the endogenous level of free SA increased significantly in all genotypes grown under late sown (high temperature stress) condition. The basal endogenous level of free SA was significantly very high in all genotypes and maximum contents were found in HD2985 while minimum was observed in HD3076 genotype. Foliar spray of SA enhanced the endogenous level of free SA significantly in all genotypes in late sown crop with maximum increase substantiated in HD2985 (33%) and HD3086 (25%) and minimum in HD3076 (12%), 2 days after foliar spray. However, 10 DAFS the free SA level reduced, but was still higher than its respective control. Foliar spray of SA also enhanced the endogenous contents of free SA in wheat genotypes grown under normal sown condition, however, total contents in respect to late sown condition was much less in all the genotypes.

4.3.4 Effects of SA (0.1mM) on gene expression under normal and high temperature stress (induced by late sowing) in different wheat genotypes at 2 DAFS at reproductive stage

The gene expression analysis of transcription factors (HSFs), HSPs, genes of different pathways and few structural genes were analysed with sqRT-PCR, the results of which are presented as under:

4.3.4.1 Expression analysis of transcription factors (HSFA2b and HSFA1a)

The results of the effect of exogenous foliar application of salicylic acid on *HSFA2b* gene expression in the flag leaf of wheat genotypes grown under control (normal sown) and high temperature stress (induced by late sowing) conditions, recorded two days after spray, are presented in plates 2 and 3, respectively. In case of *HSFA2b* gene, sqRT-PCR was performed with gene specific primers and amplicons of size 190bp were obtained in all the four genotypes in treatment and respective control. The expression of *HSFA2b* in normal sown crop was nearly same in all

Table 4.3.3.1: **Effect of SA on the endogenous level of ABA (ng g^{-1} FW) in wheat genotypes at 2 and 10 DAFS (Days after foliar spray) under normal and high temperature stress (induced by late sowing) conditions during reproductive stage.**

		Endogenous level of ABA (ng g^{-1} FW)							
		Normal sown				**Late sown**			
Time (Days)	**Genotypes**	**C**	**C+SA**	**Mean**	**%change**	**HT**	**HT+SA**	**Mean**	**%change**
2DAFS	HD2985	75.4	77.0	76.2	2.18	148.4	150.9	149.6	1.65
	HD3086	80.4	82.1	81.2	2.17	147.8	153.6	150.7	3.93
	HD3076	91.4	93.3	92.4	2.08	179.3	185.2	182.3	3.29
	HD3043	72.3	74.2	73.2	2.63	165.1	171.8	168.4	4.09
10DAFS	HD2985	78.4	79.7	79.0	1.66	198.25	202.95	200.6	2.37
	HD3086	81.4	82.7	82.0	1.66	210.8	214.8	212.8	1.90
	HD3076	93.2	94.7	94.0	1.61	224.5	231.9	228.2	3.30
	HD3043	79.2	81.0	80.1	2.27	229.55	239.85	234.7	4.49

	Factors	**Sowing (S)**	**Treatment (T)**	**S×T**	**Variety (V)**	**S×V**	**T×V**	**S×T×V**
2DAFS	C.D.	7.151	N/A	N/A	10.113	14.302	N/A	N/A
10DAFS	C.D.	5.514	N/A	N/A	7.798	11.029	N/A	N/A

C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid

Table 4.3.3.2: **Effect of SA on the endogenous level of total SA (µg g^{-1} FW) in wheat genotypes at 2 and 10 DAFS (Days after foliar spray) under normal and high temperature stress (induced by late sowing) conditions during reproductive stage.**

Time (Days)	Genotypes	Endogenous level of total SA (µg g^{-1} FW)							
		Normal sown				Late sown			
		C	C+SA	Mean	%change	HT	HT+SA	Mean	%change
2DAFS	HD2985	2.90	3.45	3.17	19.17	4.92	5.76	5.34	17.19
	HD3086	2.93	3.25	3.09	11.11	4.72	5.20	4.96	10.17
	HD3076	2.42	2.60	2.51	7.44	4.49	4.98	4.73	10.80
	HD3043	2.70	2.89	2.80	7.04	4.31	4.76	4.53	10.32
10DAFS	HD2985	2.97	3.15	3.06	6.06	4.80	5.33	5.06	11.05
	HD3086	2.55	2.77	2.66	8.63	4.63	4.98	4.81	7.56
	HD3076	2.69	2.98	2.84	10.78	4.33	4.99	4.66	15.24
	HD3043	2.63	3.11	2.87	18.25	4.17	4.67	4.42	11.99

	Factors	Sowing (S)	Treatment (T)	S×T	Variety (V)	S×V	T×V	S×T×V
2DAFS	C.D.	0.194	0.194	N/A	0.275	N/A	N/A	N/A
10DAFS	C.D.	0.128	0.128	N/A	0.181	0.255	N/A	N/A

C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid

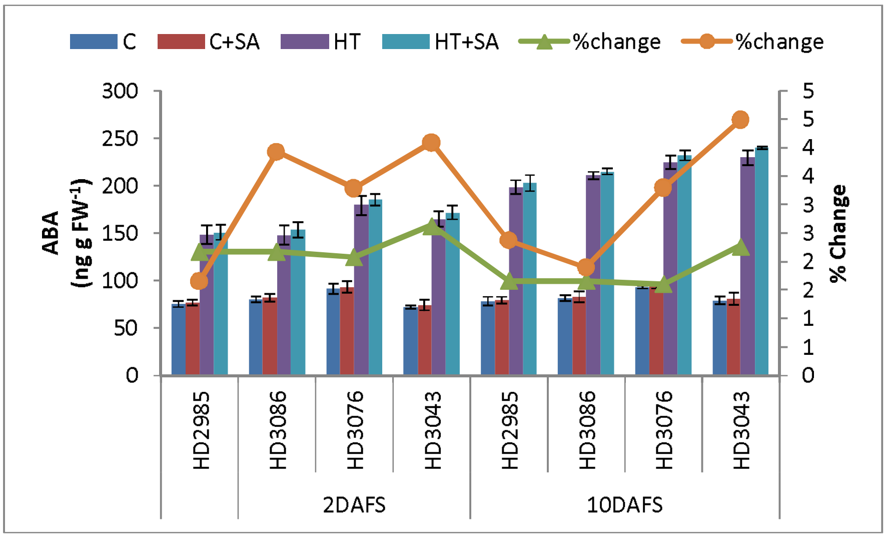

Fig 4.3.3.1: Effect of SA on the endogenous level of ABA (ng g^{-1} FW) in wheat genotypes at 2 and 10 DAFS (Days after foliar spray) under normal and high temperature stress (induced by late sowing) conditions during reproductive stage. (C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid)

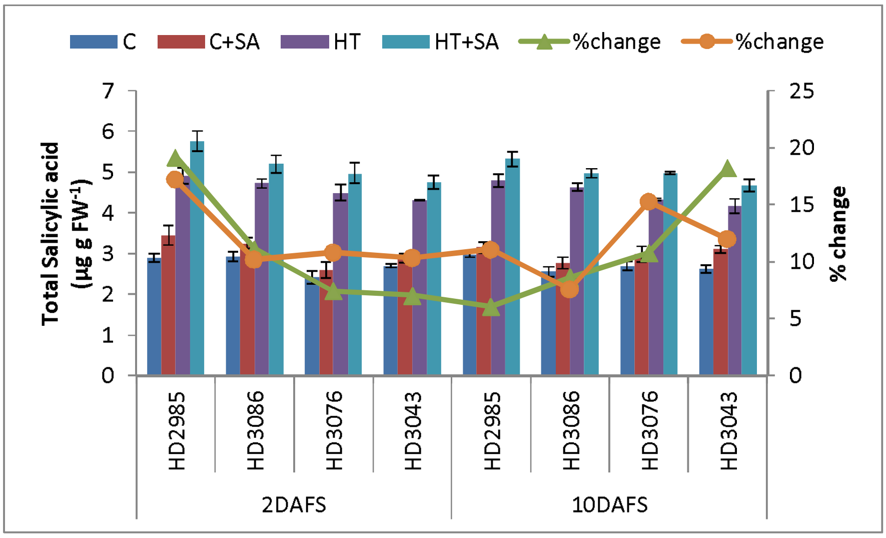

Fig 4.3.3.2: Effect of SA on the endogenous level of total SA (µg g^{-1} FW) in wheat genotypes at 2 and 10 DAFS (Days after foliar spray) under normal and high temperature stress (induced by late sowing) conditions during reproductive stage. (C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid)

Table 4.3.3.3: Effect of SA on the endogenous level of free SA (µg g^{-1} FW) in wheat genotypes at 2 and 10 DAFS (Days after foliar spray) under normal and high temperature stress (induced by late sowing) conditions during reproductive stage.

		Endogenous level of free SA (µg g^{-1} FW)							
		Normal sown				Late sown			
Time (Days)	**Genotypes**	**C**	**C+SA**	**Mean**	**%change**	**HT**	**HT+SA**	**Mean**	**%change**
2DAFS	HD2985	0.543	0.67	0.6065	23.39	2.18	2.89	2.54	32.57
	HD3086	0.53	0.635	0.5825	19.81	2.11	2.66	2.38	25.83
	HD3076	0.4	0.445	0.4225	11.25	1.53	1.71	1.62	11.76
	HD3043	0.521	0.625	0.573	19.96	2.13	2.51	2.32	18.12
10DAFS	HD2985	0.55	0.565	0.5575	2.73	2.20	2.41	2.30	9.79
	HD3086	0.52	0.535	0.5275	2.88	2.11	2.31	2.21	9.48
	HD3076	0.412	0.435	0.4235	5.58	1.52	1.61	1.56	6.27
	HD3043	0.53	0.54	0.535	1.89	2.00	2.11	2.06	5.50

	Factors	**Sowing (S)**	**Treatment (T)**	**SXT**	**Variety (V)**	**S×V**	**T×V**	**S×T×V**
2DAFS	C.D.	0.073	0.073	0.103	0.103	0.145	0.145	N/A
10DAFS	C.D.	0.087	N/A	N/A	0.122	0.173	N/A	N/A

C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid

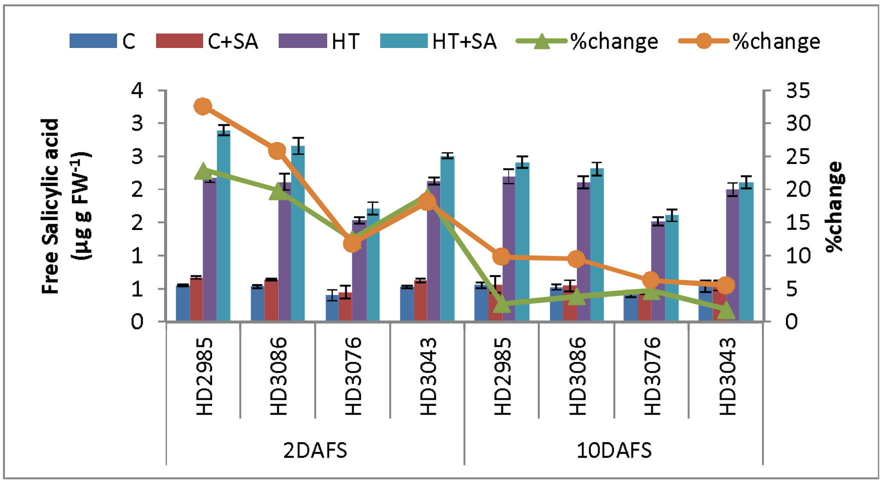

Fig 4.3.3.3: Effect of SA on the endogenous level of free SA (µg g^{-1} FW) in wheat genotypes at 2 and 10 DAFS (Days after foliar spray) under normal and high temperature stress (induced by late sowing) conditions during reproductive stage. (C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid)

genotypes except for HD3076, which exhibited relatively higher expression amongst genotypes under normal sown condition. The salicylic acid treatment in normal sown wheat genotypes did not change the expression of HSFA2b. In late sown (high temperature stress conditions) wheat genotypes the expression of HSFA2b was induced in all genotypes, however, the fold change in expression were higher in HD 2985, HD3043 and HD 3086 and lower in HD3076 compared to respective control (normal sown). The application of salicylic acid in late sown wheat genotypes induced the expression in all genotypes; however, the maximum increase occurs in HD2985 then in HD3086.

In case of *HSFA1a* gene, sqRT-PCR was performed with gene specific primers and expected amplicons of size 200 bp were obtained in all the four genotypes in treatment as well as respective control. The expression of *HSFA1a* in normal sown crop was higher in HD2985 and HD3086 compared to HD3076 and HD3043. The salicylic acid treatment in normal sown wheat genotypes did not change the expression of *HSFA1a*. In wheat genotypes experiencing high temperature stress (late sown) the expression of *HSFA1a* was induced only in HD2985 and HD 3086 while HD3043 and HD3086 genotypes showed very less increase in its expression. The application of salicylic acid in late sown wheat genotypes induced the expression of *HSFA1a* in tolerant genotypes; however, the maximum increase occurs in HD2985 then in HD3086. The expression of gene *HSFA1a* in wheat genotypes HD3043 and HD3076 did not change considerably on exogenous application of salicylic acid.

4.3.4.3 Expression analysis of large HSP (HSP101) and small HSPs (HSP16.9 and HSP17.8)

In case of *HSP101* gene, sqRT-PCR was performed with gene specific primers and expected amplicons of size 200 bp were obtained in all the four genotypes in treatment and respective control (plate 4 and 5). The expression of *HSP101* in normal sown crop was higher in HD3076 and HD3043 compared to HD2985 and HD3086 (plates 4 and 5). The salicylic acid treatment in normal sown wheat genotypes did not change the expression of *HSP101* in any wheat genotypes. In wheat genotypes under high temperature stress conditions (late sown) the expression of *HSP 101* was induced in all genotypes. The application of salicylic acid in late sown wheat genotypes also induced its expression in all genotypes;

however, the maximum increase occurs in HD2985 while minimum change was observed in HD3043 from its respective control.

In case of *HSP16.9* gene, sqRT-PCR was performed with gene specific primers and expected amplicons of size 200 bp were obtained in all the four genotypes in treatment and respective control (plate 4 and 5). The expression of *HSP16.9* in normal sown crop was higher in HD2985 compared to other genotypes of wheat. The salicylic acid treatment did not change the expression of *HSP16.9* in normal sown wheat genotypes. Under high temperature stress conditions (late sown) in wheat genotypes the expression of *HSP16.9* induced only in HD2985 compared to its respective control, and expression was not induced in other wheat genotypes. The application of salicylic acid in late sown wheat genotypes induced the expression of *HSP16.9* in HD2985 only and did not change the expression significantly in other genotypes.

In case of *HSP17.8* gene, sqRT-PCR was performed with gene specific primers and expected amplicons of size 195 bp were obtained in all the four genotypes in treatment and respective control (plate 4 and 5). The expression of *HSP17.8* in normal sown crop was higher in HD2985 and HD3086 compared to HD3076 and HD3043 (plates 4 and 5). The salicylic acid treatment in normal sown wheat genotypes did not change the expression of *HSP17.8*. Under high temperature stress conditions (late sown) genotypes the expression of *HSP17.8* was induced in all genotypes. The application of salicylic acid in late sown wheat genotypes did not induce considerable expression in any genotypes.

4.3.4.6 Expression analysis of Isochorismate synthase (ICS)

The *TaICS* expression study was done in two wheat genotypes. In case of *TaICS* gene, sqRT-PCR was performed with gene specific primers and expected amplicons of size 191 bp were obtained in two genotypes in treatment as well as in its respective control (plate 6). The expression of *TaICS* in normal sown crop was higher in HD2985 compared to HD3076. The salicylic acid treatment in normal sown wheat genotypes did not change the expression of *TaICS*. In wheat genotypes under high temperature stress conditions (late sown) the expression of *TaICS* was induced in both HD2985 and HD 3076, however, the expression of *TaICS* was more in HD2985 as compared to HD3076. The application of salicylic acid in late sown

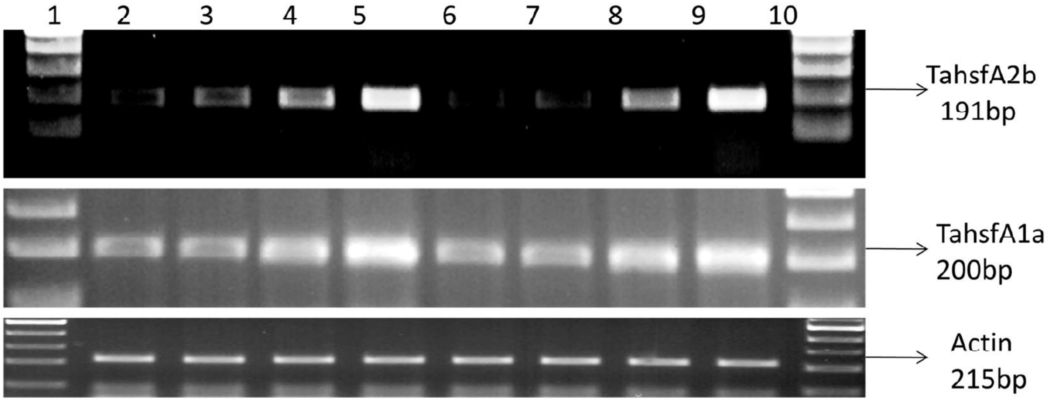

Plate 2: sqRT-PCR expression analysis of *TahsfA2b* and *TahsfA1a* genes in wheat flag leaves 2 day after SA spray. Lane -1& 10, 100 bp DNA marker, Lane 2 control (normal sown), Lane 3 (C+SA), Lane 4 (HT (late sown), Lane 5 (HT+SA) of HD 2985 and Lane 6 control (normal sown), Lane 7 (C+SA), Lane 8 (HT (late sown), Lane 9 (HT+SA) of HD 3086

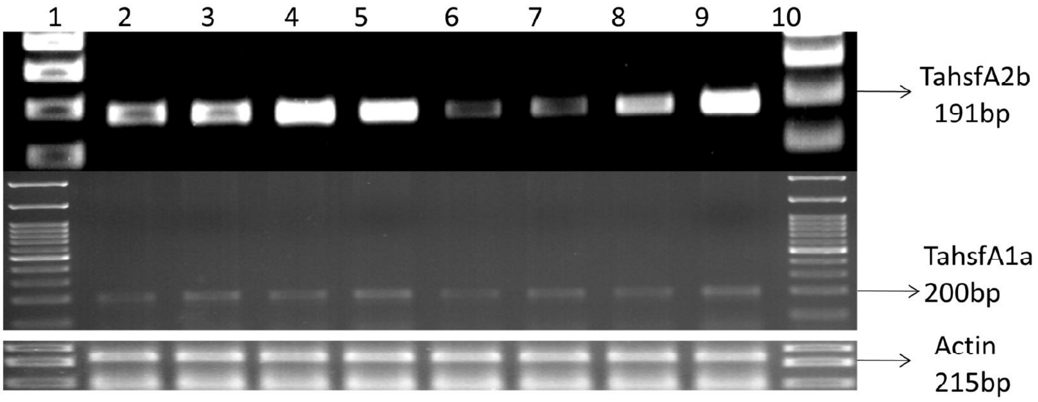

Plate 3: sqRT-PCR expression analysis of *TahsfA2b* and *TahsfA1a* genes in wheat flag leaves 2 day after SA spray. Lane -1& 10, 100 bp DNA marker, Lane 2 control (normal sown), Lane 3 (C+SA), Lane 4 (HT (late sown), Lane 5 (HT+SA) of HD 3076 and Lane 6 control (normal sown), Lane 7 (C+SA), Lane 8 (HT (late sown), Lane 9 (HT+SA) of HD 3043.

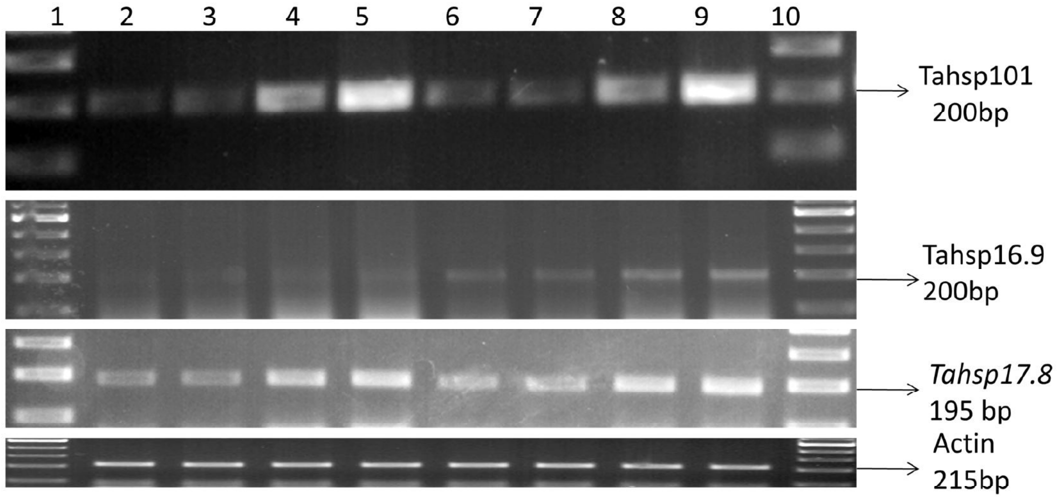

Plate 4: sqRT-PCR expression analysis of *Tahsp101. Tahsp16.9* and *Tahsp17.8* genes in wheat flag leaves 2 day after SA spray. Lane -1& 10, 100 bp DNA marker, Lane 2 control (normal sown), Lane 3 (C+SA), Lane 4 (HT (late sown), Lane 5 (HT+SA) of HD 2985 and Lane 6 control (normal sown), Lane 7 (C+SA), Lane 8 (HT (late sown), Lane 9 (HT+SA) of HD 3086.

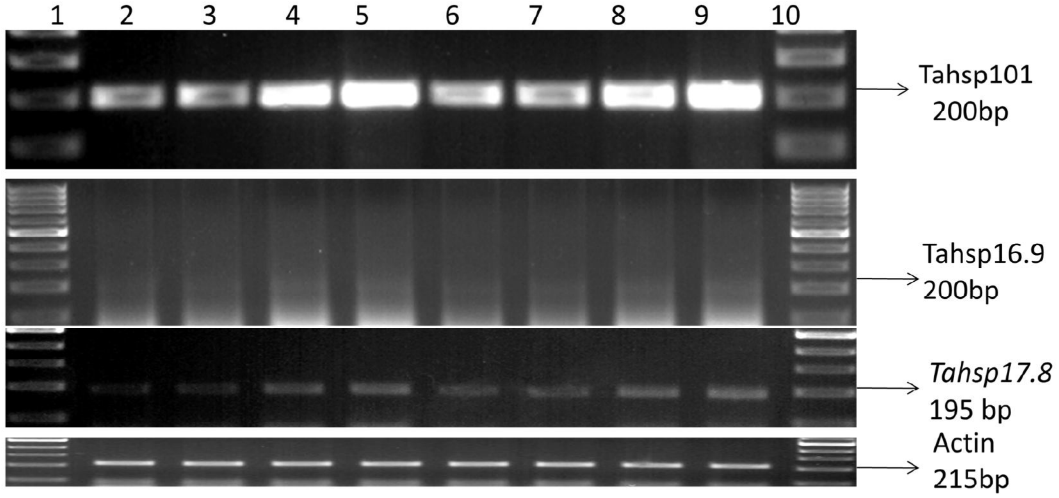

Plate 5: sqRT-PCR expression analysis of *Tahsp101, Tahsp16.9* and *Tahsp17.8* genes in wheat flag leaves 2 day after SA spray. Lane -1& 10, 100 bp DNA marker, Lane 2 control (normal sown), Lane 3 (C+SA), Lane 4 (HT (late sown), Lane 5 (HT+SA) of HD 3076 and Lane 6 control (normal sown), Lane 7 (C+SA), Lane 8 (HT (late sown), Lane 9 (HT+SA) of HD 3043.

wheat genotypes did not change the expression of *TaICS* gene in any genotypes noticeably.

4.3.4.7 Expression analysis of 9-cis-epoxycarotenoid dioxygenase (*NCED*)

The *TaNCED* expression study was done in two wheat genotypes. In case of *TaNCED* gene, sqRT-PCR was performed with gene specific primers and expected amplicons of size 200bp were obtained in all genotypes in treatment as well as in its respective control (plate 6). The *TaNCED* gene expressed in both the wheat genotypes i.e. HD3076 and HD2985 grown under normal sown conditions, however the expression was more in HD3076. The salicylic acid treatment in normal sown wheat genotypes did not change the expression of *TaNCED* in any genotypes. In wheat genotypes under high temperature stress conditions (late sown) the expression of *TaNCED* was induced in both the genotypes, however, the expression of *TaNCED* was more in the case of HD3076 as compared to HD2985. Not much change in the expression of *TaNCED* gene was observed in any of the late sown wheat genotypes on application of salicylic acid.

4.3.4.8 Expression analysis of fructan -6-exohydolase (*6-FEH*)

The *Ta6-FEH* expression study was done in two wheat genotypes. In case of *Ta6-FEH* gene, sqRT-PCR was performed with gene specific primers and expected amplicons of size 190 bp were obtained in all genotypes in treatment and respective control (plate 6). The expression of *Ta6-FEH* in normal sown crop was identical in both the wheat genotypes i.e. HD3076 and HD2985. The salicylic acid treatment in normal sown wheat genotypes did not change the expression of *Ta6-FEH* in any genotypes. In wheat genotypes under high temperature stress conditions (late sown) the expression of *Ta6-FEH* was induced in both the genotypes, however, the expression of *Ta6-FEH* was uniform in both the genotypes. The application of salicylic acid in late sown wheat genotypes did not change the expression of *Ta6-FEH* gene to a great extent in any of the genotypes.

4.3.5 Effects of foliar spray of SA (0.1mM) on yield and yield attributes in four wheat genotypes) under normal and high temperature stress (induced by late sowing).

4.3.5.1 Grain yield (Grain weight per plant)

Result of the effect of exogenous SA on grain yield (cropping season 2013-14) of four wheat genotypes recorded in control (normal sown) and temperature stressed plant (induced by late sowing) are presented in Table 4.3.5.1 (A) and Fig 4.3.5.1 (A). In comparison to normal sown condition, the grain yield decreased significantly in all genotypes grown under late sown (high temperature stress) condition with maximum decrease recorded in HD3076 (57%) and minimum in HD2985 (43%) and HD3086 (46%). Under normal condition, grain yield per plant was higher in HD3086 and minimum in HD3076, however, under stress condition, yield was higher in HD2985 and minimum in HD3076. Application of SA increased the grain yield but it was non-significant in all genotypes in both normal and late sown conditions. The results of grain yield per plant during cropping season 2014-15 are presented in Table 4.3.6.1. (A) and Fig 4.3.6.1. (A) clearly indicate that test weight were not significantly affected by application of SA.

4.3.5.2 Harvest index (HI)

Result of the effect of exogenous SA on harvest index (%) (cropping season 2013-14) of four wheat genotypes recorded in control (normal sown) and temperature stressed plant (induced by late sowing) are presented in Table 4.3.5.1 (B) and Fig 4.3.5.1 (B). In comparison to normal sown condition, HI decreased significantly in all genotypes raised under late sown (high temperature stress) condition with maximum decrease observed in HD3076 (31%) and minimum in HD2985 (14%) and HD3086 (16%). Under normal condition HI was higher in HD3086 and minimum in HD3076, however, under stress condition, HI of both tolerant genotypes was equivalent and minimum in HD3076. Application of SA did not affect the HI significantly in any of the genotypes grown under both normal and late sown conditions. The results of harvest index of cropping season 2014-15 is presented in Table 4.3.6.1 (B) and Fig 4.3.6.1 (B) clearly indicate that test weight were not significantly affected by application of SA.

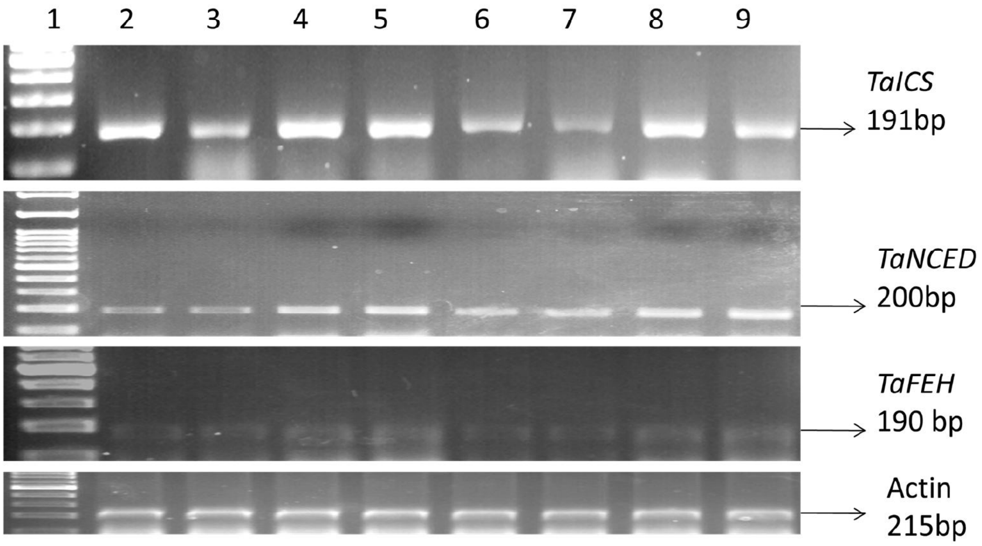

Plate 6: sqRT-PCR expression analysis of *TaICS, TaNCED* and *TaFEH* genes in wheat flag leaves 2 day after SA spray. Lane -1, 100 bp DNA marker, Lane 2 control (normal sown), Lane 3 (C+SA), Lane 4 (HT (late sown), Lane 5 (HT+SA) of HD 2985 and Lane 6 control (normal sown), Lane 7 (C+SA), Lane 8 (HT (late sown), Lane 9 (HT+SA) of HD 3076.

4.3.5.3 Test weight

Result of the effect of exogenous SA on test weight (g) (cropping season 2013-14) of four wheat genotypes recorded in control (normal sown) and temperature stressed plant (induced by late sowing) are presented in Table 4.3.5.1. (C) and Fig 4.3.5.1 (C). In comparison to normal sown condition, the test weight decreased significantly in all genotypes grown under late sown (high temperature stress) condition with maximum decrease observed in HD3076 (33%) and minimum in HD2985 (22%) and HD3086 (23%). Under normal condition test weight was higher in HD2985. Under stress condition, test weight of HD2985 was higher and minimum in HD3076. Application of SA did not affect the test weight significantly in any genotypes grown under normal as well as late sown conditions. The results of test weight of cropping season 2014-15 is presented in Table 4.3.6.1 (C) and Fig 4.3.6.1 (C) clearly indicate that test weight were not significantly affected by application of SA.

Table 4.3.4.1: Effect of SA on (A) Grain yield (g $plant^{-1}$) (B) harvest index (%) (C) and test weight (g) of wheat genotypes grown in normal and late sowing (high temperature stress) condition (2013-14).

(A)

Genotypes	**Grain yield (g $plant^{-1}$)**							
	C	**C+SA**	**Mean**	**% change**	**HT**	**HT+SA**	**Mean**	**% change**
HD2985	17.57	17.87	17.72	1.71	9.67	10.35	10.01	7.07
HD3086	18.67	18.80	18.73	0.71	9.63	10.19	9.91	5.78
HD3076	14.57	14.60	14.58	0.23	7.13	7.23	7.18	1.32
HD3043	15.97	16.10	16.03	0.83	8.00	8.31	8.15	3.84
Factors	**Sowing (S)**	**Treatment (T)**	**S×T**	**Variety (V)**	**S×V**	**T×V**	**S×T×V**	
C.D.	0.752	N/A	N/A	1.063	N/A	N/A	N/A	

(B)

Genotypes	**Harvest index (%)**							
	C	**C+SA**	**Mean**	**% change**	**HT**	**HT+SA**	**Mean**	**% change**
HD2985	47.46	47.02	47.24	-0.92	40.80	41.17	40.99	0.90
HD3086	49.44	49.03	49.24	-1.79	41.33	42.25	41.79	1.89
HD3076	43.82	43.08	43.45	-1.70	33.86	33.47	33.66	-1.14
HD3043	45.42	44.60	45.01	-1.25	37.52	37.60	37.56	0.21
Factors	**Sowing (S)**	**Treatment (T)**	**S×T**	**Variety (V)**	**S×V**	**T×V**	**S×T×V**	
C.D.	1.167	N/A	N/A	1.651	N/A	N/A	N/A	

(C)

Genotypes	**Test weight (g)**							
	C	**C+SA**	**Mean**	**% change**	**HT**	**HT+SA**	**Mean**	**% change**
HD2985	44.17	45.50	44.84	3.01	35.24	35.91	35.57	1.89
HD3086	43.33	43.68	43.50	0.81	33.73	34.33	34.03	1.78
HD3076	42.57	42.59	42.58	0.06	29.00	29.19	29.10	0.67
HD3043	37.80	38.00	37.90	0.52	27.27	27.65	27.46	1.40
Factors	**Sowing (S)**	**Treatment (T)**	**S×T**	**Variety (V)**	**S×V**	**T×V**	**S×T×V**	
C.D.	1.409	N/A	N/A	1.993	N/A	N/A	N/A	

C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid

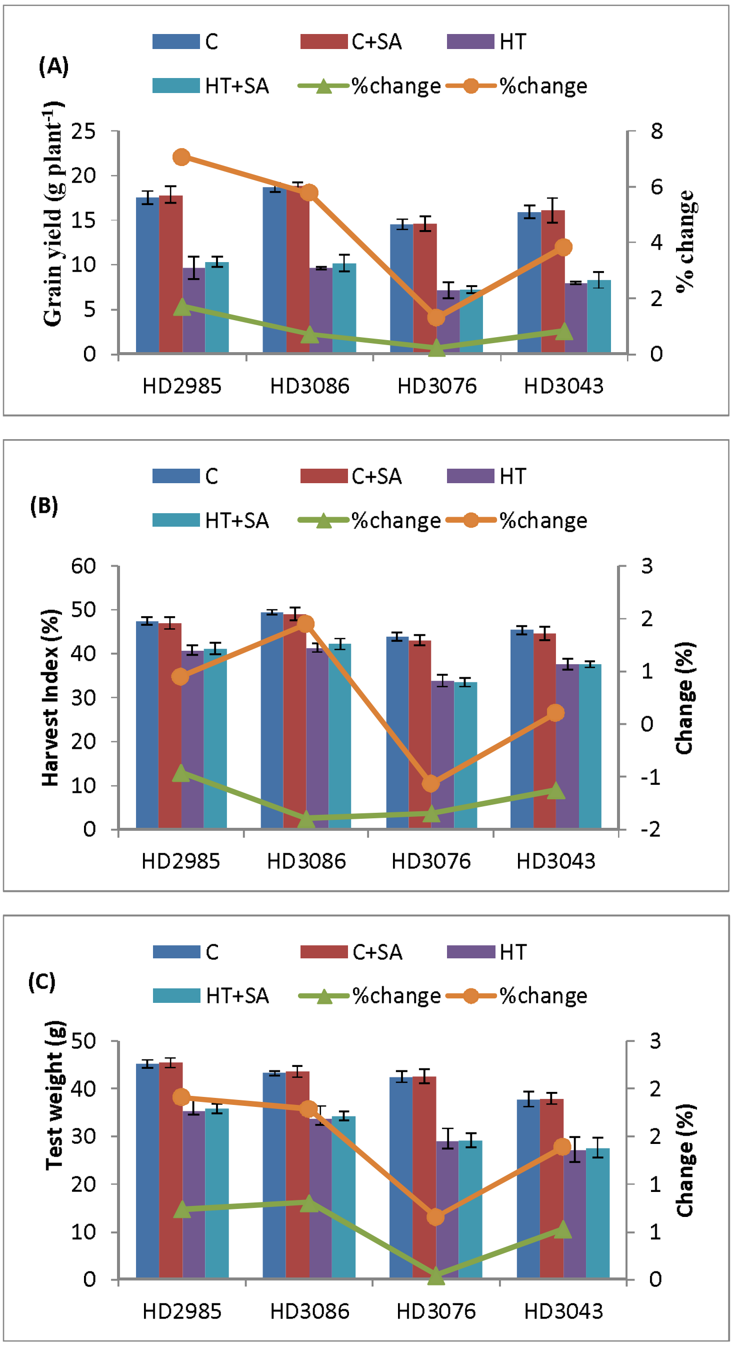

Fig 4.3.5.1: Effect of SA on (A) Grain yield (g plant^{-1}) (B) harvest index (%) and (C) test weight (g) of wheat genotypes grown in normal and late sowing (high temperature stress) condition (2013-14). (C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid)

Table 4.3.5.2: **Effect of SA on (A) grain yield (g plant^{-1}) (B) harvest index (%) (C) and test weight (g) of wheat genotypes grown in normal and late sowing (high temperature stress) condition (2014-15).**

(A)

Genotypes	Grain yield (g plant^{-1})							
	C	**C+SA**	**Mean**	**% change**	**HT**	**HT+SA**	**Mean**	**% change**
HD2985	17.933	18.113	18.023	1.00	10.107	11.083	10.595	9.66
HD3086	18.123	18.167	18.145	0.24	9.683	10.54	10.1115	8.85
HD3076	14.12	14.173	14.1465	0.38	6.003	6.327	6.165	5.40
HD3043	16.353	16.47	16.4115	0.72	7.563	8.11	7.8365	7.23
Factors	**Sowing (S)**	**Treatment (T)**	**S×T**	**Variety (V)**	**S×V**	**T×V**	**S×T×V**	
C.D.	0.794	N/A	N/A	1.123	N/A	N/A	N/A	

(B)

Genotypes	Harvest index (%)							
	C	**C+SA**	**Mean**	**% change**	**HT**	**HT+SA**	**Mean**	**% change**
HD2985	47.18	46.143	46.6615	-2.21	40.44	40.57	40.505	0.32
HD3086	48.37	48.84	48.605	-1.84	40.333	40.377	40.355	0.12
HD3076	43.887	42.78	43.3335	-2.52	30	30.03	30.015	0.09
HD3043	44.787	43.213	44	-3.55	35.157	35.293	35.225	0.37
Factors	**Sowing (S)**	**Treatment (T)**	**S×T**	**Variety (V)**	**S×V**	**T×V**	**S×T×V**	
C.D.	1.077	N/A	N/A	1.524	2.155	N/A	N/A	

(C)

Genotypes	Test weight (g)							
	C	**C+SA**	**Mean**	**% change**	**HT**	**HT+SA**	**Mean**	**% change**
HD2985	45.173	45.5	45.3365	0.72	35.117	35.967	35.542	2.42
HD3086	42.8	43.11	42.955	0.72	32.647	32.867	32.757	0.67
HD3076	42.403	42.54	42.4715	0.32	28.363	28.787	28.575	1.49
HD3043	38.197	37.8	37.9985	-1.04	29.657	30.107	29.882	1.52
Factors	**Sowing (S)**	**Treatment (T)**	**S×T**	**Variety (V)**	**S×V**	**T×V**	**S×T×V**	
C.D.	1.426	N/A	N/A	2.017	2.853	N/A	N/A	

C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid

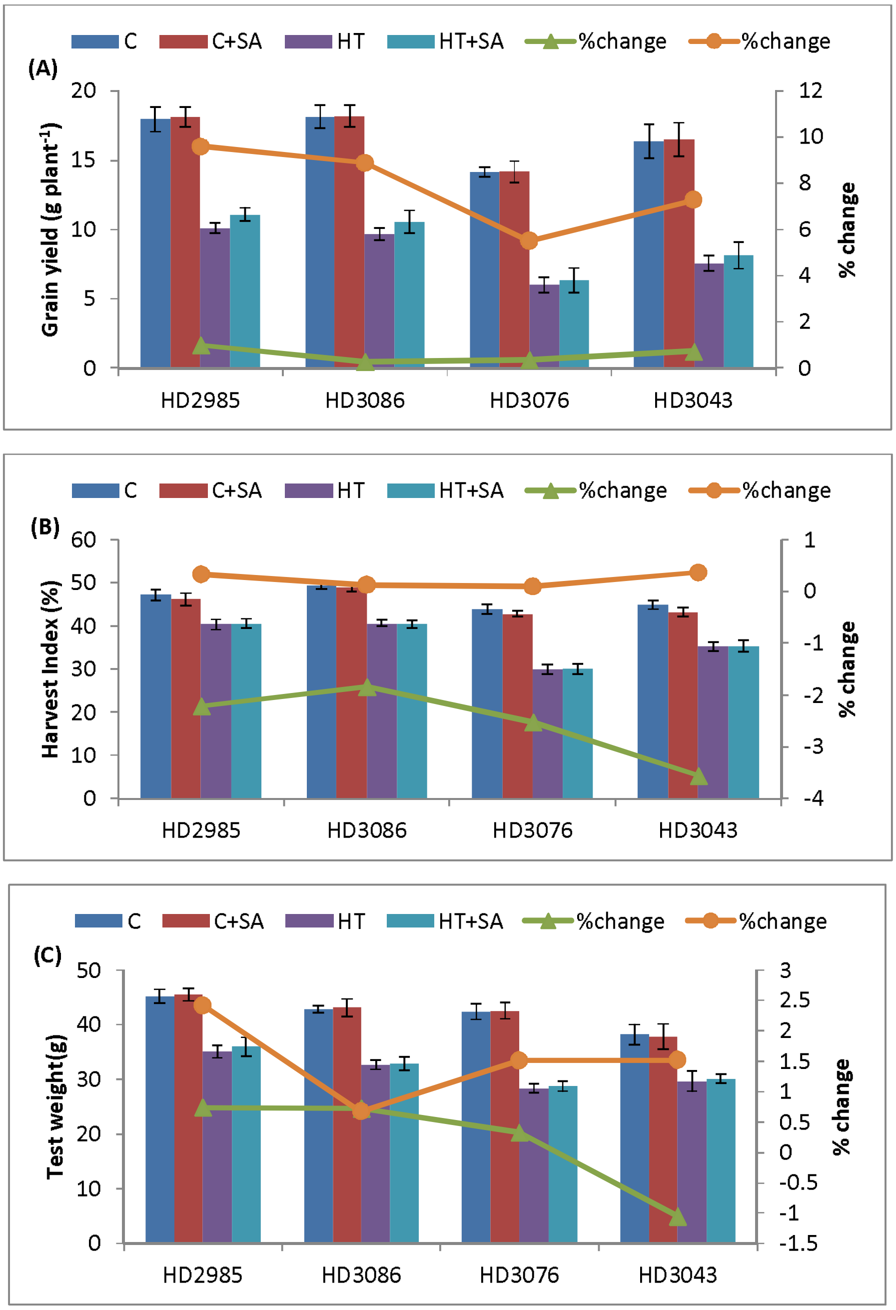

Fig 4.3.6.1: Effect of SA on (A) Grain yield (g plant^{-1}) (B) harvest index (%) and (C) test weight (g) of wheat genotypes grown in normal and late sowing (high temperature stress) condition (2014-15). (C = Control; C+SA = Control + salicylic acid; HT = High Temperature; HT+SA = High temperature + salicylic acid)

5. DISCUSSION

Wheat is one of the most important staple food crops of the world including India in terms of the harvested area, human nutrition and grown primarily for its grain. High temperature stress during reproductive phase is very detrimental for wheat as it reduces the productivity of wheat crop. It is predicted that in near future the incidence of the terminal heat stress for wheat likely to be increase. Exogenous application of signalling compounds play an important role in minimising the negative effects of high temperature stress mainly by reducing damage caused by oxidative stress (Larkindale, 2004). Induced thermotolerance has been reported when plants were pre-treated with salicylic acid (SA) (Dat et al., 1998a; Larkindale and Knight, 2002). Dat et al. (1998b) have also reported that pre-treatment with SA and mild accumulation of hydrogen peroxide in plants may carry out a signalling function during high temperature stress.

In recent years salicylic acid (SA) has been the focus of intensive research due to its function as a signal transducer or messenger under stress conditions and induction of different anti-stress programs (Klessig and Malamy, 1994). The important protective action of SA probably reflects its ability to induce the expression of genes coding not only for PR-proteins, but also induced synthesis of heat shock proteins in tobacco plants (Burkhanova et al., 1999). The influence of exogenous SA depends on several aspects, comprising, the species and developmental stage of the plant, the mode of application, and the concentration of SA and its endogenous level in the specified plant (Horvath et al., 2007; Ashraf et al., 2010; Hayat et al., 2010). The effective protective effect of SA against various environmental stresses was concentration dependent (Horvath et al., 2007).

However, the scientific information about the role of exogenously foliar applied SA under prolonged heat stress in wheat plant particularly at reproductive stage is either lacking or still not clear. Secondly, whether the positive effect of SA is long lasting or transient in nature is indistinct. Thirdly, if it is long lasting then again it is doubtful whether the positive effect culminates into increase in yield. Therefore, the present study was done to understand the role of salicylic acid in terminal heat stress tolerance of wheat. In this regard extensive studies were done at seedling, vegetative and reproductive levels and three independent experiments were formulated. The first experiment was performed on wheat seedlings (10days old) in laboratory condition, to identify the optimum salicylic acid concentration based on plant growth and development of wheat genotypes under study. The second experiment was performed in 30 days old wheat plant, with single wheat genotype (HD3086) to see the response of optimum dose (identified in first experiment) of SA to heat stress during vegetative stage. The basic idea of second experiment was to confirm whether optimum dose of SA, which was identified in first experiment, was capable of making some positive changes in physiological, biochemical and molecular parameters or not under heat stress. It was observed that 0.1mM SA concentration effectively/positivity modulated the physiological as well as biochemical traits and gene expression in wheat plants. Finally, in the third experiment same concentration of SA (0.1mM, confirmed in first and second experiment) was foliar applied during reproductive stage of four wheat genotypes (relatively heat stress tolerant and susceptible) which were grown in two rabi seasons during 2013-14 and 2014-15, and during normal sowing and late sowing (to induce high temperature stress during reproductive phase).

First experiment was performed for identification of SA dose in petri plate grown plants with six different concentrations of SA. The study showed that high temperature stress and higher concentration of SA severely inhibited the growth of wheat seedlings, as indicated by significantly decreased in plant heights, root lengths, and shoot length of wheat seedlings as reported in Fig 4.1.1. Foliar applied lower concentration (0.1mM and 0.25 mM) of SA partially restored growth and significantly counteracted the effects of heat stress in wheat seedlings by increasing plant height, roots length and shoot length, however, it was still lower than the plant control plant (grown under ambient conditions). Our findings are consistent with several previous reports. Stevens et al. (2006) showed under saline condition application of 0.1mM SA through rooting media enhanced the growth of tomatoes plant. Kang et al. (2013) have also reported that 0.5 mM SA significantly reduced NaCl-induced growth inhibition in wheat seedlings; however in our experiment 0.1mM gave best result under high temperature stress. Shakirova et al. (2003) have also reported that an important contribution to the growth- stimulating effect of SA is an enhancement of cell division and extension of cells in wheat when pre sowing treatment were done with 0.05mM SA. The observed declines in plant growth of wheat plants under control as well as heat stress condition after application of higher concentration of SA (beyond 0.5mM) might be the result of excessive accumulation of ROS. The previous report on morphological and physiological responses in seedling stage implied that SA could modify the pace of physiological metabolisms and modulate the intricate pathways of regulation under abiotic stress conditions.

In second experiment was done with single genotype. It was observed that optimum concentration of SA which was identified in first experiment (0.1mM SA) effectively/positivity modulated the physiological as well as biochemical traits and gene expression in leaves of 30 days old plant. The result presented in Fig 4.2.1 revealed that pre-treatment with SA (0.1mM), decreased the level of H_2O_2 and TBARS contents in leaves of 30 days old plant (vegetative stage) and enhanced the activity of SOD and APOX in SA treated heat stressed plant compared to without SA treated heat stressed plant (Fig 4.2.2). However the CAT activity decreased in SA treated plant. We have also measured the total chlorophyll, photosynthesis rate and efficiency of PSII and result presented in Fig 4.2.3 indicted that SA application helped in maintenances of higher chlorophyll, Pn and Fv/Fm compared to heat stressed without SA treated plant. This shows that photosynthetic machinery was protected from heat stress upon pre-treatment with SA. Similar finding were also reported by Fahad et al. (2012) in tomato under salt stress and Khan et al. (2013) in wheat seedling under heat stress.

The third experiment was done under varied and prolonged heat stress condition in four wheat genotypes and observations were recorded at two different stage to see whether positive response are long lasting or transient in nature. In third experiment salicylic acid dose (0.1mM) identified and confirmed in first and second experiment were foliar sprayed in four wheat genotypes (two tolerant and two relatively susceptible) at reproductive stage and observation recorded 2 and 10 DAFS in normal as well late sown crop. The response of biochemical and physiological traits were analysed two and ten days after spray (DAFS).

High temperature stress (induced by late sowing) significantly increased TBARS contents (Table 4.3.1.1 and Fig 4.3.1.1) and decreased the membrane stability index (Table 4.3.1.2 and Fig 4.3.1.2) of wheat leaves in all genotypes compared to control (normal sown). This shows that ROS generated during high temperature stress caused damaged to membrane by enhancing the lipid peroxidation. However, SA application ameliorated the adverse effect of high temperature stress on membrane stability index as well as TBARS contents. It indicted that foliar spray of SA may have a signalling function that plays a role in the stimulation of heat tolerance in wheat plants as indicated by the decreases in TBARS contents. Similar to our findings, Fahad et al. (2012) also reported increase in MSI of maize leaf under salinity stress in response to 10^{-5}M salicylic acid. Wang et al. (2014) have also showed, 0.3mM SA application decreased lipid peroxidation and increased MSI in wheat leaf under heat and high light stress conditions. However, 10 DAFS the content of TBARS increased even in SA treated plant of all genotypes although it was still lower than respective control. Similarly the MSI value was also decreased in all genotypes and maximum decrease observed in HD3076. When we compare response of SA, 2 DAFS and 10 DAFS then it indicate that the effect of SA is not long lasting.

Antioxidant enzymes like SOD, CAT, and APX are considered major enzymes for dissipation pathways of surplus photon energy during environmental stress (Asada, 1999). SOD is the most efficient in averting cellular damage by transforming superoxide anion to H_2O_2. CAT perform role in eliminating the bulk of H_2O_2 produced during photorespiration, and APX is also a chief enzyme that converts H_2O_2 to H_2O (Sharma and Dubey, 2007). It is also reported that plants containing high concentrations of antioxidants demonstrate substantial resistance to oxidative injury caused by activated oxygen species (Garratt et al., 2002).

Catalase is one of important antioxidant enzymes takes the responsibility for removing the bulk of H_2O_2 generated during stress conditions. However, in our study, the CAT activity declined both in normal as well in late sown crop after 2DAFS and it remains low even after 10 DAFS (Table 4.3.1.4 and Fig 4.3.1.4). However maximum decrease observed in late sown crop. Dat et al. (1998b) reported that pre-treatment with SA caused mild accumulation of H_2O_2 and helped the plant during acclimation to abiotic stress and concluded that hydrogen peroxide may perform a signalling. Other worker also reported that accumulation of H_2O_2 in response to exogenous SA treatment has also been observed by (Rao et al., 1997). Wang and Li (2006) also reported in grape, that SA application declined the CAT activity and promptly increased SOD and POD activities together in grape leaves.

Similar to our finding, Fahad et al. (2012) also reported accumulation of H_2O_2 and decrease in CAT activity in maize seedling under salinity stress.

In general antioxidant enzyme activity after foliar application of SA on reproductive stage of wheat plants enhanced the activities of SOD, POX, GR and APOX not only in late sown crop (high temperature stress conditions), but also improved their activities in normal sown crops in all genotypes. Enhancement of enzyme activities were more tolerant genotypes compared to the susceptible genotypes and more in late sown condition compared to normal sown condition. The results presented in Table 4.3.1.5, Fig 4.3.1.5 and Table 4.3.1.3, Fig 4.3.1.3 clearly indicate the foliar application of SA enhanced the SOD and POX activities, respectively, 2DAFS and maximum increase observed in HD2985 under high temperature stress condition. However, the activity decreased when measured 10 DAFS of SA, still activity of these enzymes was higher than control plant. The results are similar with Wang et al. (2006) who have also observed in grape, that SA application (0.1mM) promptly increased SOD and POD activities in grape leaves. Contrast to our finding Wang et al. (2014) have also reported that only slight enhancement of SOD activity in response of 0.3mM SA application in wheat plant under short term heat stress.

Results present in Table 4.3.1.6, Fig 4.3.1.6 and Table 4.3.1.7, Fig 4.3.1.7, clearly shows that the antioxidant enzyme activity of GR and APOX enhanced greatly under high temperature stress as well after application of SA in HD2985. Among antioxidant enzymes the activity of APOX enhanced greatly upon application of SA. Similar to our finding Wang et al. (2014) have also reported that maximum enhancement of APOX in response of 0.3mM SA application in wheat plant under short term heat stress condition. However similar to other enzymes the activity of APOX and GR also decreased 10 DAFS in all genotypes. Most of workers reported that the enhancement of antioxidant system upon application of SA in stressed plant and activity reached/ reduced to control level after few hours or days. However in present study in pot we have also observed reduced activity of antioxidant enzymes, but it was still higher than respective control even after 10 DAFS.

For proper functioning of plant the redox state of different cell compartment is very essential. Antioxidant metabolite like glutathione and GR are concerned with the redox state regulation as it is a part of the ascorbate–glutathione cycle. During the elimination of surplus H_2O_2, the reduced glutathione is transformed to its oxidized state, and reduced glutathione is regenerated by the activity of NADPH dependent GR. Increased activity of GR sustains the pool of reduced glutathione (Noctor et al., 2002). In the present investigation, elevated GR activity reduced the contents of oxidised glutathione following SA treatment and might helped regeneration of reduced GSH pool, which scavenged the excess H_2O_2 in flag leaves of wheat plants in all genotypes, however, it started increasing 10 DAFS (Table 4.3.1.8 and Fig 4.3.1.8) as activity of GR decreases. Decrease in oxidised glutathione and consequently accumulation of GSH has been corroborated during heat and cold stress in plants, and it has observed that alteration in the glutathione pool may act as signals to induce stress related transcription factors and gene expression concerned with heat and cold acclimation (Wang, 1995; Kocsya et al., 2001). Similar to our finding Wang et al. (2006) who have also reported that high GSH concentration in the leaves subsequent to SA treatment and during heat acclimation inferring that GSH may also induce defence gene expression to enhance high temperature tolerance. In present study SA spray reduced ascorbate level in wheat plant when recorded two DAFS and 10 DAFS (Table 4.3.1.9 and Fig 4.3.1.9). However maximum reduction observed two DAFS. It clearly shows that the ascorbate is utilised by the increased activity of APOX. Therefore, the increased activity of APOX which convert H_2O_2 to H_2O and O_2 utilizing reduced ascobate as the reductant following SA application. Results are in agreement with report of Wang et al. (2006) who also showed in grape AsA content decreased sharply when APOX activity reached maximum.

Photosystem II is alleged to play a crucial role in the response of leaf photosynthesis to environmental stress (Murata et al., 2007). Fv/Fm, Fv/Fo, ETR, and Pn are frequently utilized as sensitive indicators of plant photosynthetic performance and signify the practical measure of the functioning of oxygen evolving complex (Bakere al., 2008). Results of our study indicate that late sown induced high temperature significantly reduced the efficiency of PSII. The detail results are presented in Table 4.3.1.10 and Fig 4.3.1.10. However, foliar application of SA, maintained higher efficiency of PSII compared to respective control in all genotypes. The maximum increase was observed in tolerant genotypes. The higher value of Fv/Fm may be due to protection of PSII from ROS. This ROS might be scavenged by SA induced antioxidant systems. Secondly in the transcription level of *psbA* and *psbO* induced by application of SA, which was observed in leaves of 30 days old wheat, the enhanced gene expression might have protected the functioning of PSII. Indirectly our result (*TapsbA* gene expression which encode D1 protein) of present experiment support the finding of Zhao et al. (2011) who have also reported in proteomic study that foliar application of SA has been proven to retard the decrease of D1 protein content in wheat plants under heat and high light stress. Wang et al. (2014) have also shown induced *psbA* transcription by foliar applied SA may enhance the de novo synthesis of the new copies of D1 protein, and then improve the turnover of it and through maintaining the anti-oxidative enzyme activities, which are all beneficial for the improvement of PSII efficiency during short term heat stress.

The foliar spray of SA, maintained higher net photosynthesis rate (Pn) as compared to the respective control, under late sown induced high temperature stress condition and the detail result were presented in Table 4.3.1.11 and Fig 4.3.1.11. However, photosynthesis was not significantly affected by application of SA in normal sown wheat crop. The effect of SA was more pronounced in tolerant genotypes compared to susceptible genotypes. In general the ROS production enhanced during heat stress which damage the D1 protein associated with PSII and also damage the one or two heat susceptible protein associated with oxygen evolving complex. However, in our study I have found that the exogenous application of SA not only enhanced the activity of antioxidant enzymes, but also enhanced the expression of *TapsabA* and *TapsbO* genes, which encode the D1 protein and protein associated with oxygen evolving complex and protect the PSII and might be helping in maintenances of higher Pn compared to respective control. Rai et al. (1986) have proved that SA reverses the ABA-mediated stomatal closure which might enhanced the CO_2 assimilation. Findings of present study are in agreement with that of Wang et al. (2010) who reported that, SA did not significantly ($P < 0.05$) affect the net photosynthesis rate (Pn) of grape leaves prior to heat stress, but, SA pretreatment alleviated the reduction in Pn under heat stress, by keeping a higher Rubisco activation state and greater PSII efficiency and may also increase PSII revival. On the other hand, Khan et al. (2013) explained that SA application elevated photosynthesis even during no stress condition, and lessened the harmful outcome of heat stress in wheat.

The results presented in Table 4.3.1.12, 4.3.1.13, 4.3.1.14 and Fig 4.3.1.12, 4.3.1.13, 4.3.1.14, revealed that high temperature stress induced by late sowing significantly decreased chlorophyll a, chlorophyll b and carotenoid contents in flag leaves of wheat plants as compared to respective control (normal sown) in all genotypes. However, exogenously applied salicylic acid ameliorated the inhibitory effect of high temperature stress on chlorophyll *a* and total chlorophyll content. This protective effect of SA may be due to protection of membrane from ROS due to enhanced activity of most of the antioxidant enzymes. However, the exogenous application of SA did not affect the chlorophyll b contents when estimated two DAFS, however positive affect was observed after 10 DAFS of SA. The carotenoid contents were not significantly affected by foliar application of SA, the content was almost similar to respective control plant. When we analyzed the results in different genotypes, then it revealed that the response to SA was comparatively better/higher in tolerant genotypes compared to susceptible genotypes. Fahad et al. (2012) reported that the SA treatment under salt stress showed significant increase in total chlorophyll content as compared to the plants grown in saline condition. Favourable effect of SA on the chlorophyll content of maize leaves was also reported by Khodary (2004). In contrast to our findings, Fahad et al. (2012) reported that he SA treatment under saline condition resulted in higher carotenoid content as compared to that of saline condition alone in maize. The increased SPAD values are an indirect indicator of chlorophyll and have a significant association with photosynthesis and leaf N content (Araus et al., 1997). We have also studied the effect of exogenous SA on SPAD value in flag leaves of wheat genotypes and results presented in Table 4.3.1.15 and Fig 4.3.1.15, which revealed that SPAD values were maintained significantly higher compared to respective control in all genotypes. Similar to our results, Khan et al. (2013) also reported that SA application ameliorated the adverse effect of heat stress on SPAD value in wheat seedling.

Reserved NSC may work as a buffer to maintain a uniform rate of grain filling, particularly when current photosynthesis is injured because of environmental stresses. However, the involvement of these stored reserves may report for only 5 to 20% of the ultimate grain yield in non-stress conditions (Shakiba et al., 1996). When photosynthetic activity is depressed by drought or heat following anthesis, grain filling turn out to be more dependent on mobilized stem reserves, which may characterize 22 to 60% of the dry matter that accumulates in the grain (Davidson and Chevalier, 1992; Blum et al., 1994). It was observed that there is variation amongst internodes in the amount of NSC that are accumulated and mobilized (Shakiba et al., 1996). Previous study demonstrated that a major portion of carbohydrates are accumulated in the penultimate internodes of stem (Scofield et al., 2009). So, it is significant to study the mobilisation pattern of NSC found in penultimate internodes during high temperature stress conditions and their response to exogenous SA in diverse wheat genotypes. In our study the NSC were measured in penultimate internodes and results are presented in Table 4.3.2.1 to Table 4.3.2.4 and Fig 4.3.2.1 to Fig 4.3.2.4 revealed that the foliar spray of SA did not significantly affect the accumulation of maximum NSC (in anthesis +10 days sample) and mobilised NSC in normal as well as late sown plants (high temperature stress). The reason behind this may be short term positive effect of SA on wheat genotypes. Contrary to our finding, recently González et al. (2014) observed that exogenous application of 1 mM SA (very high concentration) applied in growth media (PEG treated) induced accumulation of fructo-oligosaccharides (FOS) in several degrees of polymerization in stems of *Agava tequilana*. However, in present study high temperature stress significantly reduced the maximum accumulation of NSC in penultimate internodes. The mobilised NSC were also reduced in late sown crop (high temperature stress) compared to normal sown crop in both the year. High temperature stress reduced the maximum NSC content by 27 to 30 per cent under late sown condition and the mobilized NSC content was decreased by 12 to18 per cent through all genotypes. This may be largely due to decline in photosynthesis and diminished photosynthate. In accordance to our result Johnson et al. (1984) have also reported that during stress conditions, the build-up of NSC in the stem may be much less owing to restrained photosynthesis and decreased photosynthate. In present study, remobilisation efficiency (RE) of NSC were also measured and detail results were presented in Table 4.3.2.2 & Table 4.3.2.4 and Fig 4.3.2.2 & Fig 4.3.2.4 clearly showed that the exogenous application of SA did not significantly affect the RE in any genotypes. However, the RE of NSC was higher in tolerant genotypes (19 to 20%) compared to susceptible genotypes (11 to 15%) under high temperature stress condition. This may be helped the tolerant genotypes to maintain higher grain yield and test weight compared to susceptible genotypes under late sown condition (High temperature stress). In accordance to our results, Talukder et al. (2013) also reported the maximum WSC content and mobilised WSC was decreased and RE was increased even by short term heat stress in all wheat genotypes.

The function of salicylic acid (SA) in the control of abscisic acid (ABA) biosynthesis and ABA accumulation is controversial even though both plant growth regulators accumulate in tissues experiencing abiotic and biotic stress conditions (Horváth et al., 2015). So, it is essential to explore the effect of exogenous SA on endogenous level of ABA under high temperature stress. Hostile environmental condition results in sharp variation in the balance of phytohormones coupled with accumulation of ABA and with the decrease in the level of growth activating hormones such as IAA and cytokinins (Jackson, 1997). Jiang et al. (2010) have reported that an antagonistic interaction of abscisic acid (ABA) with salicylic acid (SA) signalling pathways in rice. Fahad et al. (2012) have also proved that the lower ratio of ABA/IAA is because of the balance between the two phytohormones stimulated by SA and is advantageous for plant growth promotion during salinity stress in maize. The results of our research that are presented in Table 4.3.3.1 and Fig 4.3.3.1 also show that high temperature stress significantly enhanced the endogenous level of ABA in flag leaves of wheat genotypes and that the susceptible genotypes had higher ABA levels than the tolerant genotypes which might adversely influence the physiological traits like Pn and subsequently affect the yield of the susceptible genotypes than the tolerant genotypes under late sown conditions (high temperature stress). Contrasting to the results of Shakirova et al. (2003), we did not find any significant variation in the endogenous level of ABA in any of the genotypes after exogenous application of SA. This substantiates that the when appropriate concentration of SA was applied exogenously, it functioned autonomously without disturbing the endogenous levels of ABA or it might have induced the level of IAA and cytokinins (not studied in this experiments) and keeping ABA/IAA ratio lower, as reported by several workers (Sakhabutdinova et al., 2003; Fahad et al., 2012) and helped in recommencement or maintenance of physiological traits under stress condition as well.

Salicylic acid content is said to rise in plant tissues experiencing biotic and abiotic stresses reported by several workers. Molina et al., (2002) observed 51 and 35 per cent rise in free and conjugated SA after 4 h of salt treatment in tomato cell suspension. Wang et al. (2006) have also reported in grape leaves that free SA as well as total SA concentrations sharply increased to 530% and 500% compare to control sample within first hour of heat acclimation and then sudden decreased over the next 11 h. It has been reported by several worker that a number of environmental stimuli like pathogen infection, high temperature, heavy metal contamination, or the utilization of direct oxidative agent may apparently, increase it up to the range of µg/g FW which results into 10–50-fold increase (Pal et al., 2005). On commencement of congenial environmental conditions, very low concentrations (10–100 ng/g FW) of SA was observed from the leaves of *Arabidopsis*, tobacco and maize (Szalai and Janda, 2009).

Very few or no information available regarding level of SA in flag leaves (reproductive stage) of wheat under control and stressed condition. So, it becomes important to know the effect of exogenous applied SA on endogenous level of SA and basal level of SA during both the conditions. The results of our research that are presented in Table 4.3.3.2 and Table 4.3..3.3 and Fig 4.3.3.2 and Fig 4.3.3.3 shows that the foliar application of SA to wheat plants brought about highly significant increase in total as well free SA in normal as well as in late sown plants. The basal level of free as well as total SA was very high in late sown plants compared to normal sown plants. However, the free SA which was enhanced following foliar application decreased after 10 days of foliar spray, this shows that exogenously applied SA after induction of stress related response got transformed into bound form. This indicates that SA act as signalling molecules and after induction of activity of antioxidant enzymes as well HSPs free SA converted into bound form. Secondly, it also indicates the positive effect of SA is correlated with endogenous level of free SA. Similar to our findings, in a heat acclimation experiments, Wang et al. (2006) have also reported that free SA concentrations rapidly increase within hour of heat acclimation and then afterwards decreased in grape leaves.

Nover et al. (2001) recognised 15 Hsfs in *Arabidopsis thaliana* and more than 21 in *Solanum lycopersicum*, all of them perform critical functions in stress reaction (Scharf et al., 1998). Recently in wheat total 56 TaHafs (Heat shock factors) are identified (Xue et al., 2014) which are classified into A, B, and C classes of which a number of TaHsfs are constitutively expressed. On incidence of heat stress, the transcript levels of A2 and A6 members come about as dominant Hsfs, revealing a momentous regulatory role all through the heat stress. In fact, the interaction of HsfA2 with HsfA1 is essential for the co-localization of HsfA2 inside the nucleus (Scharf et al., 1998). HsfA2 is alleged to be the most proficient heat inducible transcription factor for the stress response and forms the principal Hsf all through the heat stress (Mishra et al., 2002). Salicylic acid perform very important role in binding of HSfs to thr promoter of heat shock gene and also in trimer formation of Hsfs during heat stress condition (Wahid et al., 2007). In our study the gene expressions analysis of heat shock transcription factors (*TahsfA1a* and *TahsfA2b*) were done in flag leaves of normal as well late sown (High temperature stress) wheat genotypes 2 days after foliar spray of SA. The results are presented in plate 2 and 3 showed that the expression of *TahsfA2b* increased very significantly under temperature stress in all genotypes. SA alone did not significantly affect the expression of *TahsfA2b*, however heat stress plus SA (exogenous application of SA in late sown crop) induced the gene expression of *TahsfA2b* in all genotypes. The maximum fold change/increase (accumulation of transcript) occurs in HD2985, HD3086 and HD3043. The expression was comparatively less in HD 3076. Likewise, the expression of *TahsfA1a* did not change merely by SA application, however, SA plus heat stress (exogenous application of SA in late sown crop) enhanced the expression in HD2985 and HD3086. Apart from increase in antioxidant enzyme activity, enhanced expression of HSFs upon SA application might induced the expression of HSPs and helped the plant to mitigate the high temperature stress even under prolonged high temperature stress. Similar to our finding Synman et al. (2008) have also revealed similar response of hsfA, hsfA2 and hsfB1 to SA in tomato seedlings under short term heat stress condition. Krishna, (2011) have also reported that tolerant wheat genotypes (seedling) having higher levels of heat stress induced expression of heat shock transcription factors *HSFA2b*, *HSFA4a* and *HSF8*.

To deal with heat stress, plants generate numerous heat shock proteins (HSPs), which act as molecular chaperones regulating the folding, localization, accumulation and degradation of protein molecules in plant and animal species. They perform an extensive task in a lot of cellular processes, which confer tolerance to compound environmental stress situations (Swindell et al., 2007). HSPs are able to maintain the standard folded state of proteins, disintegrate misfolded proteins, stabilize polypeptide strands and avert protein inactivity (Xue et al., 2010). The major HSPs detected in diverse eukaryotes can be categorised into six structurally discrete classes: small HSPs (sHSPs), Hsp60, Hsp70, Hsp90, Hsp100 and ubiquitin (Efeoğlu, 2009). All organisms produce HSPs from all of the above mentioned classes (Park and Bong, 2002). HSP100 families was expressed under temperature stress, and the expression increased with duration of stress (Wang et al., 2004). They remove non-functional and harmful polypeptides arising due to misfolding, denaturation or aggregation to maintain cellular homeostasis. HSP100 have been reported in several plant species, including wheat (Agarwal et al., 2001). Various studies have indicated the role of these proteins in conferring thermotolerance under heat stress (Lee et al., 2007). Transformants expressing *HSP101* showed enhanced tolerance to high temperature (Katiyar- Agarwal et al., 2003). *A. thaliana* plants under-expressing their own HSP100 proteins were found to lack basal as well as induced thermo-tolerance (Nieto-Sotelo et al., 2002), thus explaining their impact in plant survival. SA induced synthesis of heat shock proteins in tobacco plants (Burkhanova et al., 1999).

In present study the gene expressions analysis of heat shock proteins (*TaHSP101,TaHSP16.9* and *TaHSP17.8*) were done in flag leaves of normal as well late sown (high temperature stress) wheat genotypes 2 days after foliar spray of SA. The results of gene expression study were presented in plate 4 and 5. High temperature stress (late sowing conditions) alone induced the expression of large HSP (HSP101) and foliar application of SA under high temperature stress condition further induced the expressions of HSP101 in all genotypes however, maximum expression occurred in resistant genotype (HD2985 and HD3086). The expression of *TaHSP101* gene was also expressed in HD3043 and HD3076 but it was relatively less compared to HD2985. The expression of *TaHSP70* (plate 1) in wheat genotypes HD3086 leaves (leaves collected from 30 days old wheat plant) were induced under heat stress, nevertheless, application of SA plus heat stress induced the expression of *TaHSP70* very much. Implying that the SA-mediated induction of *TaHSP101* and *TaHSP70* may be due to induction of the expression of different ‘A’ members of Hsfs by SA. Similar to our results, in heat shock-stressed tomato seedlings, SA application caused enhanced expression of Hsfs and binding of Hsf to DNA (Snyman and Cronje, 2008).

The abundance and heterogeneity of sHSPs is exclusive to plants and propose that they assist distinctive physiological role in plant-acquired stress tolerance (Sun and Montagu, 2002; Wang et al., 2004). Transgenic rice plants over-expressing *OsHSP17.8* gene showed enhanced thermotolerance as well as increased resistance to UV-B irradiation (Murkami, 2004). In our study the small HSPs, the HSP16.9, expression was induced under high temperature stress (late sown condition) only in HD2985 and results are presented in plate 4 and 5. The foliar treatment of SA slightly induced the expression in HD2985. However, in other genotypes the expression was not affected by exogenous application of SA .In accordance to our finding Krishna, (2011) have also reported that tolerant wheat genotypes maintained higher levels of expression of small heat shock protein (*sHSP*) like *17.3, 16.9* and high molecular weight *HSP70, HSP80* and *HSP101*.

Regarding SA biosynthesis, latest research illustrate that the phenylalanine pathway cannot portray for all SA in plant cells, suggesting that there is an additional chief avenue for SA biosynthesis captivating the chloroplasts, where SA is synthesised from chorismate via isochorismate in processes catalysed by the isochorismate synthase (ICS) and isochorismate pyruvate lyase (IPL) enzymes (Wildermuth, 2006). Nevertheless, IPL encoding gene or protein has not been identified in plant (Mura, 2014). Moreover, the role of shikimate pathway in SA synthesis of juvenile pea plants was demonstrated (Szalai et al., 2011). SA accumulation in *Nicotiana benthamiana* that relied on ICS activity: total SA levels, was augmented more in wild-type plants than in plants with silenced ICS expression subsequent to UV irradiation treatment (Catinot et al., 2008). In present investigation the gene expressions analysis of *TaICS* gene (isochorismate synthase) were done in flag leaves of normal as well late sown (High temperature stress) wheat genotypes, 2 days after foliar spray of SA. The results of gene expression study were presented in plate 6. Foliar application of salicylic acid in normal and late sown wheat genotypes (high temperature stress conditions) did not change the expression of *TaICS* gene (isochorismate synthase) of SA biosynthesis pathways in wheat genotypes. High temperature stress (late sown condition) induced the expression of *TaICS* in both the genotypes; however, the maximum expression occurred in the resistant genotype (HD2985) as compared to the susceptible genotype (HD3076). The enhanced expression of *TaICS* in tolerant genotypes may bring about elevated basal level of total as well as free endogenous SA under high temperature stress (late sown) condition compared to the susceptible genotype. Pal et al. (2013) observed similar gene expression levels for the *CS* gene (chorismate synthase) in the leaves and roots of wheat plants on real-time PCR analysis, which changed after Cd treatment.

Reports suggest abscisic acid (ABA) accumulation during abiotic stresses (Chinnusamy et al., 2004). In contrast to cold-sensitive lines, the cold-tolerant rice accumulated less ABA in response to cold treatment, which may be attributed to lower ABA biosynthesis as well as faster turnover of ABA (Oliver et al., 2007). ABA is also recognized to be engaged with the response to heat stress (Toh et al., 2008). The first committed step in ABA synthesis is catalyzed by 9-cis-epoxycarotenoid dioxygenase (NCED; Schwartz et al., 1997) that plays a regulatory function in several plant species (Qin and Zeevaart, 1999). Moreover, the gene encoding zeaxanthin epoxidase (ZEP) is also known to regulate the ABA levels (Frey et al., 1999). The role of salicylic acid (SA) in the control of abscisic acid (ABA) biosynthesis is controversial although both plant growth regulators may accumulate in tissues under abiotic and biotic stress conditions (Havarth et al., 2015). In our study the gene expressions analysis of *TaNCED* were done in flag leaves of normal as well late sown (High temperature stress) wheat genotypes, 2 days after foliar spray of SA. The result of gene expression study were presented in plate 6. The expression of *TaNCED* was not affected by exogenous application of SA both under normal as well as high temperature stress (late sown conditions). Under high temperature stress condition the expression of *TaNCED* was induced in tolerant as well as in susceptible genotypes, however maximum increase occurred in HD3076 (susceptible genotype) than in tolerant genotypes (HD2985). Greater expression of *TaNCED* indicate more ABA, which may possibly bring about closure of stomata and less carbon assimilation and ultimately impinge on the growth and yield of crops. In contrast, Horváth et al. (2015) reported that hardening of tomato plants to salinity stress with 10^{-4} M SA resulted in an up-regulation of ABA biosynthesis gene 9-*cis*-epoxycarotenoid dioxygenase (*SlNCED1*) in the roots tissue.

The grain filling in wheat also depends on remobilisation of stored carbohydrates from stem and leaf sheath, particularly the dependency on reserved carbohydrates increased, when existing photosynthetic supply from leaf and ear decline during biotic and abiotic stress condition. (Blum, 1994: Plaut et al., 2004).

The main reserved form of carbohydrates in the stem of wheat is fructan (Goggin and Setter, 2004). The flow of carbon into fructan is mainly governed by enzyme sucrose: sucrose 1-fructosyltransferase (1-SST) and hydrolysis of fructan is catalyzed by fructan exohydrolases (FEHs) during remobilization of stored carbohydrates. The result of gene expression study were presented in plate 6. The expression of *Ta6-FEH* was induced under heat stress, which could lead to hydrolysis of stored carbohydrates and enhanced remobilisation efficiency under stress conditions. The foliar application of SA did not affect the expression of *Ta6-FEH* in any genotypes, however; the expression of *Ta6-FEH* was induced under high temperature stress condition which might be responsible for enhanced remobilisation efficiency of under late sown condition. At enzymatic level report already suggest that the activity of enzyme increases under stress condition. However, at molecular no information available in the literature on the effects of foliar application of SA on expression of *Ta6-FEH*. Recently (In 2014) one group have reported that 1mM SA enhance the *SST* expression (fructan synthesizing gene) in *Agave* species, however they have also not checked the expression of FEH gene.

Reports have revealed that collective heat and high light stress caused injurious affect on in the oxygen evolution complex in PSII and D1 protein in wheat plants (Zhao et al., 2011). Excess ROS generated during stress condition impairs D1 protein (Yamamoto et al., 2008) and impedes the restoration of photo-damaged PSII by restraining the *de novo* synthesis of D1 protein (Murata et al., 2007). In present study (In 2nd experiment) the gene expressions analysis of *TapsbA* and *TapsbO* were done in vegetative leaves of 30 days old plants of wheat genotypes (HD3086) grown under control, heat stress and heat stress plus SA treated sample. The result of gene expression study were presented in plate 1. Foliar application of SA in wheat genotype under high temperature stress enhanced the expression (accumulation of transcript) of both *TapsbA* and *TapsbO* genes in leaves and expression was more as compared to heat stressed plant without SA treatment, however, the expression of both the genes was less than the control plants (not experiencing heat stress and without SA treatment). Therefore, it can be deduced that foliar application of SA can shield the PSII complex from heat-injury via enhanced transcription of the *psbA* gene (encoding D1 protein) and *psbO* gene (encoding oxygen evolving complex protein), which permit prompt functional retrieval of PSII from heat stress. Identical results were also obtained by Wang et al. (2014) in real time PCR analysis of psbA gene in wheat under very short term heat and high light stress in response to foliar application of SA. Similarly, Zhao et al. (2011) also communicated, at proteome level exogenous SA treatment may impede the disintegration of D1 protein and maintain PSII functioning in heat and high light stress.

Very few or no information available in the literature on the effects of foliar application of SA on grain yield and its attributes under high temperature stress condition. The findings of our experiments were presented in Table 4.3.5.1 and table 4.3.6.1 and Fig 4.3.5.1 and Fig 4.3.6.1 shows that foliar application of exogenous SA at reproductive stage did not significantly affect the grain yield, test weight and HI in normal as well as late sown wheat crop in both cropping seasons. This might be due to positive effect of SA on physiological traits and other biochemical traits were not maintained for longer duration (after 10 DAFS). Opposite to our finding Shakirova et al. (2003) have reported pre-treatment (seed priming) with SA (0.05mM) were characterized by big ear sizes, greater mass of 100 seeds and superior grain yield and reduces the detrimental action of salinity in wheat.

From the present study, it can be concluded that the exogenous application of SA (0.1mM) positively modulated the physiological, biochemical trait as well the transcription level of genes of HSFs, large molecular weight HSPs analysed 2 days after foliar application, however, positive effect was not consistent as it decreased when analysed 10 days after foliar spray. This clearly shows the response of SA is not long lasting specifically in our experiment or is transient in nature and it is also evident from yield data, which is not significantly enhanced.

6. SUMMARY AND CONCLUSION

High temperature stress during reproductive phase is very detrimental for wheat as it reduces the productivity of wheat crop. It is predicted that in near future the incidence of the terminal heat stress for wheat likely to be increase. In plants, amid internal factors, hormones accomplish a primary role in regulating growth and development. Salicylic acid (SA) has lately been documented as a plant hormone. It is a natural phenolic compound and act as an endogenous signal molecule that plays a crucial task in the regulation of plant growth, development, interactions with other organisms, and responses to environmental stress. The outcome of exogenous SA depends on various factors for instance the species and developmental stage of the plant, the mode of application, and the concentration of SA and its endogenous level in the specified plant. However, the scientific information about the role of exogenously foliar applied SA under prolonged heat stress in wheat plant particularly at reproductive stage is either lacking or is still not clear.

The present investigation entitled “Role of salicylic acid in terminal heat stress tolerance of wheat (*Triticum aestivum* L.)” was conducted with two objectives (1) To understand the role of salicylic acid (SA) on the physiological and biochemical traits in contrasting sets of wheat genotypes under prolonged high temperature stress (2) to understand the physiological and molecular mechanisms of salicylic acid regulated changes in hormonal homeostasis under terminal heat stress response.

Before initiating the experiments related to response of SA under prolonged heat stress condition in four different wheat genotypes during reproductive stage, two small experiments were performed under *in-vitro* conditions. The first experiment was performed for the identification of optimum SA concentration for wheat plants under study. The basic idea behind the second experiment was to confirm whether the optimum dose of SA, which was identified in first experiment, was capable of making positive alterations in the physiological, biochemical and molecular parameters or not under heat stress. Finally, pot culture experiment (third experiment) was conducted in completely randomized design (CRD) with three replications using four wheat varieties, two relatively tolerant (HD2985 and HD3086) and two susceptible (HD3076 and HD3043). The experiments were performed at pot culture facility of Division of Plant Physiology, IARI, in the year of 2013-14 and 2014-15. The staggered sowing strategy was used for induction of high temperature stress. The sowing was done at two dates during both the cropping season: normal (20th November, 2013), late (9th January, 2014) and normal (20th November, 2014), late (9th January, 2015) to induce varied and prolonged high temperature stress conditions during reproductive stage and grain filling stage. At the onset of anthesis, foliar application of salicylic acid (SA) (0.1mM) was made with water sprayed on the control plant. The observations related to physiological traits (Photosynthesis, Fv/Fm, pigment contents, SPAD value, lipid peroxidation, memebrane stability index etc), antioxidant enzyme activities; non–antioxidant metabolites and endogenous level of ABA, total SA and free SA were estimated in flag leaves at 2 and 10 days after foliar spray of SA. The sqRT-PCR based expression analyses of several genes (HSfs, HSPs, ICS, NCED, FEH, psbA, and psbO) were also done. The yield and its attributes were also estimated in both SA treated and control samples of both the sowing conditions. During stress condition, when photosynthate supply decreased the grain filling in wheat also depend on NSC present or stored in stem. Therefore, we have also measured the non-structural carbohydrates (NSC) in penultimate internodes and mobilised NSC and remobilisation efficiency (RE) were calculated in both SA treated and control sample.

Summary of results obtained in the present investigation are presented below:

- The first experiment was performed only for identification of optimum SA concentrations based on growth response of wheat genotypes. Different concentrations of SA was used for this study. Results indicated that only lower concentrations (0.1mM and 0.25 mM) of SA was able to restore the growth (root, shoot and plant height) and significantly counter the detrimental effects of heat stress on wheat seedlings.
- Among antioxidant enzymes the activity of APOX was higher in late sown wheat genotypes (temperature stress conditions induced by late sowing) with maximum increase recorded in the tolerant genotypes. The foliar application of SA further improved the APOX activity when estimated two days after foliar spray of SA, however maximum increase occurred in HD2985, HD3086, and HD3043 while minimum increase was observed in HD3076.

- Ten days after foliar spray, APOX activity decreased in all genotypes, except HD2985, which still was maintained higher than its respective control.
- Among the antioxidant enzymes, the activity of catalase (CAT) decreased in all genotypes upon foliar application of SA in both the sowing conditions. However, maximum decrease occurred in HD3076 after 2 days of foliar spray.
- The foliar application of SA significantly increased activities of SOD and GR in the late sown (high temperature stress induced by late sowing) as well as in normal sown wheat genotypes, with maximum increase noted in the late sown genotypes. Under high temperature stress conditions maximum increase occurred in HD2985 followed by HD 3086 two days after foliar spray of SA.
- Ten days after foliar spray of SA, the activity of SOD and GR decreased significantly in all genotypes, but it was still slightly higher than its respective control especially in the late sown tolerant genotypes.
- Oxidised glutathione and reduced ascorbate contents were reduced after foliar spray of SA as compared to its respective control under high temperature stress in all genotypes two days after foliar spray and these contents drastically increased 10 days after foliar spray of SA. This indicated that both antioxidant metabolites were utilized by the increased activity of GR and APOX.
- The foliar application of SA significantly reduced the TBARS contents (an indicator of lipid peroxidation) in all genotypes under high temperature stress (late sown condition), with maximum decrease in TBARS contents evinced in tolerant genotypes and HD3043 while minimum decrease was observed in HD3076 two days after foliar spray. This may be due to excess ROS scavenged by increased antioxidant activity.
- Foliar spray of SA maintained significantly higher MSI than its respective control in tolerant genotypes and HD3043 and minimum MSI was recorded in HD3076 two days after foliar spray. Ten days after foliar spray the influence of SA decreased in all genotypes.
- Foliar spray of SA, maintained significantly higher total chlorophyll as well chlorophyll a contents even 10 days after foliar spray in late sown tolerant genotypes compared to its respective control.
- Foliar spray of SA did not significantly affect the carotenoid contents.

- Photosynthesis and photochemical efficiency (Fv/Fm) were also maintained higher in all genotypes under high temperature stress (late sown condition) 2 days after spray. However, maximum increases were observed in tolerant genotypes.
- Under high temperature stress (late sown condition) the remobilisation efficiency (RE) of NSC/WSC was higher compared to the normal sown crop, which was higher in tolerant genotypes than the susceptible ones. Foliar application of SA did not significantly affect the RE in any genotypes. This was also evident from gene expression study of *Ta6-FEH* (fructan exohydrolase, responsible for hydrolysis of stem reserved carbohydrates). The expression of *Ta6-FEH* induced under heat stress condition, was not affected by application of SA.
- Mobilised NSC was higher in normal sown wheat genotypes than in the late sown (high temperature stress condition) genotypes, however foliar application of SA did not significantly affect the MNSC in any of the genotypes.
- Exogenous foliar application of SA, did not significantly affect the endogenous level of ABA, however the endogenous level of ABA was higher in late sown (High temperature stress) genotypes compared to the genotypes grown under the normal sown conditions. This was also evident from gene expression study; the expression of *TaNCED* was not affected by exogenous application of SA, though the expression was induced under high temperature stress condition. Among the genotypes the ABA level was comparatively higher in the susceptible genotype (HD3076).
- Foliar spray of salicylic acid significantly induced the endogenous level of total as well as free SA in all genotypes 2 days after foliar spray, with maximum increase observed in tolerant compared to the susceptible genotype. The basal endogenous level of free SA was very high in the genotypes grown under late sown (high temperature stress) condition compared to those under normal sown conditions. The endogenous level of free SA decreased 10 days after foliar spray in all genotypes. The gene expression analysis of *TaICS* clearly shows that the expression was induced under high temperature stress condition compared to normal sown, however exogenous application of SA did not affect the gene expression significantly.

- The expression of transcription factors (*TahsfA2b* and *TahsfA1a*) was induced in the genotypes grown under high temperature (late sown) and treated with SA, in all genotypes, 2 days after foliar spray, with maximum induction observed in HD2985, HD3086 and HD3043. Foliar spray of SA also induced the expression of *TaHSP101* in late sown genotypes particularly in tolerant genotypes. This induction may be attributed to higher expression of different Hsfs. The exogenous application of salicylic acid caused slight increase in the expression of *TaHSP16.9* in HD2985 under high temperature (in late sown) conditions.
- Grain yield per plant, 1000 grain weight were improved but it was non-significant, this shows that the positive affect of SA is not consistent or was transient in nature.
- From the overall results it was apparent that the exogenous application of SA (0.1mM) positively modulated the physiological, biochemical trait as well as the gene expression pattern in wheat genotypes when measured 2 days after foliar application, however the positive effect was not consistent as it decreased when analysed 10 days after foliar spray.
- The results of foliar application of SA on 30 days old wheat plant (HD3086) (vegetative stage) exposed to heat stress clearly shows that the physiological traits like photosynthesis rate, photochemical efficiency and total chlorophyll and biochemical traits like SOD, APOX activity, and MSI were higher in SA treated heat stressed plant compared to those measured in plants without SA treatment and grown under heat stress. The TBARS and hydrogen peroxide contents decreased in SA treated heat stressed plant compared to without SA treated heat stressed plant. A reduction in catalase activity was observed upon SA treatment.
- Gene expression study of *TaHSP70* and few structural genes *TapsbA* (encoding D1 protein) and *TapsbO* (encoding a protein associated with oxygen evolving complex) were done in leaves of 30 days old wheat plant (HD3086). It is evident from the results that the expression of these genes was higher in heat stress plus SA treated genotypes as compared to heat stressed without SA treated genotypes. This explains that the SA protects the PSII by increased

turnover/ de novo synthesis of these heat susceptible proteins associated with PSII and helps in the maintenance of higher photosynthesis rate.

From the present study, it can be concluded that the exogenous application of SA (0.1mM) positively modulated the physiological, biochemical trait as well the gene expression of HSFs, HSPs analysed 2 days after foliar application, however, positive effect on physiological and biochemical trait was not consistent as it decreased when analysed 10 days after foliar spray. This clearly shows the response of SA is not long lasting specifically in our experiment or is transient in nature and it is also evident from yield data, which is not significantly enhanced.

ABSTRACT

Wheat (*Triticumaestivum*L.) is very sensitive to high temperatureand experiences heat stress to varying degrees at different phenological stages, but heat stress during the reproductivephase is more detrimental than during the vegetative phase.In wheat, grain filling also relies on mobilisation of reserve carbohydrates particularly under stress condition from the stem to the developing grains. SA is a natural phenolic compound and acts as an endogenous signal molecule that plays a crucial task in the regulation of plant growth, development, interactions with other organisms, and responses to environmental stress. Therefore, in order to test the possibilities of foliar application of salicylic acid in improving the phenology, photosynthesis, remobilisation of carbohydrates and yield under normal and late sown high temperature condition and to analyse the SA mediated change in physiological, biochemical, molecular alternations in relation to high temperature stress. In this regard extensive studies were doneand three independent experiments were formulated.The first experiment was performed for identification of SA concentration for wheat plants under study. The wheat seedling (10 days old) grown in the incubator was treated with different concentrations (6) of SA and it was observed that onlylower concentration (0.1mM and 0.25 mM) of SA was able to reinstate growth and significantly counter the detrimental effects of heat stress (39±2^{o}c) on wheat seedlings. In second experiment biochemical, physiological as well as a few gene expression patterns were analysed in leaves of 30 days old (vegetative stage) wheat genotype (HD3086) and it was observed that the exogenous application of SA (0.1mM) efficiently guarded thephysiological traits, by causing an increase in antioxidant enzyme activities and inductionof gene expression of Hsps and photosynthesis genes (*TaHSP70*, Ta*psbA* and Ta*psbO*) while decreasing the H_2O_2 and TBARS contents in wheat plants subjected to heat stress (39±2^{o}c) during vegetative stage. Finally, the SA concentration 0.1mM were used in the third experiment to authenticate the response of SA under prolonged high temperature stress at reproductive stage wheat genotypes and observation were recorded at two and ten days after foliar spray of SA. A pot culture experiment was conducted with four wheat genotypes, two relatively tolerant (HD2985 and HD3086) and two susceptible (HD3076 and HD3043) and staggered sowing were done to induce high

temperature stress at reproductive stage. Observations were recorded as TBARS content, MSI, photosynthetic pigments, photosynthetic rate, photochemical efficiency, antioxidant enzymes activity (catalase, peroxidase, superoxide dismutase, glutathione reductase and ascorbate peroxidase), non-enzymatic metabolite (oxidised glutathione and reduced ascorbate),endogenous level of ABA, total SA, free SA, non-structural carbohydrates, remobilisation efficiency (RE), grain yield, test weight and harvest index were recorded two and ten days after foliar spray of SA. Values of all above parameters were reduced under late sown high temperature stress condition while some other parameters like lipid peroxidation (TBARS content), endogenous level of SA and ABA, RE of NSC and activities of antioxidant enzymes increased. Interestingly, the application of SA in general maintained the higher values of all these parameters in tolerant genotypes compared to susceptible genotypes and reduced the lipid peroxidation and catalase activity. In present study gene expression analysis of transcription factors (*TahsfA2b* and *TahsfA1a*), small and large HSPs (Tahsp101, Tahsp16.9 and Tahsp17.8) and expression of biosynthesis pathways of SA and ABA (*TaICS*, *TaNCED*) fructan hydrolysing enzyme (*Ta6-FEH*) were done in leaves two days after foliar spray of SA. Results of gene expression study clearly revealed exogenous application of SA plus high temperature enhanced the expression of both transcription factors and large HSP and it was higher in HD2985,HD3086 and HD3043 compared to HD3076. The expression of *TaICS, TaNCED, Ta6-FEH* and small HSps were not affected significantly by application of SA. However high temperature stress induced expression of all the genes compared to normal sown plants and expression was higher in tolerant genotypes. From present study, it can be concluded that the exogenous application of SA (0.1mM) positively modulated the physiological and biochemical traits and transcription level of Hsfs and large molecular weight HSP analysed two days after foliar application. However the positive effect on most of physiological and biochemical traits were not consistent as it decreased when analysed ten days after foliar spray. This clearly shows the response of SA is not long lasting specifically in our experiment or is transient in nature and it is also evident from yield data, which is not significantly enhanced.

गेहूँ (*ट्रिटीकम एस्टाइवम* एल.) की अन्त ताप प्रतिबल सहनषीलता में सैलीसिलिक अम्ल की भूमिका

सार

गेहूँ (*ट्रिटीकम एस्टाइवम* एल.) उच्च तापमान को प्रति अत्यन्त संवेदनषील फसल है और विभिन्न घटना विज्ञानी अवस्थाओं पर तापमान प्रतिबल के विभिन्न स्तरों का अनुभव करती है किन्तु कायिक अवस्था की तुलना में जनन अवस्था के दौरान तापमान प्रतिबल इसके लिए अधिक अहितकर है। गेहूँ में दाना भरने की प्रक्रिया भी आरक्षित कार्बोहाइड्रेट्स के तने से विकासशील दानों तक प्रवाहीकरण, विशेष रूप से प्रतिबल परिस्थिति में, पर निर्भर करती है। सैलीसिलिक अम्ल (एस.ए.) एक प्राकृतिक फीनोलिक पदार्थ है जो एक अन्तर्जात सिगनल अणु के रूप में कार्य करता है और पादप वृद्धि नियमन, विकास, अन्य जीवों के साथ पारस्परिक क्रियाओं एवं पर्यावरण प्रतिबल के प्रति अनुक्रियाओं में निर्णायक भूमिका निभाता है। इसलिए, सामान्य एवं पछेती बुवाई किए गए गेहूँ में उच्च तापमान परिस्थिति में, घटना विज्ञान, प्रकाष संशलेषण, कार्बोहाइड्रेट्स के पुर्नप्रवाहीकरण में सुधार करने हेतु सैलीसिलिक अम्ल के पर्णीय छिड़काव की संभावना ज्ञात करने तथा पादप कार्यिकी, जैव रासायनिक तथा उच्च तापमान प्रतिबल के संदर्भ में आण्विक परिवर्तन में एस.ए. मीडिएटेड परिवर्तन ज्ञात करने के उद्देश्य से यह अध्ययन किया गया। इस संदर्भ में विस्तृत अध्ययन किया गया और स्वतंत्र रूप से तीन प्रयोगों का फार्मूलेश्न किया गया। प्रथम प्रयोग, अध्ययन के अन्तर्गत गेहूँ के पौधों हेतु एस.ए. सान्द्रता की पहचान हेतु किया गया। उष्मायित्र (इनक्यूबेटर) में उगाए गए गेहूँ के छोटे पौधों (10 दिन आयु के) को एस.ए. की विभिन्न सान्द्रताओं (6) के साथ उपचारित किया गया और यह देखा गया कि केवल कम सान्द्रता के एस.ए. (0.1 मिली मोल एवं 0.25 मिली मोल) में ही वृद्धि बहाल हो सकी और उसने गेहूँ की पौध पर ताप प्रतिबल (39±2 डिग्री सेल्सियस) के निर्णायक प्रभावों का महत्वपूर्ण रूप से सामना किया। द्वितीय प्रयोग में, 30 दिन आयु के (कायिक अवस्था) गेहूँ जीनप्ररूप (एच.डी. 3086) की पत्तियों में जैव रासायनिक, पादप कार्यिकीय तथा साथ ही कुछ जीन अभिव्यक्ति ढ़ंगों का विष्लेषण किया गया तथा यह देखा गया कि कायिक अवस्था के दौरान, ताप प्रतिबल (39±2 डिग्री सेल्सियस) दिए गए गेहूं के पौधों में प्रति ऑक्सीकारक एन्जाइम सक्रियताओं एवं Hsps प्रकाश संशलेषण जीन्स (Ta HSP70, Tapsb A एवं Tapsb O) की जीन अभिव्यक्ति के प्रेरण में बढ़ोतरी कर तथा H_2O_2 एवं TBARS अंश में कमी कर, एस.ए. (0.1 मिली मोल) एस.ए. के बहिर्जात अनुप्रयोग ने सक्षम रूप से पादप कार्यिकीय गुणों की रक्षा की। अंततः एस.ए. की 0.1 मिली मोल सान्द्रता का, जीनप्ररूपों की जनन अवस्था पर दीर्घकालीन उच्च तापमान प्रतिबल के अन्तर्गत एस.ए. की अनुक्रिया की यर्थाथता ज्ञात करने के लिए तृतीय प्रयोग किया गया तथा एस.ए. के पर्णीय छिड़काव के बाद दो एवं दस दिन पर निरीक्षण रेकार्ड किए गए। गेहूँ चार जीन प्ररूपों, दो अपेक्षाकृत सहनषील (एच.डी.–2985 एवं एच.डी.–3086) एवं दो सुग्राही (एच.डी.–3076 एवं एच.डी.–3043) के साथ एक पॉट कल्चर प्रयोग किया गया तथा जनन अवस्था पर उच्च तापमान प्रतिबल के प्रेरण हेतु भिन्नकालिक बुवाई की गई। एस.ए. के पर्णीय छिड़काव के दो एवं दस दिन पश्चात, TBARS अंश, एम.एस.आई., प्रकाश संशलेषण संबंधी वर्णक, प्रकाश संशलेषण –दर, प्रकाश–रासायनिक क्षमता, प्रतिऑक्सीकारक एन्जाइम सक्रियता (कैटेलेज, परॉक्सीडेज, सुपर ऑक्साइड डिसम्यूटेज, ग्लूटाथायोन रिडक्टेज एवं एस्कॉर्बेट परॉक्सीडेज), नॉन–एंजायमेटिक उपापचयज (ऑक्सीकृत ग्लूटाथायोन एवं अपचयित एस्कॉर्बेट), ए.बी.ए. का अंतर्जात स्तर, कुल एस.ए., मुक्त एस.ए., असंरचनात्मक कार्बोहाइड्रेट्स, पुर्नप्रवाहीकरण क्षमता (आर.ई.), दाना उपज, परीक्षण भार एवं कटाई सूचकांक के रूप में निरीक्षण रेकार्ड किए गए। पछेती बुवाई उच्च तापमान प्रतिबल परिस्थिति के अन्तर्गत उपयुक्त सभी

प्राचलों के मानों में कमी आई जबकि कुछ अन्य प्राचलों यथा, लिपिड परॉक्सीडेषन (TBARS अंश), एस.ए. एवं ए.बी.ए. के अन्तर्जात स्तर, एन.एस.सी. का आर.आई. एवं प्रतिऑक्सीकारक एन्जाइम्स की सक्रियता में बढ़ोतरी हुई। आश्चर्यजनक रूप से, सुग्राही जीनप्ररूपों की तुलना में सहनशील जीनप्रारूपों में एस.ए. के अनुप्रयोग से इन सभी प्राचलों के उच्चतर मान बने रहे और उसने लिपिड परॉक्सीडेषन एवं कैटेलेज सक्रियता में कमी की।

प्रस्तुत अध्ययन में, एस.ए. के पर्णीय छिड़काव के दो दिन पश्चात पत्तियों में, ट्रांसक्रिप्षन कारकों (TahsfA2b एवं TahsfA1a), छोटे एवं बृहद HsPs (Tahsp 101, Tahsp 16.9 एवं Tahsp 17.8) तथा एस.ए. एवं ए.बी.ए. (TAICS, TaNCED) फ्रक्टन हाइड्रोलायसिंग एन्जाइम (Ta6-FEH) के जैव संशलेषण पाथवेज की अभिव्यक्ति का जीन अभिव्यक्ति विश्लेषण किया गया। जीन अभिव्यक्ति अध्ययन के परिणामों ने स्पष्ट रूप से दर्शाया कि उच्च तापमान सहित एस.ए. का अनुप्रयोग ट्रांसक्रिप्षन कारकों एवं बड़े HSP दानों में बढ़ोतरी करता है तथा यह एच.डी.–3076 की तुलना में एच.डी.–2985, एच.डी.–3086 एवं डी–3043 में अधिक था। एस.ए.के. अनुप्रयोग से TaICS, TaNCED, Ta6-FEH एवं छोटे HSPs की अभिव्यक्ति पर कोई महत्वपूर्ण प्रभाव नहीं हुआ। वैसे सामान्य बुवाई किए गए पौधों की तुलना में सहनशील जीनप्ररूपों में सभी जीन्स की उच्च तपमान प्रतिबल प्रेरित अभिव्यक्ति अधिक थी। इस अध्ययन से यह निष्कर्ष निकाला जा सकता है कि एस.ए. (0.1 मिली मोल) के बहिर्जात अनुप्रयोग ने, प्रणीय छिड़काव के दो दिन पश्चात विशलेषित किए गए कार्यिकीय एवं जैव रासायनिक गुणों एवं HSPs एवं अधिक अणुभार वाले HSP के ट्रांसक्रिप्षन स्तर को घनात्मक रूप से परिवर्तित किया। वैसे, अधिकांश कार्यिकीय एवं जैव रासायनिक गुणों पर यह घनात्मक प्रभाव स्थिर नहीं था क्योंकि पर्णीय छिड़काव के दस दिन पश्चात विष्लेषण करने पर इसमें कमी पायी गयी। यह स्पष्ट रूप से दर्शाता है कि एस.ए. की अनुक्रिया, विशेष रूप से हमारे द्वारा किए गए इस प्रयोग में, दीर्घकालीन स्थाई नहीं है या परिवर्तनषील प्रकृति की है जैसा कि उपज–आंकड़ों से भी पता चलता है जिनमें महत्वपूर्ण रूप से बढ़ोतरी नहीं हुई।

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