BIOCHEMICAL ASPECTS OF DROUGHT TOLERANCE
INDUCED BY SEED PRIMING IN WHEAT

BY

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M. Phil. Biochemistry (UAF)
2004-ag-25

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY
IN
BIOCHEMISTRY

Department of Chemistry and Biochemistry
Faculty of Sciences
University of Agriculture, Faisalabad, Pakistan
2012
DECLARATION

I hereby declare that the contents of the thesis, entitled “Biochemical aspects of drought tolerance induced by seed priming in wheat” are product of my own research and no part has been copied from any published source (except the references, standard mathematical and genetic models / equations / formula / protocols etc.). I further declare that this work has not been submitted for award of any other diploma/degree. The University may take action if the information provided is found inaccurate at any stage.

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Acknowledgements

All praises belong to ALLAH SUBHANATALA who enabled me to successfully my doctoral degree, and at least revealed some of the facts hidden in universe created by KUN FAYAKON I also pay highest respect to Last Prophet Hazrat Muhammad (Sallah laho alahay allay hi wasalam) who conveyed the ALLAH,s message of learning and thinking to mankind.

I am grateful for the financial assistance by Higher Education Commission (HEC) Islamabad, Pakistan, under the Indigenous 5000 PhD Fellowship Scheme. Without HEC grant, I may not able to fulfill this manuscript.

I feel matter of great honor and pleasure for me to express deep sense of devotion and sincerest feeling of gratitude to my respected supervisor Prof. Dr. Munir A. Sheikh, Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad, for his kind supervision, keen interest, valuable suggestions and sympathetic attitude throughout my research. I want to give special appreciation to him for his greatness, kindness and extremely friendly behavior which are really rare.

My thanks are due for Prof. Dr. Amer Jamil, Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad and Prof. Dr. Shahzad M. A. Basra, Chairman, Department of Crop Physiology, University of Agriculture, Faisalabad, the members of my supervisory committee for making valuable improvements, skilled suggestions and precious advices in the completion of this manuscript.

I express my profound gratitude to my affectionate late father Mr. Abdul Hameed for his sacred prayers, continuous encouragement and unforgettable sacrifices with great patience throughout his life but left me alone when I was in the start of my journey of educational career. Now I am going to be PhD degree holder but he is not alive to see these days. I have nothing to say more than that may ALLAH rest his soul in heaven always Amin!

I am indebted to all my family members especially my dearest mother Shameem Akhter who always have been a source of encouragement, shelter , support and just like the backbone of me every success and achievement in life. Her prayers, encouragement,
immense love and mellifluous affection towards me are an asset of my life. May Allah give her a longer, happier and healthy life Amin!

I have no words to express my humble obligation to my brother for his placidity and efforts towards the enhancement of the script of this research work impeccable.

I want to express my humble appreciation to my sweet sisters whose hands always rose for my success. They not only pray for me but also encourage me in all fields of life. All my achievements of life, especially academic may not be possible without their untiring efforts.

I cannot forget the corporation and support extended by my friends. I am exceedingly lucky to have these wonderful friends who shared all the good and bad times of my Ph.D. research tenure and have always boosted my morale especially in times of difficulty. I am thankful to my friends for their moral support and prayers.

“THANKS”
Is Not Enough
“Thanks” is just a portion
Of deep appreciation
For sharing so much goodness
From your heart,
Your kindness is such a source
Of joy and inspiration
That “Thanks” is not enough
It’s just a start.
ARRUJE HAMEED
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ABSTRACT

Water stress is the major abiotic factor that limits crop productivity. Among various strategies, seed priming is low cost, easy low risk approach to improve the abiotic stress tolerance in crop plants. The effects of seed priming with varying concentrations of chitosan (0.1, 0.25 and 0.50 %), sodium nitroprusside (SNP) (75, 100 and 125μM) and sodium silicate (20, 40 and 60mM) on physiological and biochemical attributes in seeds, seedlings and wheat plants under non-stress, osmotic stress by poly ethylene glycol (PEG) and drought were appraised in this study. Seed priming with chitosan, SNP and sodium silicate promoted the activities of proteases, soluble proteins, hydrolases, α-amylase, α-naphthyl acetate esterase activities in the seeds. Moreover, tested seed priming treatments induced enhancement in antioxidant enzymes. Hydropriming induced modulations in seed biochemical processes were generally different and less prominent as observed by other tested priming treatments. In general, tested seed priming treatments brings out the production or activation of enzymes in the seeds that are required for the degradation and mobilization of seeds reserves and defense response. In seed germination experiments, osmotic stress induced by 15 % PEG-6000 adversely affected the seed germination attributes while chitosan SNP and sodium silicate seed priming improved the germination (FGM, GE, VI, GR, MGT and GI) and promoted the early seedling growth under non-stress and osmotic stress conditions.

In seedling experiments, chitosan, SNP and sodium silicate priming generally resulted in promotion of early seedling establishment and synchronized growth along with better biochemical and physiological attributes. Osmotic stress increased the peroxidase, protease, α-amylase activities, total soluble proteins, malondialdehyde (MDA) contents, reducing and total sugars while dropped the relative water content in the leaves. Chitosan seed priming improved the osmotic stress tolerance of seedlings evident from adjusted antioxidants activities (POD, CAT, SOD) soluble sugars, improved CMS and leaf RWC and reduced lipid peroxidation. Similarly, SNP priming significantly improved the CMS, RWC, TPC, proteins and reducing sugars while reduced the hydrolases (protease, α-amylase) activities and lipid peroxidation in seedlings providing evidence for lesser osmotic stress induced injury and improvement in stress tolerance. Moreover, sodium silicate (60mM) priming enhanced the POD, TPC, CMS, RWC and reducing sugars while decreased the CAT, protease, α-amylase and SOD activities and MDA content in leaves under osmotic stress indicating improvement in stress tolerance.

In pot experiments, drought stress adversely affected the biochemical, physiological processes, and yield in wheat plants grown from non-primed seeds. In wheat plants grown from seeds primed with chitosan, SNP or sodium silicate antioxidants (CAT, POD, SOD and TPC), osmoprotectants (GB, proline and sugars), CMS, leaf water relations (WP, OP, TP and RWC) and pigments generally improved while hydrolyzing enzymes and lipid peroxidation decreased under drought stress. Chitosan SNP and sodium silicate priming improved the grain yield, 100 grain weight and plant biomass grown under drought stress. In conclusion, tested seed priming treatments successfully improved the seed germination and performance and alleviated the adverse effects of osmotic and drought stress in wheat seedling and plants respectively.
CHAPTER 1

1 INTRODUCTION

Multiple stresses in natural habitats or fields can affect the plants (Ishag and Mohamed, 1996). Abiotic stresses are constantly creating problems during our efforts for crop improvement (Tardieu and Tuberosa, 2010). Stresses such as drought, salinity, heat and low temperature are responsible for creating considerable loss in world food supply (Farooq et al., 2009a). Among abiotic stresses, drought is a major abiotic factor that extensively limits the production of crop plants (Ramachandra et al., 2004; Araus et al., 2008).

Plants show simultaneously multigenic responses to tolerate abiotic stresses, which are very difficult to manage. Genes responsible for stress tolerance are either up regulated or synthesized in plants. Drought stress simultaneously caused osmotic stress, oxidative stress, cellular water loss and severe damage to plant organs (Poltronieri et al., 2011). Low water availability to wheat plants at any stage adversely effects the crop growth and development and finally results in reduced crop yield (Dicken and Wright 2008).

Drought stress either temporarily or permanently but adversely affects a number of morphological, physiological and biochemical processes in crop plants. Deleterious effects of drought stress on crops may include altered plant metabolism in higher plants and maize (Lawlor and Cornic, 2002; Chimenti et al., 2006;), impaired enzyme activities in rice and maize (Hong and Ji-yun 2007; Xu et al., 2008), reduction in plant biomass of brassica species and pea plants (Ashraf and Mehmood, 1990; Arshad et al., 2008), reduced solute accumulation in wheat (Khan et al., 1999) or a combination of all these factors.

Under stressful environment, alterations in morphological, physiological and biochemical characters occur in plants that leads to improvement in stress tolerance (Sakamoto and Murata, 2002; Suralta and Yamauchi 2008; Xu et al., 2008). Plants transfer signals from roots to other parts for activation of defensive genes (Chaves and Oliveira, 2004; Ramachandra et al., 2004; Siopongco et al., 2008), including the synthesis and
accumulation of substances/ions such as Ca$^{2+}$, salicylic acid, abscisic acid, jasmonic acid, ethylene that play specific roles in signaling cascades (Liu et al., 2010). Jasmonic acid or abscisic acid signaling pathways activated in plants to upstream the stress responsive genes that lead to production of defense proteins. These proteins activate the defense system of plants for abiotic as well as biotic stresses (Hughes et al., 2009). Proteins like dehydrins and late embryogenesis abundant proteins (LEA proteins) activated in drought stressed plants for the assimilation of drought tolerance. Only tolerant varieties can accumulate these types of defense proteins in their cells (Hu et al., 2010).

Among various cultivated crops of Pakistan, wheat is the major cultivated cereal crop of Rabi season. It almost covers 70 % area of cultivated land. Late maturity of basmati varieties of rice and delayed picking of cotton results in delayed sowing of wheat up to late December (Nayyar & Iqbal, 2001). Reduction in yield of wheat is primarily due to poor and erratic germination because of low temperature and less water availability to the crop at the time of sowing (Khan et al., 2010a).

The average yield of wheat is quite low mainly due to shortage of water. Even in cereal crops, low degree water stress at crucial stages may result in substantial yield reduction (Jalal, 2011). The extent of stress tolerance in plants depends upon the cultivar, growth, duration and intensity of stress (Araus et al., 2002; Mark and Antony, 2005).

Crop breeding is one of the ways used for increasing yield under stresses by producing more stress tolerant crop plants. However, success in breeding has many limitations. These include (i) stress tolerance is a multigenic character and simultaneous selection of all these genes is very hard task (Richards, 1996; Yeo, 1998; Flowers et al., 2000); (ii) elimination of undesirable gene during breeding is very uncertain (Richards, 1996); finally (iii) reliable procedures for their selection in field conditions are missing (Ribaut et al., 1997).

Among various strategies, seed priming is very valuable, low cost, easy approach and has limited risk to overcome the abiotic stresses in crop plants (Wahid and Shabbir, 2005; Iqbal and Ashraf, 2006). Priming technique used widely to enhance germination and seedling growth in almost all crop plants (McDonald, 2000). Pre-sowing seed treatments have been shown to enhance emergence percentage and stand establishment under non-
stressed conditions (Khan, 1992; Afzal et al., 2005) and have potential in stress full environments (Basra et al., 2006). Improved germination is necessary for establishing a proper crop stand and better yield, but numerous constraints have immense impediments (Wahid et al., 2008).

Priming is of many types i.e. hydroprining, halopriming, antioxidant priming, osmopriming and hormonal priming etc. in which seeds are treated with H$_2$O, chemicals, antioxidants, osmotica and hormones respectively, that results in pre-germination changes. Especially under stress, these changes show intense effects on germination index and seedling emergence (Ashraf and Foolad, 2005). Moreover, many plant hormones as priming agent or any other type of seed pre sowing treatments have reported to enhance the crop stand establishment (Miyoshi and Sato, 1997; Basra et al., 2006).

At genetics level, many of the genes activate after seed priming i.e. late embryogenesis abundant proteins (LEA proteins) and globulins (Capron et al., 2000; Gamboa-deBuen et al., 2006). Priming also improves the antioxidant activities in primed seeds (McDonald, 1999; Wang et al., 2003; Hsu et al., 2003). Antioxidant enzymes including superoxide dismutase, catalase, and peroxidase have reported to express during seed priming (Bailly et al., 2000; Chiu et al., 2006).

Antioxidants activate in seeds by H$_2$O$_2$ under oxidative stress to overcome the effects of oxidants (Wahid et al., 2007). Many of the metabolic changes can occur in primed seeds i.e. reduced malondialdehyde contents (Bailly et al., 1998, 2000), altered ratio of unsaturated to saturated fatty acids (Walters et al., 2005), increased soluble sugars, enhanced alpha amylase activity and other related features (Mwale et al., 2003; Farooq et al., 2006).

Naturally, to face drought stress, crop plants accumulate osmolytes (glycine betain, proline), antioxidants (Catalase, Peroxidase, Superoxide dismutase, etc) and hormones (salicylic acid, gibberellins, cytokinin, etc). The accumulation of all these substances increase after seed priming that could help the crop plant to withstand drought stress (Hong-Bo Shao et al., 2007; Farooq et al., 2009a).
Seed priming efficiently reduces the adverse effects related to drought stress and results in better and synchronized crop stand. Reduced germination index and final germination percentage highly improved after priming with different seed treatments (Abbasdokhta, 2011).

Chitosan belongs to carbohydrate family with structural similarity to cellulose as well as chitin. Sea fish, shrimps and cramps provide chitosan mainly or chitin after deacetylation transformed to chitosan. Chitosan acts as germination promoter and its application to plants, improves their growth and yield (Sheikh and Malki, 2011).

Sodium silicate is an anhydrous white powder of sodium metasilicate, commonly called as silicon. Silicon synchronizes the crop growth and yield. Its application can improve plant height, leaf area, dry mass and yield of crops under drought stress (Gong et al., 2003; Singh et al., 2006).

Sodium nitroprusside (SNP), a nitric oxide (NO) donor enhances the germination and biochemical status of crop plants under abiotic stresses. SNP application at various stages of plant growth and development has proved to be very effective in improvement of relative water contents, photosynthesis, oxidant and antioxidants status, gas exchange characteristics and many other processes related to drought stress (Tian and Lei, 2006).

Therefore, we conducted the present study to explore the role of SNP, sodium silicate and chitosan seed priming in improving germination, growth and biochemical parameters in wheat at seed, seedling and mature plant stages.

**Aims and Objectives:**

1. To investigate the biochemical changes in the seeds as a result of priming treatments with special emphasis on modulations in antioxidant defense potential.
2. To investigate the possible use of sodium silicate, SNP and chitosan as seed priming tools to ameliorate drought tolerance in wheat.
2 REVIEW OF LITERATURE

Pakistan lies in area of arid and semi arid lands of climatic conditions. Agriculture of this area depends on rains. Any reduction in rainfall or low availability of water to the plants leads to drought stress. Drought stress shows many deleterious effects to the plants that must be counter balance by the plants by use of different approaches (Mollasadeghi et al., 2011).

Wheat (*Triticum aestivum L.*) is the second important crop on the globe and the major staple crop of Pakistan that can limit the world’s food quality. Pakistan is at 5th position for the total yield of wheat in world (Anonymous, 2007). During its life cycle, wheat has to face many stresses (Shao Hong-Bo et al., 2006). Among them, drought is one that curtails its emergence and yield. Wheat is most probably cultivated at drought prone areas (Hong-Bo Shao et al., 2007; Farooq et al., 2009a). Drought stress level up to which crop can tolerate may change with respect to wheat genotypes. Different genotypes can activate their defense entirely in a different way at similar level of drought (Shao Hong-Bo et al., 2006).

2.1 Drought stress impact on crops:

Drought, the condition of low water availability to plants is responsible for many adverse effects that range from physiological to molecular levels. Some of drought stress effects and the extent to which they can affect the plants are discussed below.

2.1.1 Plant water status:

Water is an essential element of plant, which is required at all stages of plant growth and development. Any decline in water status of plants resulted in drought stress. Water relations i.e. water potential, osmotic potential and turgor potential are important parameters to evaluate drought tolerance in plants. Relative water content (RWC) has characterized as an indicator of drought stress in wheat leaves (Merah, 2001). Water deficit condition highly influenced water relations and resulted in sever drop of plant
water status. Grover et al., 2004 reported decline in RWC and decrease in water relations immediately after abiotic stress development in plants.

Among water relations, the leaf water potential has reported as a reliable parameter with regard of plant response to drought stress. Water potential significantly curtailed during drought stress in brassica seeds (Singh et al., 1990). While relative water content (RWC) negatively affected by stresses especially by drought stress is considered as better indicator of water status as compared to water potential achieved by plants under drought stress mitigation (Sinclair and Ludlow, 1985; Payam, 2011).

Yürekli et al., 2001 has reported the drought stress induced decline in leaf relative water content (RWC). Similarly, rose plants (Hibiscus rosa-sinensis) with lowered RWC and water relations under drought stress have been reported (Egilla et al., 2005). Reduced stomatal conductance in wheat and rice plants has reported to be responsible for maintaining plant water status under drought stress (Siddique et al., 2001; Abbate et al., 2004).

2.1.2 Photosynthesis:
Drought adversely affects the photosynthetic activity of plant cell that leads to reduce the grain yield. Impaired photosynthesis in crop plants is one of the reasons for food scarcity (Wahid and Rasul, 2005). During drought stress, the stomatal conductance of plant cell reduces that leads to limited CO₂ availability to plant. Due to low concentration of CO₂, rubisco, the key enzyme of photosynthesis acts as oxygenase rather than carboxylase and results in over production of reactive oxygen species (ROS). Reactive oxygen species with lots of adverse effects; act as signaling molecules to activate antioxidant defense systems (Griffiths and Parry, 2002).

Photosynthetic pigments i.e. chlorophyll and caratinoides has reported to reduce under drought stress. Their amount in plants correlates with the grain yield under stress (Jaleel et al., 2009). Impaired photosynthesis also results in over production of ROS due to which antioxidant defense system of plants is retarded (Reddy et al., 2004).

2.1.3 Stomatal conductance:
Drought stress adversely affects the photosynthesis a basic process of plant growth and development (Chaves and Oliveira, 2004). Stomata closure is the major effect of drought
stress that leads to decreased transpiration rate and reduced CO2 influx, which ultimately inhibit the Calvin-cycle even at moderate drought stress level (Horton et al., 1996). Stomatal and non-stomatal limitations, both phenomena are responsible for the reduced photosynthesis (Graan and Boyer, 1990; Shangguan et al., 1999). Limited carbon uptake in leaves under drought stress is the important feature of plants that occurs due to stomatal closure (Chaves, 1991; Cornic and Massacci, 1996).

Concomitant decline in RWC and water potential with reduced photosynthetic rate has reported in higher plants (Lawlor and Cornic, 2002). Generally, reduced stomatal conductance is considered as a main detrimental effect of declined photosynthesis under drought stress (Cornic, 2000). Stomatal closure has reported as a character in response to either a decreased leaf turgor and/or water potential in plants, or to a lowered humid environment (Maroco et al., 1997).

During drought stress, stoma of plants closes to achieve drought tolerance. Plant hormones like abscisic acid and cytokinin are responsible for the opening and closing of stomata (Turner et al., 2001; Wilkinson and Davies, 2002). However, due to stomatal closure, stomatal conductance across cell reduces and leads to impaired photosynthetic activity (Yokota et al., 2002).

Decreased RWC has reported to induce stomatal closure with parallel decline in photosynthetic rate (Cornic, 2000). Nevertheless, stomata are more efficiently responding to dehydrated roots as compared to plant water status. Dehydrating roots activate the abscisic acid signaling for stomata closure (Davies and Zhang, 1991). Thus, photosynthesis and stomatal opening has a high degree of correlation (Farquhar et al., 2001; Hubbard et al., 2001).

2.1.4 Oxidative Stress:

Oxidative stress caused by over production of ROS i.e. superoxide radical (O2-), singlet oxygen, hydrogen peroxide (H2O2); etc during drought stress is a common phenomena. ROS produced during drought stress are quenched by the antioxidant defense system of plants (Singh et al., 2012). ROS damage the macromolecules (DNA, RNA, Proteins and lipids) and finally impair the plant cell function (Foyer and Fletcher, 2001). Detrimental
effects of ROS on macromolecules include DNA damage, lipid peroxidation and oxidation of amino acid and proteins (Asada, 1999; Johnson et al., 2003). The damaged macromolecules are either repaired or replaced by de-novo synthesis. However, under intense stresses, severe damage to macromolecules impair their function that cannot be repaired and finally cell death occurs. During water deficit conditions, many scientists (Ramachandra et al., 2000; Chaitanya et al., 2002; Mano, 2002) have reported development of oxidative stress in plants. Drought stress induces oxidative stress by overproduction of reactive oxygen species. Although, ROS are normally produce in chloroplast during photosynthesis (Reddy et al., 2004), in mitochondria during electron transport chain (Moller, 2001), in glyoxylate cycle of peroxisomes (Fazeli et al., 2007), and in the plasma membrane (Sairam et al., 2005) but their concentration within cell increases during stress like drought.

2.1.5 Osmotic stress:

Osmotic stress results in rapid decline in growth of most of the plants (Flowers, 2004; Ashraf, 2004). Osmotic stress results in reduction of leaf chlorosis, antioxidants, plant growth, development, and hormonal imbalance (Mittler, 2002; Munns, 2002; Ashraf et al., 2010; Iqbal and Ashraf, 2010) but reduction in growth depends upon the duration and level of stress and plant tissue types (Cony and Trione, 1998; Meloni et al., 2003). Similarly, many scientists has reported the reduced growth of leaves and stems, leaf area, number of tillers, development of new leaves, lateral buds, branches formation and continued root growth under osmotic stress (Spollen and Sharp, 1991; Munns et al., 2006; Taiz and Zeiger, 2006; Munns and Tester, 2008). Reduction in growth usually occurs due to altered biochemical and physiological responses of plants (Ashraf and Harris, 2004). Reduced plant biomass with parallel decrease in chlorophyll contents, gaseous exchange characteristics and water potential has reported in pea plants (Noreen et al., 2010). Moreover, reduced growth with reduced relative water content, phenolics and malondialdehyde, leaf osmotic potential and antioxidant activities has also reported in turnip (Noreen et al., 2010). Actually, ionic imbalance accomplishes the reduced leaf water potential in plants (Zhu, 2001; Meloni et al., 2008; Munns and Tester, 2008).
Osmotic stress is also one of the consequences of drought stress, usually caused by over accumulation of salts within cell that reduce water status of plant cell. Ultimately, osmotic stress changes the whole biochemistry of plant cells. Poly ethylene glycol (PEG) is most commonly used to create osmotic stress in plants because it is not naturally produced in the plant tissue nether penetrate into cell from the media. PEG eventually destroys the normal emergence, growth, biochemical attributes and yield of plants including wheat (Pei et al., 2010).

2.1.6 Cell membrane injury and lipid peroxidation:
Drought stress very adversely affects the cell membranes. Cell membrane loses their stability and integrity under drought stress. One measure of cell membrane injury is the level of lipid peroxidation product MDA that drastically increases during drought stress. Increased lipid peroxidation in stressed crops can be reduced by seed enhancement treatments from which most economical is seed priming (Farooq et al., 2006; Yang et al., 2009).
Drought stress induced decrease in cell membrane stability that indicates the lipid peroxidation actually occurs due to reactive oxygen species (Dhindsa et al., 1981; Pastori and Trippi, 1992; Baisak et al., 1994; Menconi et al., 1995). Decreased cell membrane stability index of leaves with increasing extent of drought stress has reported (Sairam and Saxena, 2000).

2.1.7 Pigment composition:
Photosynthetic pigments are the key components of photosynthetic machinery. Drought stress most commonly results in reduction of photosynthetic pigments (Farooq et al., 2009a; Jaleel et al., 2009). There are several reports on reduction of chlorophyll contents under drought stress in many crop plants including sunflower (Reddy et al., 2004; Kiani et al., 2008), cotton (Massacci et al., 2008) and wheat (Khan et al., 2009). Carotenoids not only act as accessory pigments but also have some antioxidant property to quench triplet chlorophyll produced in photosynthesis during drought stress (Farooq et al., 2009a).
2.1.8 Soluble sugars:
Sugars that synthesize starch and cellulose are responsible for the enhancement of plant growth and development (Gupta and Kaur, 2005). Glucose, the most important sugar activates a lots of stress responsive genes in abiotic stresses (Price et al., 2004). Usually, soluble sugars increase in plants under abiotic stresses (Gill et al., 2001) including drought stress (Prado et al., 2000; Siddique et al., 2000).

The amount of soluble sugars has reported to enhance in wheat leaves under water deficit conditions (Munns and Weir, 1981; Kameli and Loesel, 1993). Soluble sugars are also considered as an important constituent of osmotic adjustment in response to drought stress (Turner and Jones, 1980; Kameli and Loesel, 1995, 1996).

2.1.9 Soluble proteins:
Soluble protein content of plants usually decreases after drought stress development (Singh and Usha, 2003). Some specific proteins like dehydrins, late embryogenesis abundant proteins and drought responsive proteins are induced in plants during drought stress as defense proteins. Many other proteins like antioxidants (superoxide dismutase, catalase, peroxidase, protease, glutathione reductase and amylase) are also activated in plants to reduce the deleterious effects of drought stress (Tzvi et al., 2000; Ashraf et al., 2003; Katam et al., 2007). These defense proteins have reported to increase in drought tolerant groundnut varieties in response of drought stress (Katam et al., 2007).

2.1.10 Germination and yield:
Drought stress can result into sever reduction of germination and growth in crop plants (Harris et al., 2002; Kaya et al., 2006). Drought stress causes many changes in plant cell development and growth (Kaya et al., 2006; Hussain et al., 2008). Drought stress has reported to result in reduction of germination and growth in pea (Okcu et al., 2005), alfalfa (Zeid and Shedeed, 2006) and rice seedlings (Manikavelu et al., 2006). Reduction in turgor pressure of cell results in impairment of cell growth under drought stress (Taiz and Zeiger, 2006).

Yield reduction in crop plants has reported as the major factor of abiotic stresses (Rehman et al., 2005, Bouman, 2007; Munns and Tester, 2008; Reynolds and Tuberosa, 2008; Blum 2009). About 17 % yield losses in crop plants due to drought stress have
recorded (Ashraf et al., 2008; Rehman et al., 2005). Drought stress adversely affects the yield depending upon stress level, time duration and causes of stress development that result into reduced plant yield (Plaut, 2003). Reduced dry weight in wheat (Wardlaw and Willenbrink, 2000), reduced grain yield and spikes numbers per plant in maize (Cattivelli et al., 2008) and reduced grain yield in soybean, cotton and pearl millet (Frederick et al., 2001; Pettigrew, 2004; Yadav et al., 2004) have observed under drought stress.

2.2 Improvement of drought tolerance:
Plants perform many of the functions to cope drought stress at physiological, morphological and biochemical grounds. Drought tolerance is the phenomena of better plant growth and development under limited water supply. Plants maintain water relations in proper way (Zhou et al., 2007), shed their leaves to reduce transpiration (DaMatta, 2004), develop extensive and prolific root system to extract water from depth of soils under limited water supply (Kavar et al., 2007) to establish drought tolerance.

2.2.1 Improvement of antioxidants:
For removal of ROS produced during oxidative stress, plants have inbuilt antioxidant defense system that consists of antioxidants i.e. ascorbic acid and glutathione, carotenoids and antioxidative enzymes like SOD, CAT, POD and glutathione reductase (Hasegawa et al., 2000; Gong et al., 2005; Wahid, 2007). However, antioxidant enzymes have proved to be more effective for removal of ROS as compared to non-enzymatic antioxidants (Farooq et al., 2008). Moreover, Fazeli et al., 2007 proved that peroxidase more effectively scavenge free radicals than catalase in plants.

SOD is the first line of defense against oxidative stress and detoxifies superoxide radical (Basra et al., 2006; Hong-Bo Shao et al., 2007). Therefore, increased superoxide dismutase activity in plants is the confirmation of stress tolerance (Pan et al., 2006; Hameed et al., 2010). Oxidative damage reduces in plants after intensive action of enzymatic and non-enzymatic antioxidative defense.

2.2.2 Improvement of cell membrane stability:
Cell membranes are most commonly affected by drought stress. Development of cell membrane stability is one of the features to establish drought tolerance (Bajji et al.,
Scientists also proved this trait to be the most suitable for establishment of drought tolerance in different plant varieties at genetic level (Tripathy et al., 2000; Dhanda et al., 2004). Cell membrane stability highly improves by accumulation of osmolytes like proline, glycine betaine, glutamate, sorbitol, mannitol, trehalose, sucrose and inorganic ions under drought stress (Folkert et al., 2001; Gigon et al., 2004). Higher membrane stability due to lower lipid peroxidation has also reported in tolerant wheat and maize genotypes (Pastori and Trippi, 1992; Kraus et al., 1995).

2.2.3 Improvement of osmotic adjustment:

One of the silent feature of drought tolerance in plants is osmotic adjustment during osmotic as well as drought stress. It is achieved by the production and accumulation of different osmolytes i.e. reducing sugars (sucrose, mannose and trehalose), iminoacids (proline), quaternary ammonium compounds (glycine betaine, alanine betaine) and sugar alcohols (sorbitol, mannitol) usually in higher concentrations than normal (Mahajan and Tuteja, 2005). It can keep the plants in stage of normal growth and function under drought stress and is responsible for improved grain yield (Subbarao et al., 2000). Among compatible solutes, proline is one that freely accumulates in higher plants and bacteria (Wahid and Close, 2007), in pea (Alexieva et al., 2001), in maize (Guan et al., 2009) and in petunia (Yamada et al., 2005) under drought stress.

Similarly, quaternary ammonium compound, the glycinebetaine functions as compatible solute is equally important for plants, bacteria and animals (Wahid et al., 2007). Glycine betain concentration increases under drought stress in most of plants especially in crop plants (Quan et al., 2004). In response to glycine betaine concentration, many of the stress responsive genes are activated. Therefore, glycine betaine participates as signaling molecule for activation of stress tolerance genes (Subbarao et al., 2000; Sakamoto and Murata, 2002).

Trehalose, a non-reducing sugar has some function as compatible osmolytes in plants during abiotic stresses. Trehalose is effective against cell membrane damage, stabilizes macromolecules in their native structure under drought stress and effectively maintains water status of plants (Wingler, 2002; Karim et al., 2007; Ramon et al., 2007). In general, osmotic adjustment is the key protective mechanism that plants use to withstand drought
stress. Plants maintain it by improving antioxidants status, osmolytes concentrations and cell membrane stability under drought stress.

2.3 Seed priming:
Among various strategies that scientists have developed to cope with drought, seed priming is most commonly used. Seed priming is simple technique by which remarkable results can be achieved in lesser time (Basra et al., 2006; Iqbal and Ashraf, 2006). Seed priming with different chemicals, ions, organic compounds, hormones and antioxidants has reported to have profound effects on seed biochemistry, germination, growth and yield (Ashraf and Foolad, 2005).

Seed priming is most viable and practical approach to reduce the adverse effects of drought stress mainly on seed germination, crop stand establishment, seedling vigor and growth (Harris et al., 2004, 2007). Seed priming is a valuable technique in which metabolic processes of germination starts earlier and before the radicle emergence. For this purpose, either seeds are soaked in water (hydroprimed) or in a solution of specific concentration for a specified time, however, the concentration and time duration varies for genotypes within species and crops (Harris et al., 1999; Farooq et al., 2006; Ashraf and Foolad, 2007). This approach has effectively employed for improving germination, seedling growth and yield under drought stress in many crops (Chiu and Sung, 2002; Harris et al., 2002; 2004, 2007; Khan et al., 2008).

2.3.1 Effects of seed priming on biochemical processes:
Priming is a pre-germination technique. During imbibition phase of germination, biochemical processes initiate. A number of repair mechanisms needed for seed germination are activated, including repairing of cell membranes and organelles, as well as activation of protein and enzymes needed for food reserve mobilization (McDonald, 1999). Reinero et al., 1983 reported that in Araucaria araticana (Mol.) seeds, α-amylase activity was significantly increased after imbibition for 12 to 24 h. Moreover, enzyme activity remained active until 64 h of imbibition in maize seeds (Vashisth and Nagarajan, 2010). In Euphorbia heterophylla embryos, the amount of reducing sugars increased rapidly after 36 h of imbibition (Suda and Giorgini, 2000). Sugar alcohols like raffinose,
stachyose; etc reduced during imbibition and confirmed their utilization in the germination process in cotton (Doman et al., 1982) and pea seeds (Blochl et al., 2006). Respiration rate in imbibed seeds is enhanced to produce ATP and specific biochemicals for germination, such as DNA (Bino et al., 1996) and protein (Rajjou et al., 2004). Commonly during germination, reactive oxygen species (ROS) are produced in cells (Mittler, 2002; Bailly, 2004). Balance between the amount of ROS and antioxidant system is imperative for synchronized germination. Any disturbance in this balance leads to membrane deterioration, lipid peroxidation, protein and nucleic acid degradation (McDonald, 1999; Bailly, 2004). Hence, antioxidants are crucial for the removal of ROS. During imbibition, antioxidant activities increases many fold. Superoxide dismutase (SOD) is specific for the conversion of superoxide radical to hydrogen peroxide (Halliwell and Gutteridge, 1984). Bailly et al., 2000 has reported the increased activity of SOD after 3-6 h imbibitions in sunflower seeds. At final germination stages, enhanced SOD activity in Chenopodium murale (Bogdanovic et al., 2008) and increased ascorbate peroxidase (APX) activity in excised embryos of germinating wheat and pea seeds within 12-24 h of imbibitions (De Gara et al., 1997; Pallanca and Smirnoff, 1999) have also reported.

2.3.2 Hydro priming:
Hydro priming is pre-sowing technique in which seeds are soaked with water under controlled water potential that allows only imbibition and prevents radical emergence (Bradford and Bewley, 2002). Hydropriming has extensively used to improve plant growth and germination. Previously, hydropriming has reported to improve yield in maize (Harris et al., 1999; Afzal et al., 2002), to enhance seedling emergence in rice (Farooq et al., 2009b), to improve the seedling vigor and decrease the mean germination time in wheat (Basra et al., 2003) and to improve germination percentage in corn seeds by enhancing antioxidants (Chiu and Sung, 2002). Antioxidant enzyme (SOD and APX) activities have reported to be enhanced for improvement of germination in bitter gourd seeds after priming (Hsu et al., 2003). Moreover, increased catalase (CAT) and peroxidase (POD) enzyme activities were found to enhance chilling tolerance of maize seedlings after priming (Guan et al., 2009). Goel et al., 2003 reported that hydropriming
partially maintained germination and activities of antioxidants under artificial aging. Priming has shown to repair and build up nucleic acids, proteins and membranes in sunflower (Wahid et al., 2008) and in tomato (Van Pijlen et al., 1996) seeds. Thus, hydropriming has proved to be effective to increase the antioxidant enzymes, and repair or synthesis of proteins that play important role for enhancement of seed vigor.

2.4 Improvement of drought tolerance by seed priming:
Primed seeds as a shotgun approach can ameliorate the adverse effects of osmotic or drought stress. Seed priming responses include early germination, stable growth, improved antioxidant potential and even increase in the crop yield (Basra et al., 2006). Primed seeds usually results in enhanced germination percentage, germination index and sometimes greater vigor index (Kaya et al., 2006; Farooq et al., 2007). Naturally, to face drought stress, crop plants accumulate osmolytes (glycine betaine, proline), antioxidants (Catalase, Peroxidase, Superoxide dismutase, etc) and hormones (salicylic acid, gibberellins, cytokinin, etc). The accumulation of all these substances increases after seed priming that could help the crop plant to withstand drought stress (Hong-Bo Shao et al., 2007; Farooq et al., 2009a). Potassium ions help in osmotic adjustment while calcium ions activate the signaling cascade to overcome the side effects of drought stress. All these and lots more can be used as priming tools to improve drought tolerance (Basra et al., 2006).

Seed priming induces the biochemical changes in seeds to enhance the production of antioxidants and other germination enhancing proteins that results in early germination, enhanced germination percentage, energy of germination, germination index and vigor index (Khan et al., 2011). Seed priming efficiently reduces the adverse effects related to drought stress and results in better and synchronized crop stand. Reduced germination index and percentage was highly improved after priming with different agents (Abbasdokhta, 2011).

Plants have inbuilt defense systems to reduce cellular and sub-cellular damages occurred due to reactive oxygen species (Liebler et al., 1986; Elstner, 1987; Larson, 1988). Activities of antioxidant enzymes i.e. CAT, POD and SOD etc have reported to increase
after seed priming under stressful conditions (Basra et al., 2006; Hong-Bo Shao et al., 2007; Farooq et al., 2009a).

2.4.1 Use of plant hormones as priming agents:
Plant growth hormones applied as seed priming agent or as foliar application highly improves the growth of plants against a variety of abiotic stresses. Plant growth substances like gibberellins, salicylic acid, indole acetic acid (IAA), cytokinin, brassinosteroids and abscisic acid modulate the response to drought stress (Farooq et al., 2009a).

Gibberellinic acid, the growth promoter increases the length and fresh weight of hypocotyl (Taiz and Zeiger, 2006) and enhances the photosynthetic activity and stomatal conductance (Kumar et al., 2001) under drought stress.

Brassinosteroids and abscisic acid increases yield, water potential and chlorophyll content in plants under drought stress (Zhang et al., 2004). Jasmonates act as signaling molecule; trigger the production of drought tolerant genes and enhances glycine betaine contents in pear plant (Gao et al., 2004).

Jasmonic acid application to maize seedlings results in increased activities of abscisic acid hormone and antioxidants (Li et al., 1998). Drought tolerance of wheat has reported to improve after salicylic acid treatment due to increased catalase activity and high accumulation of proline (Shakirova et al., 2003; Horvath et al., 2007; Korkmaz et al., 2007).

2.4.2 Use of osmoprotectants as priming agents:
Osmoprotectants have been exogenously applied to plants for the improvement of stress tolerance (Ashraf and Foolad, 2007). Osmolytes like proline and glycine betain can enhance the antioxidant defense, protect biomolecules from oxidative damage and results in increased defense response (Shao Hong-Bo et al., 2006). Glycinebetaine as priming agent or in foliar spray has reported to improve the growth and development of plants (Hussain et al., 2008), increase the achene numbers (Azam et al., 2005), better water status and stomatal conductance (Sakamoto and Murata, 2002) and enhance the antioxidants level (Ma et al., 2007) under stress.
Glycine betaine (GB) which acts as osmoprotectant increased under abiotic stresses to cope the adverse effects of stresses in tolerant genotypes. Increased amount of GB has resulted in increased yield of plants under abiotic stresses i.e. drought stress (Sarwar et al., 2006; Khan et al., 2010b). Proline application to plants under drought stress has resulted in increased accumulation of proline to get drought tolerant genotypes (Yamada et al., 2005). Polyamines highly improved the stress tolerance in plants by improving plant growth (Kubis, 2003; Liu et al., 2004; Liu et al., 2007a; Yang et al., 2007).

2.5 Use of chitosan as priming agent:
Chitosan is a cation polysaccharide and a derivative of chitin that isolated from marine animal’s tissue. It belongs to carbohydrate family with structural similarity to cellulose as well as chitin. Mainly Sea fish, shrimps and cramps provide chitosan or alternatively chitin after deacetylation transformed to chitosan. Chitosan structural nitrogen dissolves in soil water solution and provides this micronutrient for improved growth and development of plants (Sheikh and Malki, 2011).
Chitosan is linear polysaccharide, consists of repeating units of glucosamine with inserted nitrogen. Chitosan is not soluble in water but soluble in acidic solutions as that of acetic acid and citric acid.

(Source: Sheikh and Malki, 2011)
Chitosan application activates the jasmonic acid signaling pathway in plants and provides resistance to diseases. Chitosan relives the inhibitory effects of salicylic acid on jasmonic acid pathway and activate defensive genes for disease resistance in plant (Doares et al., 1995).

(Source: Doares et al., 1995)

Chitosan has proved itself as an excellent fertilizer. Chitosan application to plants resulted in many essential reactions of growth and development. Chitosan has been reported to have antiviral, antimicrobial, antioxidant activities and its application as priming agent or in growing media can improve the stress tolerance (Marcello and
Franco, 2008; Yang et al., 2009). Chitosan is an exogenous elicitor of defense responses in plants, as its application resulted in accumulation of phenolic compounds in crops (Bautista-Banos et al., 2006).

2.5.1 Effects on plant water status:

Plant water status that consists of relative water contents and water relations is very important feature during drought stress mitigation that must remain at normal level. Only tolerant crops can maintain water status during drought stress. However, chitosan proved itself as an effective polysaccharide under low water availability that can reduce transpiration and maintain plant water status by closing stomata of plants (Farouk et al., 2011; Farouk and Amany, 2012).

2.5.2 Effects on antioxidants:

Oxidative stress develops in plants as constituent of drought stress and retards the normal function of plant cell organelles. Plant cell metabolism totally changes under oxidative stress. Reactive oxygen species produced in cell during in unusual concentration must be removed from the cell. In built, antioxidant defense system of plants cannot function properly under drought stress (Hameed et al., 2010). Chitosan has some sort of antioxidative properties in it and can act as scavenger of ROS by enhancing the antioxidative pool of plant cells. Increased CAT, POD, SOD enzyme activities have been reported in low temperature stressed maize plants after chitosan priming treatment (Guan et al., 2009).

2.5.3 Effects on cell membrane stability and lipid peroxidation:

Cell membrane damage during drought stress has been reported to reduce after seed priming that resulted into up regulation of many related genes by the H$_2$O$_2$ signaling pathway. Detoxification of toxic compounds results into the improvement of membrane stability (He et al., 2009). Cell membrane stability is one of the features of plant cell that are adversely affected by drought stress. To overcome this aspect of stress, chitosan application can improve the cell membrane stability under drought stress (Yang et al., 2009). Chitosan as a germination promoter reduces the MDA contents of plants under abiotic stresses especially drought. One measure of cell membrane leakage is the MDA
content of cell that instantly decreases after chitosan application to plants under stress (Yang et al., 2009; Guan et al., 2009).

2.5.4 Effects on pigments:
Photosynthetic pigments of plants are adversely affected by drought stress. Stomatal closure not only reduces the CO₂ flux in plant cells but also results in reduced production of photosynthetic pigments during drought stress. However, chitosan application highly improves the pigment composition mainly chlorophyll contents of cereal crops (Sheikh and Malki, 2011). Similarly, chitosan application to radish plant has been reported to result in increased contents of chlorophyll a, chlorophyll b and total chlorophylls under cadmium stress (Farouk et al., 2011).

2.5.5 Effects on germination parameters:
Better seedling growth and improved germination primed seeds is an established fact. Chitosan has been reported to enhance the germination index and seedling growth under stress. Chitosan acts as plant growth and germination promoter. Chitosan application improves the germination of plants under drought stress. Reduction in the mean germination time and enhancement in germination index after chitosan treatment actually results in promotion of early seedling establishment and synchronized growth (Suchada et al., 2007; Guan et al., 2009).

2.5.6 Effects on yield and yield parameters:
Grain yield of plant is the measure of its productivity, which is badly affected under both biotic and abiotic stresses. Drought mainly results into high degree of reduction in grain yield in cereal crops especially in case of wheat. Total grain yield and yield components of wheat plants are severely affected under drought stress. However, these effects of drought stress have been reported to reverse after chitosan treatment (Mahmood et al., 2012). Chitosan application either as priming agent or in growing medium of cereals has resulted in high degree improvement of yield and yield parameters. Under drought stress, yield and other related attributes highly increased after chitosan priming in rice seedlings (Suchada et al., 2007).
2.6 Use of sodium nitroprusside as priming agent:

Sodium nitroprusside (SNP), a NO donor activates the signaling cascade for stress tolerance in plants. Its application extensively improves the cellular as well as metabolic function of plants. It has proved to be very effective in improvement of relative water contents, photosynthesis, oxidant and antioxidants status, gas exchange characteristics and many other processes needed for drought stress mitigation in wheat seedlings (Grac and Lamattina, 2001; Tian and Lei, 2006).

Nitric oxide (NO) produced by SNP in plant cells is dependent on two ways; one is enzymatic way that depends on nitrite and other depends on amino acid arginine. Increase in the Ca\(^{2+}\) concentrations up regulates the arginine pathway. Nitric oxide increases the intracellular Ca\(^{2+}\) concentrations by increasing cGMP and phosphorylation of other proteins that in turn activate the CDPKs and MAPKs in response of osmotic stress. Nitrosylated proteins also activate the NO signals. NO and Ca\(^{2+}\) cross-talk and protein kinases leads to regulation of gene expression related to stress tolerance in plant cells under biotic and abiotic stress (Courtois et al., 2008).
2.6.1 Effects on plant water status:
Drought adversely affects the RWC and hence water status of plants. Primed seeds can easily maintain their water status under reduced water supply. Sodium nitroprusside in growing media extensively improves the water status of plants. SNP application to wheat seedlings in growing media not only improves the relative water contents but also enhanced the antioxidants, osmolytes concentration and stomatal conductance in leaves under drought or osmotic stress (Grac and Lamattina, 2001; Jinfang et al., 2008; Wang et al., 2011a).
2.6.2 Effects on antioxidants:
SNP application in growing media highly improves the antioxidants status of plants under drought stress. Key antioxidant enzymes i.e. CAT, POD and SOD has been reported to increase after SNP application in growing medium to cope with drought stress conditions in wheat plants (Tian and Lei, 2006) and in pea plants (Moussa and Mohamed, 2011). Exogenous application of SNP reduces the oxidative damage caused by salt stress in chickpea plants by enhancing antioxidants level (Sheokand et al., 2010). Moreover, SNP pre treatment in growing media of rice plants has been reported to reduce the oxidative damage by enhanced production of antioxidants i.e. superoxide dismutase, peroxidase, glutathione reductase, total phenolic contents and catalase under drought stress (Shehab et al., 2010).

2.6.3 Effects on cell membrane stability and lipid peroxidation:
Drought stress negatively affects the cell membrane stability that results into membrane leakage in almost all crop plants. SNP application to wheat seedlings has shown positive effects on the cell membrane stability that highly improved under drought stress condition (Hao et al., 2008). Lipid peroxidation highly increases in stressed plants that result in membrane leakage. However, SNP treatment to wheat plants has reported to enhance the stress tolerance by reducing lipid peroxidation (MDA) and improved membrane stability and integrity under stress. NO produced by SNP alleviates the toxic effects of lipid peroxidation with reduction of ROS produced under nickel stress in wheat seedlings (Wang et al., 2011a). Exogenous SNP treatment to chickpea plants has reported to reduce the oxidative damage by reducing lipid peroxidation under salt stress (Sheokand et al., 2010).

2.6.4 Effects on osmoprotectants:
Iminoacid proline accumulates during drought stress especially in wheat. Proline strongly acts as osmoprotectant and has a role in osmotic adjustment during drought stress. Proline also detoxifies the ROS produced during oxidative stress. Seed priming can enhance the potential of osmotic adjustment during stresses, which leads towards stress tolerance. SNP treatment in growing media has shown to enhance the leaf proline content under osmotic stress in wheat seedlings (Jinfang et al., 2008; Khan et al., 2009; Simaei et al., 2010).
Total soluble sugars increase after SNP application to rice plants under drought stress (Shehab et al., 2010).

### 2.6.5 Effects on pigments:

Chlorophyll the photosynthetic pigments of plants decreases during drought stress while SNP treatment has been reported to highly improve the chlorophyll content in wheat (Wang et al., 2011a) and both chlorophyll and total caratinoides in pea plants under drought stress (Moussa and Mohamed, 2011; Simaei et al., 2011). Due to improvement in photosynthetic pigments after exogenous application of SNP, the photosynthetic activity of wheat seedling was enhanced (Jinfang et al., 2008).

### 2.6.6 Effects on germination:

Germination parameters can be highly affected under drought stress in crops. Many growth substances, hormones, osmoprotectants has been used to improve germination under stressed conditions. In this decade, sodium nitroprusside (SNP) a nitric oxide (NO) donor is being used for this purpose that efficiently promotes germination upon its application to seed. Rice seeds primed with SNP germinated well and showed high performance under salt stressed conditions (Noman et al., 2010). SNP acts as germination promoter and enhances the germination responsive genes to improve the germination in wheat (Sen, 2010). It also enhances the seed vigor index under abiotic stress by enhancing germination promoting proteins (Duan et al., 2007).

### 2.6.7 Effects on yield and yield parameters:

Plants yield is most important component that must remain constant under stress to produce stress tolerant varieties. Many strategies have been used to improve yield of plants, one of which is seed priming. SNP pre treatment to crops proved to be very effective in improvement of yield and yield components under stressful environment. After sodium nitroprusside application, grain yield and plant biomass has been reported to increase under cadmium stress in chickpea and under drought stress in pea seedlings (Kumari et al., 2010; Moussa and Mohamed, 2011).
2.7 Use of sodium silicate as priming agent:

Silicon is most abundant and essential element of crop plants. It gives strength, mineral nutrition, resistance to stresses and improves early seedling growth (Gong et al., 2003). However, most of its effects on plants under drought stress are yet not well understood and much extensive research is needed to provide information in this aspect. Sodium silicate, a derivative of silicone can improve the water balance by increasing root endodermal silification (Farooq et al., 2009a). Silicone as sodium silicate can improve the germination, growth, antioxidant enzymes activities and lipid membrane stability during drought stress in wheat (Pei et al., 2010).

![Na-O-Si-]

(Source: Wikipedia)

2.7.1 Effects on plant water status:
Seed priming can highly improve the decreased water relations and disturbed plant water status during water stressed conditions. Sodium silicate (commonly called as silicone) application can improve the decreased water potential and RWC under drought stress. This increase in water status of plant results in better plant growth under drought stress (Gong et al., 2003). Shoot water uptake increases after sodium silicate application in sorghum plants that leads to higher relative water content, photosynthetic activity, better stomatal conductance and increased dry matter accumulation (Hattori et al., 2005).

2.7.2 Effect on antioxidants:
Sodium silicate treatment can reduce the oxidative injury by enhancing the production of antioxidants to quench the ROS. Sodium silicate treatment in wheat stimulates the
activities of antioxidants (SOD, POD and CAT) under stress (Ali et al., 2012). Sodium silicate treatment lower down the oxidative stress by enhancement of antioxidant production (glutathione reductase, catalase, peroxidase, and superoxide dismutase) to reduce the drought stress effects in wheat, barley and soy bean (Liang et al., 2003; Gong et al., 2005; Miao et al., 2010; Wang et al., 2011b).

2.7.3 Effects on cell membrane stability and lipid peroxidation:
Cell membrane stability is a drought tolerance index in plants. Sodium silicate application has been shown to result in better cell membrane stability under drought or osmotic stress (Pei et al., 2010). Sodium silicate not only deposits in the plant cells but also actively reduces the MDA contents and enhances the activities of antioxidants in plants (Liang et al., 2003; Miao et al., 2010). Due to reduction in lipid peroxidation the cell membrane stability improves during abiotic stresses (Liang et al., 2007; Pei et al., 2010; Wang et al., 2011b).

2.7.4 Effects on osmoprotectants:
Plants accumulate osmoprotectants like proline and glycine betaine to overcome the deleterious effects of different abiotic and biotic stresses. Osmotic adjustment of plants under stress is the common phenomena. Sodium silicate has been reported to profoundly increase the accumulation of proline and glycine betaine in potato and wheat plants under drought stress (Carlos et al., 2009; Ahmad and haddad, 2011).

2.7.5 Effects on pigments:
Impaired photosynthesis results in drastic decrease in concentration of pigments in plants. Photosynthetic pigments especially chlorophyll contents markedly decrease under abiotic stresses which reverts to normal concentration or even increases after silicone treatment. Previously it has been proved that silicone application to rose plants increases the chlorophyll contents and caratinoides under salt stress (Reezi et al., 2009). Chlorophyll contents also improve after sodium silicate treatment in wheat leaves under osmotic stress (Pei et al., 2010).
2.7.6 Effects on germination parameters:
Sodium silicate can positively affects the germination rate and development of plants under stress. Sodium silicate acts as growth stimulator in plants and can improve the tolerance to abiotic stresses (Fauteux et al., 2006). Low concentration of applied sodium silicate has been reported to result in higher germination percentage, germination index and increased yield in plants (Abro et al., 2009). In soybean seedlings, sodium silicate application enhances germination and nutrient use, which leads to better seedling development (Miao et al., 2010).

2.7.7 Effects on yield and yield parameters:
Seed priming can increase the yield and yield components of plants under drought stress. Silicone (as sodium silicate) treatment has been reported to increase the yield and synchronize the yield components in wheat plants (Pei et al., 2010). Sodium silicate application in growing media highly improves the yield of wheat and potato under drought stress (Abro et al., 2009; Carlos et al., 2009; Pei et al., 2010).
In view of above literature, it is now clear that chitosan, sodium nitroprusside and sodium silicate have many properties due to which they can improve abiotic stress tolerance in general and drought tolerance in particular in different crop plants. However, seed priming induced changes in the seeds biochemistry have not been given special emphasis and therefore should be investigated. In this view, we planned the experiments to analyze the effects of seed priming with these chemicals on biochemical changes in the wheat seeds, seedlings and mature plants. Improvement in the drought and osmotic stress tolerance by seed priming treatments was also tested.
3 MATERIALS AND METHODS

Present study was planned to investigate the possible ways for improvement of drought tolerance in wheat by use of seed priming. For this purpose, three independent experiments were conducted in Biochemistry Lab and wire house of NIAB, Faisalabad.

3.1 Experiment I: Effects of different priming treatments on wheat seed biochemistry.
Chitosan, sodium nitroprusside and sodium silicate concentrations and treatment duration for seed priming were selected after optimization. Best performing treatments were selected as priming tools to prime the wheat seeds. Firstly, to investigate the biochemical changes in wheat seeds, experiment was laid out in completely randomized design (CRD) with three replications.

3.1.1 Seed material:
The spring wheat (Triticum aestivum L. cv. AARI-2011) seeds were obtained from Wheat Section, Ayub Agriculture Research Institute (AARI), Faisalabad, Pakistan. Seed priming treatments used in the study were hydropriming, chitosan priming, SNP priming and sodium silicate priming. For hydropriming, seeds were soaked in aerated distilled water for 8 h. For chitosan priming, seeds were soaked in 0.1, 0.25 and 0.50 % aerated solutions of chitosan for 8 h. For SNP priming, seeds were soaked in 75, 100 and 125 μM aerated solutions of SNP for 8 h. Similarly, for sodium silicate priming, seeds were soaked in 20, 40 and 60 mM aerated solutions of sodium silicate for 8 h. After all priming treatments, seeds were given three washings with water and re-dried near to original weight with forced air under shade at 26 ±2 °C. Non-primed seeds were used as control for comparison.

3.1.2 Sample extraction and analysis
For extraction and estimation of enzymes and other biochemical parameters primed and non-primed control seeds were grounded in extraction buffer specific for different enzymes/biomolecules and centrifuged at 15,000×g for 20 min at 4 °C. The supernatant was separated and used for the quantification of different enzyme activities.
3.1.3 Biochemical analysis:
Seed priming induced biochemical changes in the seeds were analyzed. For this purpose, different biochemical parameters were measured in primed and non-primed seeds according to the following methods.

3.1.3.1 Total soluble protein content:
Quantitative protein estimation was performed by method of Bradford (1976) using bovine serum albumin (BSA) as standard. For protein estimation in seed, 5 µl of supernatant and 95 µl 150mM NaCl were mixed with 1.0 ml of dye reagent (100 mg Coomassie Brilliant Blue G-250 dye was dissolved in 50 ml 95 % ethanol and 100 ml 58 % (w/v) phosphoric acid and dilute to one litter) and the mixture was left for 5 min to form a protein dye complex. Then, the absorbance was measured at 595 nm by using spectrophotometer (HITACHI, U2800). Different standards (05-50 µg with 5 µg interval) of bovine serum albumin were prepared in 100 µl 150mM NaCl. Absorbance at 595 nm was measured 5 min after mixing the standards with 1.0 ml of dye reagent. A standard curve was prepared (Figure 3.1) by plotting the concentration on x-axis against their absorbance at 595 nm on y-axis. A simple linear regression equation was also calculated.

![Figure 3.1: Standard curve for total soluble proteins estimation.](image)

\[
y = 0.0414x + 0.0867 \\
R^2 = 0.9873
\]
3.1.3.2 Superoxide dismutase (SOD)

Seed samples (0.5 g) were homogenized in extraction buffer consisting of 50 mM potassium phosphate, pH 7.8, 0.1% (w/v) BSA, 0.1% (w/v) ascorbate, 0.05% (w/v) β-mercaptoethanol as described by Dixit et al., (2001). The activity of SOD was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) following the method of Giannopolitis and Ries (1977). The reaction solution (3 ml) contained 50 µM NBT, 1.3µM riboflavin, 13mM methionine, 75nM EDTA, 50mM potassium phosphate buffer (pH 7.8) and 20-50 µl enzyme extract. The test tubes containing the reaction solution were irradiated under a light (15W fluorescent lamps) at 78 µmolm$^{-2}$ s$^{-1}$ for 15 min. The absorbance of the irradiated solution at 560 nm was determined with a spectrophotometer (Hitachi U-2800, Tokyo, Japan). One unit of SOD activity was defined as the amount of enzyme, which caused 50% inhibition of photochemical reduction of NBT.

3.1.3.3 Catalase (CAT) and peroxidase (POD)

For the estimation of CAT and peroxidase, seeds (0.5 g) were homogenized in medium composed of 50 mM potassium phosphate buffer, pH 7.0 and 1 mM dithiothreitol (DTT). Activities of peroxidase (POD) and catalase (CAT) were measured using the method of Chance & Maehly (1955) with some modifications. For measurement of POD activity, assay solution (3 ml) contained 50mM potassium phosphate buffer (pH 7.0), 20mM guaiacol, 40mM H$_2$O$_2$ and 0.1 ml enzyme extract. The reaction was initiated by adding the enzyme extract. Increase in absorbance of the reaction solution at 470 nm was recorded after every 20 s. One unit POD activity was defined as an absorbance change of 0.01 units min$^{-1}$. For measurement of CAT activity, assay solution (3 ml) contained 50mM phosphate buffer (pH 7.0), 5.9mM H$_2$O$_2$ and 0.1 ml enzyme extract. The reaction was initiated by adding the enzyme extract. Decrease in absorbance of the reaction solution at 240 nm was recorded after every 20 s. An absorbance change of 0.01 units min$^{-1}$ was defined as one unit CAT activity. Enzyme activities were expressed on seed weight basis.
3.1.3.4 Protease activity

For estimation of protease activity, samples were extracted in 50mM potassium phosphate buffer pH 7.8. Protease activity was determined by the casein digestion assay described by Drapeau and others (1974). A series of tubes were equilibrated with 2.0 ml of 1% casein at 37°C for 5 minutes. To all the tubes, 100μl of protease extracts was added and mixed well. A reagent blank was also included. Exactly ten minutes after adding sample, reaction was stopped by adding 2.0 ml TCA solution and mixed well. Tubes were then allowed to stand for ten minutes and then reaction solution was filtered to remove the precipitate formed during reaction. The absorbance of filtrate was measured at 280 nm. By this method one unit is that amount of enzyme, which releases acid soluble fragments equivalent to 0.001 A280 per minute at 37°C and pH 7.8. Enzyme activity was expressed on seed weight basis.

3.1.3.5 Malondialdehyde (MDA) content

The level of lipid peroxidation in the seed was measured in terms of malondialdehyde (MDA, a product of lipid peroxidation) content determined by the thiobarbituric acid (TBA) reaction using method of Heath and Packer (1968) with minor modifications as described by Dhindsa et al., (1981) and Zhang and Kirkham (1994). Seeds were homogenized in 5 ml 0.1% TCA. The homogenate was centrifuged at 10 000 g for 5 min. To 1 ml aliquot of the supernatant, 4 ml 20 % TCA containing 0.5% TBA were added. The mixture was heated at 95 °C for 30 min and then quickly cooled in an ice-bath. After centrifuging at 10 000 g for 10 min the absorbance of the supernatant at 532 nm was read and the value for the non-specific absorption at 600 nm was subtracted. The MDA content was calculated by using extinction coefficient of 155 mM⁻¹ cm⁻¹.

3.1.3.6 Amylase activity:

The amylase activity was determined by the modified method as reported by the Varavinit et al., (2002). Seeds (0.5 g) were extracted in phosphate buffer. For amylase estimation, 1 ml of seed extract was added to 1 ml of 1% starch solution. Then this mixture was incubated for 3 min. After incubation 1ml DNS reagent (prepared by adding 1 g of 3, 5- dinitrosalicylic acid and 30 g of sodium potassium tartrate tetrahydrate in 50 ml distilled water followed by addition of 20 ml of 2 N NaOH and finally diluted to
100 ml was added and placed in boiling water bath for 15 min. The reaction mixture was then cooled to room temperature and 9 ml distilled water was added to each tube. After mixing, absorbance of solution was measured at 540 nm using spectrophotometer (HITACHI, U2800). Different standards (0.4-2 mg ml\(^{-1}\) with 0.4 mg ml\(^{-1}\) interval and 4-10 mg ml\(^{-1}\) with 2 mg ml\(^{-1}\) interval) of maltose were prepared in 2 ml distilled water. After mixing the standards with 1.0 ml of DNS reagent, tubes were boiled in boiling water bath for 15 min. Then absorbance of all standards was measured at 540 nm. A standard curve was prepared (Figure 3.2) by plotting the concentration on x-axis against their absorbance at 540 nm on y-axis. A simple linear regression equation was derived for calculation of amylase activity in the samples.

![Standard curve for α-amylase estimation.](image)

\[
y = 0.2135x + 0.1114
\]

\[R^2 = 0.9855\]
3.1.3.7 Reducing sugars:
Reducing sugars were determined by dinitrosalicylic acid method (Miller, 1972). Seeds (0.5 g) were extracted in phosphate buffer. For estimation of sugars, 0.2 ml of seed extract was added to 1.8 ml distilled water and 1 ml DNS reagent and placed in boiling water bath for 15 min. The mixture was then cooled to room temperature and 9 ml distilled water was added to each tube, mixed well and absorbance of solution was measured at 540 nm using spectrophotometer (HITACHI, U2800). Different standards (0.4-2 mg ml\(^{-1}\) with 0.4 mg ml\(^{-1}\) interval and 4-10 mg ml\(^{-1}\) with 2 mg ml\(^{-1}\) interval) of glucose were prepared in 2ml distilled water. After mixing the standards with 1.0ml of DNS reagent, tubes were boiled in boiling water bath for 15 min. Then absorbance of all standards was measured at 540 nm. A standard curve was prepared (Figure 3.3) by plotting the concentration on x-axis against their absorbance at 540 nm on y-axis. A simple linear regression equation was derived for calculation of sugar concentration in the samples.

\[
y = 0.5067x + 0.0723 \\
R^2 = 0.9626
\]
3.1.3.8 **Total soluble Sugars:**

The amount of total soluble sugars in seed samples was estimated by phenol sulphuric acid reagent method described by Dubois *et al.*, (1951). Seeds (0.5 g) were extracted in phosphate buffer, 1 ml of seed extract was mixed with 0.5 ml of 5 % phenol solution and then 2.5 ml of 96 % sulphuric acid was added rapidly. Each tube was gently agitated during acid addition and then allowed to stand in a water bath at 26-30 °C for 20 minutes. Then absorbance of coloured solution was measured at 490 nm using spectrophotometer (HITACHI, U2800). Different standards (0.1 to 3 mg ml⁻¹ with 0.5 mg ml⁻¹ interval and 3 to 6 mg ml⁻¹ with 1 mg ml⁻¹ interval) of glucose from were prepared in 4ml distilled water. After mixing the standards in phenol and acid solutions as described above, tubes were placed in water bath at 26-30 °C for 20 minutes and then absorbance was measured at 490 nm. A standard curve was prepared (Figure 3.4) using the standards. A simple linear regression equation was derived for calculation of total sugars in the samples.
3.1.3.9 Total phenolic content

A micro colorimetric method as described by Ainsworth and Gillespie (2007) was used for total phenolics assay, which utilizes Folin-Ciocalteau (F-C) reagent. A standard curve was prepared using different concentration of gallic acid and a linear regression equation was calculated. A 0.2 gram of seed sample was homogenized in 0.8ml ice cold 95% methanol using an ice cold mortar and pestle. The samples were then incubated at room temperature for 48 h in the dark. The samples were then subjected to centrifugation at 10000 g for 5min at room temperature. The supernatant was removed and used for TPC measurement. A 100µl of supernatant was mixed with 100µl of 10 % (vol/vol) F-C reagent, vortex thoroughly and then 800µl of 700mM Na₂CO₃ was added. Samples were then incubated at room temperature for 2 h. Blank corrected absorbance of samples was measured at 765nm. Phenolic content (gallic acid equivalents) of samples was determined (Figure 3.5) using linear regression equation.

![Standard curve for total phenolic contents estimation](image)

Figure 3.5: Standard curve for total phenolic contents estimation.
3.1.3.10 Sample extraction for esterase activity:
Esterase activity was determined according to method adapted from Van Asperen (1962). Seed sample (0.5 g) were homogenized in 5 ml phosphate buffer (100mM, pH 7.0), containing 1mM each of EDTA, PMSF, PTU and 20% glycerol by using chilled mortars and pestles for 20 s and then centrifuged at 10,000x g for 20 min at 4 °C. The supernatant was used for enzyme estimation. For preparation of standard curve, different standards (0.10-0.90 μM with 0.10 μM interval) of α and β-naphthol were prepared in 1000 μl distilled water. Samples and standards were analyzed for esterase activity using the procedure given below.

3.1.3.11 Estimation of esterases activity using α and β- naphthyl acetate as substrate:
First of all a substrate solution was made by adding 30mM α or β-naphthyl acetate in 1 ml acetone and added to 99 ml of phosphate buffer (40mM, pH 6.8). To make staining solution, Fast Blue BB salt 1% (w/v) in phosphate buffer (40mM, pH 6.8) and sodium dodecyl sulphate (SDS) 5% (w/v) in double distilled water were prepared separately. Staining solution was prepared by adding 2 parts of 1% solution Fast Blue BB salt into 5 parts of 5% solution sodium dodecyl sulphate (SDS). For analysis, samples were diluted by adding 10 µl of seed extracts to 990 µl phosphate buffer (40mM, pH 6.8).
Assay mixture was prepared by adding 1 ml of diluted sample into 5 ml of substrate solution and 1 ml of phosphate buffer (40mM, pH 6.8). For preparation of blank, 5 ml of substrate solution was mixed into 1 ml of phosphate buffer (40mM, pH 6.8). Finally, all the samples including blank were incubated in dark for 20 min at 30 °C with gently shaking. After 20 min, 1 ml of staining solution was added to all the samples including blank and again incubated in dark for 20 min at 30 °C with shaking. Finally, the absorbance was measured at 590 nm using spectrophotometer (U-2800, 122-003 Hitachi, Japan). Standard curves for α and β- naphthyl acetate esterase were also prepared using standards and following the same procedure (Figure 3.6).
Figure 3.6: Standard curves for estimation of α and β-naphthyl acetate esterase activities.
3.2 Experiment II: Improvement of osmotic stress tolerance in wheat by seed priming.

In second experiment, effects of chitosan, SNP and sodium silicate priming on seed germination, seedling growth and biochemistry were tested under normal and osmotic stress conditions. Seed germination and seedling vigor tests were performed on treated seeds along with controls under osmotic stress and normal conditions. Methodologies for germination test and seedling vigor are described below.

3.2.1 Seed germination test:

Germination potential of the primed and non-primed wheat seeds was estimated in accordance with the International Rules for Seed Testing by Association of Official Seeds Analyst (AOSA) (Anonymous. 1990). To test seed germination and seedling vigor under normal conditions, four replicates of 25 seeds were germinated in 12 cm diameter petri dishes at 25 °C and 5 ml deionized distilled water was applied. While to test the performance of primed seeds under osmotic stress conditions, 5 ml of 15 % polyethylene glycol (PEG-6000) solution (-3.0 Mpa) was applied in each petri dish to impose the stress. Similarly, four replicates of 25 seeds were germinated in 12 cm diameter petri dishes at 25°C in these studies. A seed was scored as germinated when coleoptile and radicle lengths reached 2-3 mm. Counts of germinating seeds were made twice a day at different time intervals (20, 28, 44, 52, 68, 76, 92 and 100 h), starting on the first day of imbibition, and terminated when maximum germination was achieved.

3.2.1.1 Mean germination time:

Mean germination time (MGT) was calculated according to the equation of Ellis and Roberts (1981).

\[
MGT = \frac{\sum D_n}{\sum n}
\]

Where n is the number of seeds, which were germinated on day D and D is the number of days counted from the beginning of germination.

3.2.1.2 Final germination percentage:

Final germination percentage was measured according to following formula.

\[
FGP = \frac{\text{No of seeds germinated on final day} \times 100}{\text{Total no of seeds sown}}
\]
3.2.1.3 Germination index:
Germination index (GI) was calculated as described by the Association of Official Seed Analysts (AOSA) (Anonymous. 1983) using the following formulae.

\[ \text{GI} = \frac{\text{number of germinated seeds} + \text{number of germinated seeds}}{\text{days of first count} + \text{days of final count}} \]

3.2.1.4 Energy of germination:
Energy of germination was recorded 4\textsuperscript{th} day after planting. It is the percentage of germinated seeds 4 days after planting relative to the total number of seeds tested (Ruan \textit{et al.}, 2002).

3.2.2 Seedling growth response and biochemical analysis:
For growth response and biochemical studies, seedlings from above said experiments for seed germination tests were allowed to continue growth after collecting the data for germination. Ten day old seedlings were harvested for comparison of growth and biochemical parameters under normal and osmotic stress conditions. Seedling root and shoot length was measured by spreading them on a scale calibrated in cm. For biochemical studies, leaves were collected from ten days old seedlings. Collected leaf samples were immediately stored at -80 °C till further analysis. For extraction of enzymes, fresh leaves (0.5 g) were ground in extraction buffer specific for different enzymes and centrifuged at 15,000×g for 20 min at 4 °C. The supernatant was separated and used for the determination of different enzyme activities. Different biochemical parameters were measured by using the following methods.

3.2.2.1 Total soluble proteins:
Quantitative protein estimation was performed by method of Bradford (1976) as described in Experiment I.

3.2.2.2 Superoxide dismutase (SOD):
SOD (EC 1.15.1.1) activity was assayed by using the method of dixit \textit{et al.}, (2001) as described in experiment I.
3.2.2.3 Peroxidase (POD) activity:
Peroxidase (POD) activity was measured using the method of Chance and Maehly (1955) and modified by Liu and Huang (2000) as described in experiment I.

3.2.2.4 Catalase (CAT) activity:
Catalase level in the samples was estimated by the following method described by Beers et al., (1952) as described in experiment I.

3.2.2.5 Protease activity:
Protease activity was determined by the casein digestion assay described by Drapeau et al., (1974) as explained in experiment I.

3.2.2.6 Malondialdehyde (MDA) contents:
The level of lipid peroxidation was determined by the thiobarbituric acid (TBA) reaction using method of Heath and Packer (1968) with minor modifications as described by Dhindsa et al., (1981) and Zhang and Kirkham (1994) as explained in experiment I.

3.2.2.7 Amylase activity:
The amylase activity was determined by the modified method reported by the Varavinit et al., (2002) as described in experiment I.

3.2.2.8 Total phenolic content:
Total phenolic content was measured by a micro colorimetric method as described by Ainsworth and Gillespie (2007) in experiment I.

3.2.2.9 Reducing sugars:
Reducing sugars were determined by dinitrosalicylic acid method (Miller, 1972) as described in experiment I.

3.2.2.10 Total soluble sugars:
The amount of total soluble sugars in leaf sample was estimated by phenol sulphuric acid reagent method by Dubois et al., 1951 as described in experiment I.
3.2.3  **Physiological parameters:**
Different physiological parameters were also studied on collected leaf samples by using the following methods.

3.2.3.1  **Cell membrane stability:**
Cell membrane stability was measured by using the following equation. Detailed are as following

\[
CMS\% = \left\{ \frac{1-(T1/T2)}{1-(C1/C2)} \right\} \times 100
\]

Where T1= Stress sample conductance before autoclaving

T2= Stress sample conductance after autoclaving

C1= control sample conductance before autoclaving

C2= control sample conductance after autoclaving

3.2.3.2  **Relative water content (R.W.C):**
The same leaf, as used for water potential, was used for relative water content (RWC). The relative water contents (RWC) were calculated with following formula:

\[
RWC = \left\{ \frac{\text{Fresh weight} - \text{dry weight}}{\text{Saturated weight} - \text{dry weight}} \right\} \times 100
\]

3.3  **Experiment III: Improvement of drought tolerance in wheat by seed priming.**
A pot experiment was conducted to check the effects of seed priming treatments on mature wheat plants under normal and drought stress conditions. For this purpose, primed (Chitosan, SNP and sodium silicate) and non primed seeds were germinated in 20×10 cm plastic pots in wire house at NIAB during normal wheat growing season (Nov 2011-April 2012) under natural conditions with three replications. Pots were protected from rain with the help of transparent polythene sheet on the wire house. Pots were filled with 4 kg of soil [sandy clay loam (45% sand, 33% silt and 22% clay) with average bulk density of 1.4 g cm\(^{-3}\)] and irrigated at soil water holding capacity. In control pots, water was maintained at soil water holding capacity throughout the experiment. While in pots in which drought stress was induced, water was maintained at 50 % soil water holding capacity after sowing. With normal agronomical practices, experiment was continued until maturity.
3.3.1 Flag leaf samples for analysis:
For biochemical and physiological studies, flag leaf samples were collected from wheat plants (98 days old) growing under normal and drought stress conditions. Collected leaf samples were immediately stored at -80 °C till further analysis.

3.3.2 Biochemical analysis:
For different biochemical analysis, leaves (0.5 g) were ground in extraction buffer specific for different enzymes or biochemical and centrifuged at 15,000×g for 20 min at 4 °C. The supernatant was separated and used for the determination of different biochemical parameters using the following methods

3.3.2.1 Total Protein Contents:
Quantitative protein estimation was performed by method of Bradford (1976) as described in Experiment I.

3.3.2.2 Superoxide dismutase (SOD):
SOD (EC 1.15.1.1) activity was assayed by using the method of dixit et al., (2001) as described in experiment I.

3.3.2.3 Peroxidase (POD) activity:
Peroxidase (POD) activity was measured using the method of Chance and Maehly (1955) and modified by Liu and Huang (2000) as described in experiment I.

3.3.2.4 Catalase (CAT) activity:
Catalase level in the samples was estimated by the following method as described by Beers et al., (1952) as explained in experiment I.

3.3.2.5 Protease activity:
Protease activity was determined by the casein digestion assay described by Drapeau et al., (1974) as explained in experiment I.
3.3.2.6 Malondialdehyde (MDA) contents:
The level of lipid peroxidation was determined by the thiobarbituric acid (TBA) reaction using method of Heath and Packer (1968) with minor modifications as described by Dhindsa et al., (1981) and Zhang and Kirkham (1994) as described in experiment I.

3.3.2.7 Amylase activity:
The amylase activity was determined by the modified method reported by the Varavinit et al., (2002) as described in experiment I.

3.3.2.8 Total phenolic content:
Total phenolic content was measured by a micro colorimetric method as described by Ainsworth and Gillespie (2007) in experiment I.

3.3.2.9 Reducing sugars:
Reducing sugars were determined by dinitrosalicylic acid method (Miller, 1972) as described in experiment I.

3.3.2.10 Total soluble sugars:
The amount of total soluble sugars in leaf sample was estimated by phenol sulphuric acid reagent method by Dubois et al., 1951 as described in experiment I.

3.3.2.11 Glycine betaine determination:
Glycine betaine was determined following the Grieve and Grattan (1983) method. Dry leaf material (1.0 g) was ground in 10 ml of distilled water and filtered. After filtration, 1 ml of the extract was mixed with 1 ml of 2M HCl. Then 0.5 ml of this mixture was taken in a glass tube and 0.2 ml of potassium tri-iodide solution was added to it. The contents were shaken and cooled in an ice bath for 90 min with occasional shaking. Then 2.0 ml of ice cooled distilled water and 20 ml of 1-2 dichloroethane (cooled at -10 °C) were added to the mixture. The two layers formed in the mixture were mixed by passing a continuous stream of air for 1-2 min while tubes were still in ice bath (4 °C). The upper aqueous layer was discarded and optical density of the organic layer was measured at 365 nm using spectrophotometer (HITACHI, U2800). Different standards (5-40 µg ml\(^{-1}\) with 5 µg ml\(^{-1}\) interval) of glycine betaine from working stock solution were prepared in 1000 µl
distilled water. After following the method, absorbance at 365 nm was measured. The concentrations of the betaine were calculated (Figure 3.7) against the standard curve.

![Graph showing the standard curve for glycine betain determination. The equation y = 0.0399x + 0.0388 and R² = 0.9963 are displayed.](image)

Figure 3.7: Standard curve for glycine betain determination.

### 3.3.2.12 Proline Determination:

Proline from the dry leaf sample was estimated according to the method of the Bates *et al.*, (1973). A sample of 0.5 g fresh leaf tissue was homogenized in 10 ml of 3 % sulfosalicylic acid and the homogenate was filtered through Whatman No. 2 filter paper. Then 2.0 ml of the filtrate was mixed with 2.0 ml acid ninhydrin solution (Ninhydrin (1.25 g) was dissolved in 20 ml of glacial acetic acid and 20 ml of 6 M orthophosphoric acid there after stored and cooled at 4 °C) and 2 ml of glacial acetic acid in a test tube. This mixture was incubated at 100 °C for 60 minutes and then cooled in an ice bath. Finally, 4.0 ml of toluene were added to the solution and mixed vigorously by passing a
Continuous stream of air for 1-2 min. The chromophore containing toluene was aspirated from the aqueous phase, warmed at room temperature and the absorbance was read at 520 nm using spectrophotometer (HITACHI, U2800) and toluene was taken as blank. The proline concentration was determined from a standard curve (Figure 3.8) and calculated on dry weight basis as follows:

\[
\text{μmole proline g}^{-1}\text{ fresh weight} = \left(\frac{\text{μg proline ml}^{-1} \times \text{ml of toluene}}{115.5}\right) / \text{(g of sample)}
\]

Figure 3.8: Standard curve for proline determination.

3.3.2.13 Pigments analysis:
The concentration of chlorophyll \((a\) and \(b)\) was determined following the method of Arnon (1949) whereas carotenoids were determined following the method of Davis (1976). Fresh flag leaf (0.2 g) were grind well and extracted in 80% acetone at -4 °C. The extract was centrifuged at 10,000 g for 5 min at 4 °C. The absorbance of the supernatant was read at 645, 663 and 480 nm using a spectrophotometer (Hitachi-HITACHI, U2800). The concentration of total chlorophyll contents, chlorophyll \(a\), \(b\) and carotenoids were calculated by the following formulae:

\[
\text{Chl a (mg/g f.wt.)} = [12.7(\text{OD 663}) - 2.69(\text{OD 645})] \times \frac{V}{1000} \times \frac{1}{W}
\]
Chl b (mg/g f.wt.) = \[22.9(\text{OD 645}) - 4.68(\text{OD 663}) \times \frac{V}{1000} \times W\]

Carotenoids (mg/g f.wt.) = \[\frac{\text{Acar}}{\text{Em}} \times 1000\]

\[\text{Acar} = \text{OD 480} + 0.114(\text{OD 663}) - 0.638(\text{OD 645})\]

Where V is the volume of the sample, W is the weight of fresh tissue taken for extraction and Em is 2500.

3.3.3 Physiological parameters:
Different physiological parameters were studied in collected flag leaf samples by using the following methods.

3.3.3.1 Cell membrane stability:
The measurements of CMS were made by following the protocol of Blum and Ebercon (1981). Briefly, samples were collected from both control and drought stressed plants. Collected leaf samples were washed thrice with deionized water to remove any electrolytes adhered on the surface. The samples were then kept in a capped vial (20 ml) containing 10 ml of deionized water and incubated in the dark for 24 h at room temperature. After that, the conductance of control (C1) and stressed (T1) samples was measured with the help of conductivity meter (YSI Model 345, Yellow Springs, Ohio). After the first measurement the vials were autoclaved for 15 min to kill the leaf tissue and release the electrolytes. After cooling to room temperature, the second conductivity reading from control (C2) and stressed (T2) samples was taken. CMS was calculated as the reciprocal of cell-membrane injury according to Blum and Ebercon (1981):

\[\text{CMS} \% = \left[\frac{1 - \left(\frac{T1}{T2}\right)}{1 - \left(\frac{C1}{C2}\right)}\right] \times 100\]

Where T1= Stress sample conductance before autoclaving
T2= Stress sample conductance after autoclaving
C1= control sample conductance before autoclaving
C2= control sample conductance after autoclaving

3.3.3.2 Relative water content (R.W.C):
The same leaf, as used for water potential, was used for relative water content (RWC). The relative water contents (RWC) were calculated with following formula:

\[\text{RWC} = \frac{(\text{Fresh weight} - \text{dry weight})}{(\text{Saturated weight} - \text{dry weight})}\times 100\]
3.3.3.3 Water relations parameters:
The determination of leaf water potential was done at early morning between 6.00 a.m. to 8.00 a.m. A fully expanded youngest leaf was excised from each plant and leaf water potential measurement was made with a Scholander type pressure chamber (Arimad-2-Japan). The same leaf that was used for the measurement of water potential was frozen for two weeks at -70°C in an ultra-low freezer. For the determination of osmotic potential, the leaf tissue was thawed and extracted by crushing the material with a glass rod. The extract was centrifuged at 8000g at -4 °C for 4 min. and the supernatant was taken for the determination of osmotic potential. Ten μl of the supernatant was placed in a vapor pressure osmometer (Wescor, 5520) and the values obtained were converted into MPa. The leaf turgor potential was calculated as the difference between osmotic potential and water potential values ($\Psi_p = \Psi_w - \Psi_s$).

3.3.3.4 Yield and yield attributes:
Finally, yield attributes i.e., grain yield per plant; 100-grain weight and plant biomass were recorded at maturity.

3.3.4 Statistical Analysis:
All experiments were conducted in triplicates and descriptive statistics was applied to analyze and organize the resulting data. Significance of data was tested by analysis of variance and Tukey (HSD) Test at $p<0.05$ and where applicable at $p<0.01$ using XL-STAT software. Values presented in graphs are mean ± SD.
CHAPTER 4

4 RESULTS

Different experiments were conducted to investigate the use of chitosan, sodium nitroprusside and sodium silicate as seed priming treatments in wheat. Studies were conducted to uncover the changes that occur in the seeds, seedlings and wheat plants as results of these priming treatments. Furthermore, the possible use of these priming treatments for improvement of osmotic stress (PEG) and drought tolerance in wheat was also tested. Research findings from these experiments are as following

4.1 Effects of different priming treatments on wheat seed biochemistry

4.1.1 Seed priming with chitosan

Biochemical changes induced in wheat seeds after priming with chitosan were analyzed. Among the antioxidant enzymes, catalase (CAT) and superoxide dismutase (SOD) and peroxidase activities (POD) significantly increased in the primed seeds as compared to non primed non-stress (Figure 4.1). Increase in catalase and peroxidase activities was gradual with increasing treatment concentration being highest in seeds treated with 0.5 % chitosan. Catalase activity was near two folds in 0.5% chitosan treated seeds as compared with non-stress. SOD activity was highest in the seeds treated with 0.1 % chitosan. However, other higher chitosan concentrations (0.25 % & 0.5 %) induced comparatively lesser increase in the SOD activity in the seeds. In contrast hydropriming diminished the SOD activity in the seeds. However, catalase and peroxidase activities were also enhanced by simple water socking of seeds.

The level of total phenolics decreased in the seeds after all priming treatments including hydropriming with the least decrease (Figure 4.2). Drop in the phenolics contents was higher with increasing concentration of chitosan. Thus lowest value of total phenolics as compare to the non-stress seeds as observed after priming with 0.5% chitosan. Seed priming with chitosan increased the MDA contents in the seeds as compared to non-primed seeds and effect was more prominent with increasing concentration of treatment.
In contrast, MDA content was lower in hyroprimed seeds as compared to non-primed non-stress.

Total soluble proteins and protease activity were significantly increased after all priming treatments as compared to non-stress (Figure 4.3). Protease activity increased to many folds in the chitosan primed seeds. As this increase in the protease activity increased with increasing chitosan concentration, highest value was recorded in seeds treated with 0.5% chitosan. Hydro priming also increased the level of protein and proteases in the seeds but the magnitude of increase was much less as compare to chitosan priming treatments.

The level of reducing sugars and total sugars significantly decreased in the seeds after priming with chitosan while non reducing sugars increased significantly in the primed seeds (Figure 4.4). Highest modulation in the sugar contents was induced by 0.5% chitosan treatment. In chitosan primed seeds, reducing sugars were 37 % higher as compared to non-primed seeds. Hydropriming induced a decline in non-reducing and total sugars in the seeds but the decrease in total sugars was less as compared to that induced by chitosan priming. Hydropriming did not influence the reducing sugars level in the seeds.

Among reserve mobilizing enzymes, α-amylase activity was significantly increased in the seeds by 0.25 % and 0.50% chitosan treatments ( Figure 4.5). Though, seed priming with 0.1% chitosan and hydropriming were not able to modulate the α-amylase activity in the seeds. Similarly, α- naphthyl acetate esterase activity was also significantly increased in the seeds primed with 0.25 % and 0.5 % chitosan but not with 0.1% treatment. In contrast, hydropriming significantly reduced the level of α- naphthyl acetate esterase activity in the seeds. Moreover, β-naphthyl acetate esterase activity was only increased after 0.50 % chitosan priming while other seed treatments could not modulated this enzyme.

### 4.1.2 Seed priming with sodium nitroprusside (SNP)

Seed priming with different concentrations of sodium nitroprusside significantly modulated the most of analyzed parameters in the wheat seeds. Antioxidants like SOD activity was significantly increased by all SNP priming treatments while POD activity was enhanced by 75 μM and 125 μM SNP treatments but not by 100 μM treatment (
Figure 4.6. Magnitude of SNP priming induced increase in the SOD activity increased with increasing concentration of treatment with highest level in seeds primed with 125 µM SNP. On the other hand, catalase activity diminished significantly by the all SNP seed priming treatments. Hydropriming of seeds significantly dropped the SOD activity, uplifted the POD activity to many folds and significantly raised the CAT activity in seeds.

All SNP priming treatments including hydropriming dropped the level of total phenolics in the seeds (Figure 4.7). With increasing concentration of SNP treatment, decline in the phenolic contents was higher. Accordingly, the lowest total phenolic contents as compare to the non-stress seeds as observed after priming with 125 µM SNP priming. Hydropriming induced the least decrease in the phenolic contents of the seeds. Seed priming with SNP increased the MDA concentration in the seeds as compared to non-primed non-stress and increase was higher with increasing concentration of SNP. On the other hand, MDA level was lower in hydroprimed seeds as compared to non-primed non-stress.

Protease activity significantly increased in the seeds by all SNP priming treatments as compared with non-primed non-stress (Figure 4.8). Highest and many folds increase in seed protease activity was induced by 125 µM SNP treatment. Hydropriming of the seeds caused the least increase in seed protease activity as compared with non-stress. In parallel to enhanced protease activity in the primed seeds, a significant increase in the soluble proteins was also observed in the seeds primed with 100 µM and 125 µM SNP. However, seed priming with 75 µM SNP was not able to induce any change in the total soluble protein contents in the seed. Total soluble proteins were also raised in the seeds by the hydropriming. Here the level of increase in proteins was same as induced by 100 µM SNP treatment while comparatively low as by 125 µM SNP treatment.

Seed priming with different concentrations of SNP significantly reduced the level of reducing and total sugars whereas increased the non reducing sugar contents in the primed seeds (Figure 4.9). The most prominent effects on overall seed sugar contents were observed after 125 µM SNP treatment. In seeds primed with 125 µM SNP, non reducing sugars increased up to 33 % higher as compared to that in non-primed seeds. Hydropriming induced a reduction in non-reducing and total sugars while did not
influence the reducing sugars level in the seeds. All concentrations of SNP tested for seed priming significantly elevated the $\alpha$-amylase activity in the seeds (Figure 4.10). Highest uplift in the $\alpha$-amylase was recorded in the seeds primed with 125 $\mu$M SNP. Among esterases, only 125 $\mu$M SNP treatment increased the level of both $\alpha$-naphthyl and $\beta$-naphthyl acetate esterase activities in the seeds. In contrast, priming with 75 $\mu$M SNP significantly decreased the level of $\alpha$-naphthyl and $\beta$-naphthyl acetate esterases as compared with non-primed non-stress. However, both esterases remained at the level of non-primed non-stress seeds after priming with 100 $\mu$M SNP. Hydropriming reduced the level of $\alpha$-naphthyl acetate esterase in the seeds while $\beta$-naphthyl acetate esterase and $\alpha$-amylase activity levels were not influenced by this priming treatment.

4.1.3 Seed priming with sodium silicate

Different concentrations of sodium silicate as seed priming considerably altered the most of analyzed biochemical constituents in the wheat seeds. Among antioxidant enzymes, SOD activity was significantly increased by all sodium silicate priming treatments (Figure 4.11). POD activity was enhanced by only 20mM sodium silicate treatment while higher concentrations did not changed the level in the seeds. Conversely, CAT activity was significantly decreased by the all sodium silicate seed priming treatments. Higher concentrations of sodium silicate caused higher decrease in the CAT activity in the seeds as compared with non-primed non-stress. Moreover, SOD activity significantly decreased while POD and CAT activities significantly increased as a result of hydropriming of seeds. Hydropriming induced boost in the POD activity was many folds as compared with the level in non-primed seeds. Seed priming with different concentrations of sodium silicate significantly reduced the level of total phenolics in the seeds (Figure 4.12). The level of total phenolics after sodium silicate seed priming was almost half as that of non-primed non-stress. Hydropriming also lowered down the phenolic contents in the seeds however, degree of reduction was much lesser as compared to that by sodium silicate priming. On the other hand seed priming with sodium silicate increased the MDA concentration in the seeds as compared to non-primed non-stress. The sodium silicate priming induced boost in MDA increased with increasing concentration of sodium silicate. On the other hand, as compared to non-primed seeds, MDA level was lowered.
down by hydropriming. As compared with non-primed non-stress, all sodium silicate priming treatments significantly increased the protease activity in seeds (Figure 4.13). The highest sodium silicate treatment (60 mM) induced the highest and many folds increase in seed protease activity. Hydropriming of the seeds also caused an increase in the protease activity in seeds, however; the magnitude of increase was low as compared to that observed after sodium silicate treatments. Along with priming induced elevation in seed protease activity, the soluble protein content also increased significantly in the seeds primed with all concentrations of sodium silicate. Higher sodium silicate concentrations also induced higher uplift in the seeds soluble proteins. Hydropriming also increased the total soluble proteins in the seeds as compared with non-primed non-stress. Though, this increase was comparatively less as induced by sodium silicate priming. Seed priming with different concentrations of sodium silicate significantly reduced the level of reducing and total sugars (Figure 4.14). Maximum drop in the reducing and total sugars was observed in the seeds primed with 40mM and 60mM sodium silicate respectively. Priming with 40mM sodium silicate increased the non reducing sugar contents in the primed seeds while other concentration did not show any effect on non-reducing sugars in seeds. Hydropriming induced a reduction in non-reducing and total sugars while did not influence the reducing sugars level in the seeds.

The α-amylase activity in seeds was significantly raised by all concentrations of sodium silicate tested for seed priming (Figure 4.15). Maximum increase in the α-amylase activity was observed in the seeds primed with 60 mM sodium silicate. Among esterases, 40 mM and 60 mM sodium silicate treatments increased the level of β-naphthyl acetate esterase activity in the seeds. However, priming with 20 mM sodium silicate diminished the β-naphthyl acetate esterase activity in the seeds as compared with non-primed non-stress. The α-naphthyl acetate esterase activity did not changed by 20 mM and 40 mM sodium silicate as priming treatments. However, priming with 60 mM sodium silicate decreased the level of α-naphthyl acetate esterase in the seeds as compared with non-primed non-stress. Hydropriming also reduced the level of α-naphthyl acetate esterase in the seeds and the reduction was more as induced by sodium silicate treatments. Although, β-naphthyl acetate esterase and α-amylase activity levels were not influenced by the hydropriming.
Figure 4.1: Peroxidase, catalase and superoxide dismutase activities in wheat seeds after priming with chitosan.
Figure 4.2: Total phenolics and MDA content in wheat seeds after priming with chitosan.
Figure 4.3: Total soluble proteins and protease activity in wheat seeds after priming with chitosan.
Figure 4.4: Sugar contents in the seeds after priming with chitosan.
Figure 4.5: Amylase, α-naphthyl and β-naphthyl acetate esterase activities in wheat seeds after priming with chitosan along with non-stress.
Figure 4.6: Peroxidase, catalase and superoxide dismutase activities in wheat seeds after priming with sodium nitroprusside (SNP) along with non-stress.
Figure 4.7: Total phenolics and MDA content in wheat seeds after priming with sodium nitroprusside along with non-stress.
Figure 4.8: Total soluble proteins and protease activity in wheat seeds after priming with sodium nitroprusside along with non-stress.
Figure 4.9: Sugar contents in wheat seeds after priming with sodium nitroprusside.
Figure 4.10: Amylase, α-naphthyl and β-naphthyl acetate esterase activities in wheat seeds after priming with sodium nitroprusside.
Figure 4.11: Peroxidase, catalase and superoxide dismutase activities in wheat seeds after sodium silicate priming.
Figure 4.12: Total phenolics and MDA content in wheat seeds after sodium silicate priming.
Figure 4.13: Total soluble proteins and protease activity in wheat seeds after sodium silicate priming.
Figure 4.14: Sugar contents in wheat seeds after sodium silicate priming.
Figure 4.15: Amylase, α-naphthyl and β-naphthyl acetate esterase activities in wheat seeds after sodium silicate priming.
4.2 Experiment 2: improvement of osmotic stress tolerance in wheat by seed priming

In these experiments, effects of seed priming with chitosan, sodium nitroprusside and sodium on seed germination, seedling growth and physio-biochemical responses of seedlings under normal (without stress) and osmotic stress (PEG) were investigated. The results from these experiments are as following

4.2.1 Seed priming with chitosan: effects on seed germination, seedling growth and physio-biochemical responses.

Seed primed with three varying levels of chitosan (0.1, 0.25 and 0.5%) were germinated in petri plates under normal and osmotic stress (15% PEG-6000) conditions. Here the results of seed germination, seedling growth and physio-biochemical responses of seedlings are discussed.

4.2.1.1 Germination and growth response

For non-primed seeds, final germination percentage did not differ under osmotic stress and non-stressed condition (Error! Reference source not found.). Final germination percentage was significantly increased after chitosan priming under osmotic stress. However, chitosan priming did not improve the final germination percentage under non-stressed condition. Under osmotic stress, highest germination percentage (95%) was observed after priming with 0.25% chitosan. Hydropriming was not able to improve the final germination percentage under non-stressed and osmotic stress conditions. For non-primed seeds, mean germination time (MGT) was significantly longer under osmotic stress as compared to non-stressed condition. Seed priming with varying levels of chitosan significantly reduced the MGT under non-stressed and osmotic stress conditions. Hydropriming of seeds reduced the MGT under osmotic stress but not under non-stressed condition.

The germination energy (%) significantly increased by 0.5% chitosan priming under osmotic stress (Figure 4.17). However, the germination energy did not improved
The germination index for non-primed seeds was significantly reduced under osmotic stress as compared to non-stress condition. All chitosan priming treatments significantly improved the germination index under non-stress and osmotic stress conditions. Seed priming with 0.5 % chitosan induced highest increase in the germination index under non-stress as well as osmotic stress conditions. Hydropriming of seeds also improved the germination index under non-stress and osmotic stress conditions.

For non-primed seeds, vigor index was significantly lower under osmotic stress as compared to non-stress condition. Priming with 0.25 % and 0.50 % chitosan significantly increased the vigor index under non-stressed condition. Moreover, all chitosan treatments significantly improved the vigor index under osmotic stress condition. Hydropriming was not able to improve the vigor index under non-stress and osmotic stress condition.

Seed priming with chitosan enhanced the germination rate as compared to non-primed non-stress seeds (Figure 4.18). Under non-stress, seed priming with 0.50 % chitosan induced the highest enhancement in the seed germination rate. Under osmotic stress, 0.25 % and 0.5 % chitosan treatments were equally effective in improving the seed germination rate. Seed priming with 0.1% chitosan also improved the germination rate, however, degree of improvement was comparatively low as by induced by higher concentrations. Hydropriming also improved the germination rate but to a lesser extent as compared to seed priming with chitosan.

Seed priming with 0.50 % chitosan increased the shoot length of seedlings as compared to seedlings from non-primed seeds (Figure 4.19). Furthermore, all concentrations of chitosan increased the shoot length under osmotic stress. Similar was also true for root length which was increased by 0.5 % chitosan under non-stress condition while by all chitosan priming treatments under osmotic stress. Hydropriming of seeds could not improve the shoot and root length of seedling under normal as well as osmotic stress conditions.
Figure 4.16: Effect of chitosan seed priming on final germination % and mean germination time (MGT) of wheat seedlings grown under non-stress and osmotic stress.
Figure 4.17: Effect of chitosan seed priming on germination energy, germination and vigor index of wheat seedlings grown under non-stress and osmotic stress.
Figure 4.18: Effect of chitosan priming on germination rate under normal and osmotic stress conditions.
Figure 4.19: Effect of chitosan seed priming on shoot and root length of wheat seedlings grown under normal and osmotic stress conditions.
4.2.1.2 Physio-biochemical responses

For non-primed seeds, leaf peroxidase activity increased under osmotic stress as compared to non-stress condition (Figure 4.20). Chitosan priming significantly increased the peroxidase activity in leaves under non-stress and osmotic stress conditions. Under non-stress condition, increase in peroxidase activity by chitosan priming increased with increasing concentration of treatment, being the highest by 0.5 % chitosan treatment. Chitosan priming induced increase in peroxidase was many folds as compared to non primed seeds. Under osmotic stress, chitosan priming also raised the peroxidase activity in leaves. The magnitude of increase in peroxidase activity by chitosan priming was comparatively less under stress as observed under non-stress condition. Hydropriming also increased the leaf peroxidase activity under non-stress condition. However, hydropriming did not alter the peroxidase activity in the leaves of seedlings grown under osmotic stress.

Osmotic stress significantly decreased the catalase activity in the leaves of seedlings grown from non-primed seeds (Figure 4.20). Chitosan priming significantly decreased the catalase activity in leaves under non-stress as well as osmotic stress conditions. Under non-stress condition, decrease in catalase activity by chitosan priming was higher by 0.25 % and 0.5 % chitosan treatments. The level of decrease in catalase activity by chitosan priming was comparatively less under stress as observed under non-stress condition. Hydropriming did not alter the catalase activity in the leaves of seedlings grown under non stress or osmotic stress conditions.

Superoxide dismutase activity was not significantly changed as a result of osmotic stress (Figure 4.21). None of the tested chitosan priming treatments modulated the SOD activity under non-stress or osmotic stress conditions. Hydropriming of seeds raised the leaf SOD activity but only under osmotic stress while did not change it under non-stress condition. Osmotic stress significantly enhanced the level of total phenolics in the leaves of seedlings grown from non-primed seeds. Chitosan priming significantly decreased the total phenolic contents of leaves under both non-stress and osmotic stress conditions. However, the level of decrease in total phenolics varied for different concentrations of chitosan. Under non-stress condition, most prominent effect was observed after 0.5 % chitosan treatment while under osmotic stress, highest dropped in total phenolics was
induced by 0.25 % chitosan. Hydropriming of seeds enhanced the level of phenolics in leaves under non-stress condition. Yet, hydropriming did not change the level of total phenolics in leaves under osmotic stress.

Osmotic stress significantly elevated the total soluble proteins in the leaves of seedlings grown from non-primed seeds (Figure 4.22). Protein content was significantly increased by chitosan priming under non-stress and osmotic stress with more prominent increase under non-stress conditions. Hydropriming of seeds also raised the level total soluble proteins in leaves of seedlings under non-stress condition while it remained at the level of non-primed non-stress under osmotic stress conditions.

The level of leaf protease activity in seedlings from non-primed seeds was significantly escalated by osmotic stress (Figure 4.22). Seed priming with varying levels of chitosan significantly increased the protease activity in leaves under non-stress and osmotic stress conditions. Increase in protease activity by chitosan priming increased with increasing concentration of treatments under non-stress condition. Thus, the highest increased in leaf protease activity was induced by 0.5 % chitosan treatment. The magnitude of increase in protease activity by chitosan priming was very high under stress as observed under non-stress condition. Seed priming with 0.25 % chitosan induced highest increase in protease activity under osmotic stress. Hydropriming did not alter the level of protease activity in the leaves of seedlings under non-stress or osmotic stress conditions.

For non-primed seeds, leaf α-amylase activity increased under osmotic stress as compared to non-stress condition (Figure 4.23). Chitosan priming significantly decreased the leaf α-amylase activity under non-stress and osmotic stress conditions. Under non-stress as well as osmotic stress conditions, drop in leaf α-amylase activity by chitosan priming increased with increasing concentration of treatment, being the highest by 0.5 % chitosan treatment. Hydropriming did not alter the α-amylase activity in the leaves of seedlings grown under non-stress condition while it elevated the level under osmotic stress.

Osmotic stress significantly raised the level of lipid peroxidation measured as MDA content in the leaves of seedlings grown from non-primed seeds (Figure 4.23). Seed priming with 0.25 % and 0.50 % chitosan significantly reduced the level of MDA in leaves under non stress and osmotic stress conditions. Seed priming with 0.1 % chitosan
did not influence the leaf MDA content under non-stress as well as osmotic stress condition. Under osmotic stress seed priming with 0.25 % chitosan was successful in undoing the stress induced increase in MDA content and bring the level back to that in non-stress. Seed priming with 0.50 % chitosan further decreased the MDA level in leaves under osmotic stress. Hydropriming of seeds raised the MDA level in leaves under osmotic stress while it remained at the level of non-primed non-stress under non-stress condition. The level of reducing sugars increased significantly under osmotic stress in the leaves of seedling grown from non-primed seeds (Figure 4.25). Seed priming with all concentrations of chitosan significantly decreased the level of reducing sugars in leaves under non stress and osmotic stress conditions. Hydropriming of seeds raised the reducing sugars level in leaves under osmotic stress whereas it remained at the level of non-primed non-stress under non-stress condition.

On the other hand, level of non reducing sugars dropped significantly under osmotic stress in the leaves of seedling grown from non-primed seeds (Figure 4.25). Seed priming with varying concentrations of chitosan significantly raised the level of non reducing sugars in leaves under non stress as well as osmotic stress conditions. Hydropriming of seeds further decreased the non reducing sugars level in leaves under osmotic stress whereas the level remained same as in non-primed non-stress under non-stress condition.

Total sugars content in the leaves significantly increased under osmotic stress in non-primed non-stress seedlings (Figure 4.25). Seed priming with 0.1 % and 0.25 % chitosan significantly lower down the level of total sugars in leaves under non stress conditions. Here, seed priming with 0.1 % chitosan induced more decrease in total sugars as compared to 0.25 % treatment. However, under osmotic stress, only 0.25 % chitosan seed priming decreased the total sugars in the leaves. Hydropriming of seeds raised the level of total sugars in leaves under non-stress condition whereas it remained at the level of non-primed non-stress under osmotic stress.

Leaf relative water content significantly dropped under osmotic stress in seedlings grown from non-primed seeds (Figure 4.25). Seed priming with 0.10 % and 0.50 % chitosan significantly improved the leaf relative water content under non stress condition while all chitosan seed priming treatments improved this trait under osmotic stress. Seed priming
with 0.25 % and 0.50% chitosan caused more improvement in leaf relative water content under osmotic stress. Hydropriming of seeds improved the leaf relative water content under osmotic stress but could not affect it under non-stress condition. Leaf cell membrane stability was increased by all chitosan seed priming treatments providing evidence for lesser osmotic stress induced injury to membrane in seedlings grown from primed seeds (Figure 4.26). Hydropriming of seeds did not improve the cell membrane stability under osmotic stress.

![Graph showing peroxidase and catalase activities in leaves of wheat seedlings grown after chitosan seed priming under normal and osmotic stress conditions.](image)

Figure 4.20: Peroxidase and catalase activities in leaves of wheat seedlings grown after chitosan seed priming under normal and osmotic stress conditions.
Figure 4.21: Superoxide dismutase activity and total phenolics in leaves of wheat seedlings grown after chitosan seed priming under normal and osmotic stress conditions.
Figure 4.22: Total soluble proteins and protease activity in leaves of wheat seedlings grown after chitosan seed priming under normal and osmotic stress conditions.
Figure 4.23: The \( \alpha \)-amylase activity and MDA content in leaves of wheat seedlings grown after chitosan seed priming under normal and osmotic stress conditions.
Figure 4.24: Sugar contents in leaves of wheat seedlings grown after chitosan seed priming under normal and osmotic stress conditions.
Figure 4.25: Effect of chitosan seed priming on leaf relative water content under non-stress and osmotic stress conditions.

Figure 4.26: Effect of chitosan priming on leaf cell membrane stability (CMS).
4.2.2 Seed priming with sodium nitroprusside: effects on seed germination, seedling growth and physio-biochemical responses.

Seed primed with three varying levels of sodium nitroprusside (75, 100 and 125 µM) were germinated in petri plates under normal and osmotic stress (15 % PEG-6000) conditions. Here the results of seed germination, seedling growth and physio-biochemical responses of seedlings are discussed.

4.2.2.1 Germination and growth response

Final germination percentage did not differ under osmotic stress and non-stressed condition in case of non-primed seeds (Figure 4.27). Final germination percentage was significantly increased after SNP priming under osmotic stress. However, only 100 and 125 µM SNP priming improved the final germination percentage under non-stressed condition. Under osmotic stress, highest germination percentage (95 %) was observed after priming with 100 µM SNP. Hydropriming was not able to improve the final germination percentage under both non-stressed and osmotic stress conditions. For non-primed seeds, mean germination time (MGT) was significantly longer under osmotic stress as compared to non-stressed condition. However, seed priming with varying levels of SNP significantly reduced the MGT under non-stressed as well as osmotic stress conditions. Hydropriming of seeds reduced the MGT under osmotic stress but not under non-stressed condition.

The germination energy (%) significantly increased by 125 µM SNP priming under osmotic stress (Figure 4.28). However, the germination energy did not improve significantly by any other SNP priming treatment under osmotic stress as well as non-stress conditions. Hydropriming was unable to improve the germination energy under osmotic stress or non-stressed condition. In non-primed seeds, germination index was significantly reduced under osmotic stress as compared to non-stress condition. All SNP priming treatments significantly improved the germination index under non-stress as well as osmotic stress conditions. Seed priming with 125 µM SNP induced highest increase in the germination index under non-stress condition while 100 µM SNP induced highest increase in germination index under
osmotic stress. Hydropriming treatment was unable to improve the germination index under both non-stress and osmotic stress conditions.

For non-primed seeds, vigor index was not significantly changed under osmotic stress as compare to non-stress condition. Vigor index was significantly improved only after 75 and 100 µM SNP priming under non-stressed condition. While in case of osmotic stress condition, all SNP treatments were equally effective in improving the vigor index. Hydropriming was not able to improve the vigor index under non-stress and osmotic stress condition.

Seed priming with SNP enhanced the germination rate as compared to non-primed non-stress seeds (Figure 4.29). Under non-stress, seed priming with 125 µM SNP induced the highest enhancement in the seed germination rate. Under osmotic stress, 100 and 125 µM SNP treatments were equally effective in improving the seed germination rate. Seed priming with 75 µM SNP also improved the germination rate, however, degree of improvement was comparatively low as induced by higher concentrations. Hydropriming also improved the germination rate but to a lesser extent as compared to seed priming with SNP.

Seed priming with 75 µM SNP increased the shoot length of seedlings as compared to seedlings from non-primed seeds under non stress condition (Figure 4.30). Furthermore, all concentrations of SNP equally and significantly increased the shoot length under osmotic stress. However, root length of seedlings under non stress condition was significantly increased after all SNP priming treatments with maximum increase in case of 100 µM SNP priming. Similarly, it was also true for root length under osmotic stress which was increased by all SNP priming treatments Hydropriming of seeds could not improve the shoot length of seedlings under normal as well as osmotic stress conditions and root length of seedling was only somewhat improved under normal condition with no change in case of osmotic stress.
Figure 4.27: Effect of sodium nitroprusside seed priming on final germination % and mean germination time under non-stress and osmotic stress conditions.
Figure 4.28: Effect of sodium nitroprusside seed priming on germination energy, germination and vigor index of wheat seedlings grown under non-stress and osmotic stress conditions.
Figure 4.29: Effect of sodium nitroprusside seed priming on seed germination rate under non-stress and osmotic stress conditions.
Figure 4.30: Effect of sodium nitroprusside seed priming on shoot length and root length of wheat seedlings grown under non-stress and osmotic stress.
4.2.2.2 Physio-biochemical responses

For non-primed seeds, leaf peroxidase activity increased under osmotic stress as compared to non-stress condition (Figure 4.31). SNP priming significantly increased the peroxidase activity in leaves under non-stress condition. Under non-stress condition, increase in peroxidase activity by SNP priming increased with all concentration of SNP treatment, being the highest by 125 µM SNP treatment. SNP priming induced increase in peroxidase activity was many folds as compared to non primed seeds. However under osmotic stress, raised peroxidase activity in leaves were significantly decreased after SNP priming treatments which were reach up to the level of control seeds. Hydropriming also increased the leaf peroxidase activity under non-stress condition. However, hydropriming did not alter the peroxidase activity in the leaves of seedlings grown under osmotic stress. Osmotic stress significantly decreased the catalase activity in the leaves of seedlings grown from non-primed seeds (Figure 4.31). Under non stress condition, SNP priming with 75 µM SNP significantly increased the catalase activity in leaves as compared to all other levels that decreased the catalase activity as compared to non primed seeds. While under osmotic stress condition, only 125 µM SNP treatment significantly enhanced the catalase activity as compared to all other SNP treatment concentrations.

Under non-stress condition, decrease in catalase activity by SNP priming was higher by 100 and 125 µM SNP treatments. The level of decrease in catalase activity by SNP priming was comparatively less under stress as observed under non-stress condition. Hydropriming increased the catalase activity in the leaves of seedlings grown under non stress and osmotic stress conditions.

Superoxide dismutase activity was significantly increased as a result of osmotic stress in non primed seeds (Figure 4.32). Under non stress condition, all tested SNP priming treatments significantly enhanced the SOD activity. However under osmotic stress, increased SOD activity was significantly decreased after all SNP priming treatments even more than the observed SOD level in leaves under non stressed conditions. Hydropriming of seeds raised the leaf SOD activity but only under osmotic stress while did not change it under non-stress condition.
Osmotic stress significantly enhanced the level of total phenolics in the leaves of seedlings grown from non-primed seeds (Figure 4.32). SNP priming significantly increased the total phenolic contents of leaves under both non-stress and osmotic stress conditions. However, the level of increase in total phenolics varied for different concentrations of SNP. Under non-stress condition, most prominent effect was observed after 75 µM SNP treatment.

Under osmotic stress, 100 and 125 µM SNP treatment significantly increased the total phenolics with highest increase in total phenolics was observed after 100 µM SNP priming treatment. Hydropriming of seeds somewhat enhanced the level of phenolics in leaves under non-stress as well as osmotic stress condition.

Osmotic stress significantly elevated the total soluble proteins in the leaves of seedlings grown from non-primed seeds (Figure 4.33). Protein content was significantly increased by all SNP priming treatments under non-stress condition. While under osmotic stress, total soluble protein contents were decreased after SNP priming. Hydropriming of seeds also raised the level of total soluble proteins in leaves of seedlings under non-stress condition while it not significantly altered the protein contents under osmotic stress conditions.

The level of leaf protease activity in seedlings from non-primed seeds was not significantly altered by osmotic stress (Figure 4.33). Seed priming with varying levels of SNP significantly decreased the protease activity in leaves under non-stress and osmotic stress conditions. The magnitude of decrease in protease activity by SNP priming was more prominent under osmotic stress as compared to non-stress condition.

Highest decrease in leaf protease activity was induced by 125 µM SNP treatment in non-stress condition. While, seed priming with 75 µM SNP induced highest decrease in protease activity under osmotic stress. Hydropriming did not alter the level of protease activity in the leaves of seedlings under non stress or osmotic stress conditions.

For non-primed seeds, leaf α-amylase activity significantly increased under osmotic stress as compared to non-stress condition (Figure 4.34). SNP priming significantly decreased the leaf α-amylase activity under non-stress and osmotic stress conditions. Under non-stress as well as osmotic stress conditions, drop in leaf α-amylase activity by SNP priming increased with increasing concentration of treatment, being the highest by
125 μM SNP priming treatment. Hydropriming did not alter the α-amylase activity in the leaves of seedlings grown under non-stress as well as osmotic stress conditions. Osmotic stress significantly raised the level of lipid peroxidation measured as MDA content in the leaves of seedlings grown from non-primed seeds (Figure 4.34). Seed priming with all SNP priming treatments significantly reduced the level of MDA in leaves under non stress and osmotic stress conditions.

Under non-stress condition, seed priming with all SNP priming treatments equally influence the leaf MDA content. Under osmotic stress, MDA content many fold decreased after SNP priming with most prominent decrease was observed in case of 100 μM SNP priming. Hydropriming of seeds raised the MDA level in leaves under osmotic stress while it remained at the level of non-primed non-stress under non-stress condition.

The level of reducing sugars significantly raised under osmotic stress in the leaves of seedling grown from non-primed seeds (Figure 4.35). Seed priming with all concentrations of SNP significantly increased the level of reducing sugars in leaves under non stress and osmotic stress conditions with maximum increase was observed after 75 and 125 μM SNP treatment under osmotic stress. Hydropriming of seeds raised the reducing sugars level in leaves under osmotic stress whereas it remained at the level of non-primed non-stress under non-stress condition.

On the other hand, level of non reducing sugars dropped significantly under osmotic stress in the leaves of seedling grown from non-primed seeds (Figure 4.35). Seed priming with varying concentrations of SNP significantly decreased the level of non reducing sugars in leaves under non stress as well as osmotic stress conditions. Hydropriming of seeds further decreased the non reducing sugars level in leaves under osmotic stress whereas the level remained same as in non-primed non-stress under non-stress condition.

Total sugars content in the leaves significantly increased under osmotic stress in non-primed non-stress seedlings (Figure 4.35). Seed priming with SNP significantly lower down the level of total sugars in leaves under non stress as well as osmotic stress conditions. Seed priming with 75 μM SNP induced more decrease in total sugars as compared to all other treatments under non-stress conditions.
However, under osmotic stress, 125 µM SNP seed priming maximally decreased the total sugars in the leaves. Hydropriming of seeds did not affect the level of total sugars in leaves under both non-stress and osmotic stress conditions whereas it remained at the level of non-primed non-stress.

Leaf relative water content remained unchanged under osmotic stress in seedlings grown from non-primed seeds (Figure 4.36). Seed priming with all SNP priming treatments significantly improved the leaf relative water content under non stress condition and all SNP seed priming treatments improved this trait more significantly under osmotic stress as compared to non-stress conditions.

Seed priming with 100 µM SNP caused more improvement in leaf relative water content under osmotic stress as compared to all other priming treatments. Hydropriming of seeds could not affect the leaf relative water content under non-stress and osmotic stress conditions. Leaf cell membrane stability was increased by all SNP seed priming treatments providing evidence for lesser osmotic stress induced injury to membrane in seedlings grown from primed seeds (Figure 4.37). Hydropriming of seeds did not improve the cell membrane stability under osmotic stress.
Figure 4.31: Peroxidase and catalase activities in leaves of wheat seedlings grown after sodium nitroprusside seed priming under non-stress and osmotic stress conditions.
Figure 4.32: Superoxide dismutase activity and total phenolics in leaves of wheat seedlings grown after sodium nitroprusside seed priming under non-stress and osmotic stress conditions.
Figure 4.33: Total soluble proteins and protease activity in leaves of wheat seedlings grown after sodium nitroprusside seed priming under non-stress and osmotic stress conditions.
Figure 4.34: MDA content and α-amylase activity in leaves of wheat seedlings grown after sodium nitroprusside seed priming under non-stress and osmotic stress conditions.
Figure 4.35: Sugar contents in leaves of wheat seedlings grown after sodium nitroprusside seed priming under non-stress and osmotic stress conditions.
Figure 4.36: Effect of sodium nitroprusside seed priming on leaf relative water content under non-stress and osmotic stress conditions.

Figure 4.37: Effect of sodium nitroprusside seed priming on leaf cell membrane stability (CMS).
4.2.3 Seed priming with sodium silicate: effects on seed germination, seedling growth and physio-biochemical responses.

Seed primed with three varying levels of sodium silicate (20, 40 and 60 mM) were germinated in petri plates under normal and osmotic stress (15 % PEG-6000) conditions. Here the results of seed germination, seedling growth and physio-biochemical responses of seedlings are discussed.

4.2.3.1 Germination and growth response

For non-primed seeds, final germination percentage did not differ under osmotic stress and non-stressed condition (Figure 4.38). Final germination percentage was significantly increased after sodium silicate priming under osmotic stress. Sodium silicate priming with 40 and 60 mM significantly improved the final germination percentage under non-stressed condition. Under osmotic stress, highest germination percentage (97 %) was observed after priming with 40 mM sodium silicate. Hydropriming was not able to improve the final germination percentage under non-stressed and only somewhat improved under osmotic stress conditions. For non-primed seeds, mean germination time (MGT) was significantly longer under osmotic stress as compared to non-stressed condition (Figure 4.38). Seed priming with varying levels of sodium silicate significantly reduced the MGT under non-stressed and osmotic stress conditions with maximum drop in MGT was observed after 60 mM sodium silicate. Hydropriming of seeds reduced the MGT under osmotic stress but not under non-stressed condition.

The germination energy (%) significantly increased by 40 mM sodium silicate priming under osmotic stress (Figure 4.39). However, the germination energy did not improved significantly by any of other sodium silicate priming treatment under osmotic stress and non-stress conditions. Hydropriming was unable to improve the germination energy under osmotic stress or non-stressed condition.

The germination index for non-primed seeds was significantly reduced under osmotic stress as compared to non-stress condition (Figure 4.39). All sodium silicate priming treatments significantly improved the germination index under non-stress and osmotic stress conditions. Sodium silicate priming with increasing concentrations gradually improved the germination index under osmotic stress. Seed priming with 60 mM sodium
silicate induced highest increase in the germination index under non-stress as well as osmotic stress conditions. Hydropriming of seeds only improved the germination index under osmotic stress conditions.

For non-primed seeds, vigor index did not alter under osmotic stress as compared to non-stress condition (Figure 4.39). Priming with 40 and 60 mM sodium silicate priming significantly increased the vigor index under non-stressed condition. Moreover, all sodium silicate priming treatments significantly improved the vigor index under osmotic stress condition. Hydropriming was not able to improve the vigor index under non-stress and osmotic stress condition.

Seed priming with sodium silicate enhanced the germination rate as compared to non-primed non-stress seeds (Figure 4.40). Under non-stress condition, seed priming with 60 mM sodium silicate induced the highest improvement in the seed germination rate. Under osmotic stress, 40 mM sodium silicate priming was maximally effective in improving seed germination rate. Seed priming with 20 and 60 mM sodium silicate were also improved the germination rate, however, degree of improvement was comparatively low as by induced by 40 mM sodium silicate. Hydropriming also improved the germination rate but to a lesser extent as compared to seed priming with sodium silicate.

Seed priming with 20 mM sodium silicate only increased the shoot length of seedlings as compared to seedlings from non-primed seeds under osmotic stress, no other treatment show any significant affect on shoot length under both stressed and non-stress conditions (Figure 4.41). Root length was increased by 20 and 60 mM sodium silicate under non-stress condition while by all sodium silicate priming treatments under osmotic stress. Hydropriming of seeds could not improve the shoot length of seedling under normal as well as osmotic stress conditions. However, root length was little bit improved after hydropriming.
Figure 4.38: Effect of sodium silicate (SS) seed priming on final germination % and mean germination time (MGT) under non-stress and osmotic stress conditions.
Figure 4.39: Effect of sodium silicate (SS) seed priming on germination energy, germination and vigor index under non-stress and osmotic stress conditions.
Figure 4.40: Effect of sodium silicate (SS) seed priming on germination rate under non-stress and osmotic stress conditions.
Figure 4.41: Effect of sodium silicate (SS) seed priming on shoot length and root length of wheat seedlings grown under non-stress and osmotic stress conditions.
4.2.3.2 Physio-biochemical responses

For non-primed seeds, leaf peroxidase activity increased under osmotic stress as compared to non-stress condition (Figure 4.42). Sodium silicate priming by 20 and 60 mM significantly increased the peroxidase activity in leaves under non-stress condition and only by 60 mM sodium silicate under osmotic stress condition. Hydropriming also increased the leaf peroxidase activity under non-stress condition. However, hydropriming did not alter the peroxidase activity in the leaves of seedlings grown under osmotic stress. Osmotic stress significantly decreased the catalase activity in the leaves of seedlings grown from non-primed seeds (Figure 4.42). Sodium silicate priming significantly decreased the catalase activity in leaves under non-stress as well as osmotic stress conditions. Under non-stress condition, decrease in catalase activity by sodium silicate priming was higher by 40 mM sodium silicate treatment. The level of decrease in catalase activity by sodium silicate priming was comparatively less under non-stress condition as observed under osmotic stress condition. Hydropriming did not alter the catalase activity in the leaves of seedlings grown under non stress condition.

Superoxide dismutase activity was significantly increased as a result of osmotic stress (Figure 4.43). All tested treatment levels of sodium silicate reduce the SOD activity under non-stress condition and 20 and 60 mM sodium silicate priming treatments reduced the increased SOD activity under osmotic stress. Hydropriming of seeds did not change the SOD activity both under non-stress and osmotic stress conditions. Osmotic stress significantly enhanced the level of total phenolics in the leaves of seedlings grown from non-primed seeds (Figure 4.43). Sodium silicate priming significantly increased the total phenolic contents of leaves under both non-stress and osmotic stress conditions. However, the level of increase in total phenolics varied for different concentrations of sodium silicate. Under non-stress condition, most prominent effect was observed after 60 mM sodium silicate treatment while under osmotic stress, highest increase in total phenolics was induced by 20 mM sodium silicate priming treatment. Hydropriming of seeds enhanced the level of phenolics in leaves under non-stress condition. Yet, hydropriming did not change the level of total phenolics in leaves under osmotic stress.

Osmotic stress significantly elevated the total soluble proteins in the leaves of seedlings grown from non-primed seeds (Figure 4.44). Protein content was significantly increased
by 40 and 60 mM sodium silicate priming under non-stress and only by 60 mM sodium silicate priming under osmotic stress. Hydropriming of seeds also raised the level total soluble proteins in leaves of seedlings under both non-stress as well as osmotic stress conditions. The level of leaf protease activity in seedlings from non-primed seeds was not altered by osmotic stress (Figure 4.44). Seed priming with varying levels of sodium silicate significantly decreased the protease activity in leaves under non-stress and osmotic stress conditions except for 60mM sodium silicate priming that significantly increased the protease activity under osmotic stress. Hydropriming did not alter the level of protease activity in the leaves of seedlings under non stress condition.

For non-primed seeds, leaf α-amylase activity increased under osmotic stress as compared to non-stress condition (Figure 4.45). Sodium silicate priming significantly decreased the leaf α-amylase activity under non-stress and osmotic stress conditions. Under non-stress as well as osmotic stress conditions, drop in leaf α-amylase activity by sodium silicate priming increased with increasing concentration of treatment, being the highest by 60 mM sodium silicate treatment. Hydropriming did not alter the α-amylase activity in the leaves of seedlings grown under non-stress condition while it elevated the level under osmotic stress.

Osmotic stress significantly raised the MDA content the product of lipid peroxidation measured in the leaves of seedlings grown from non-primed seeds (Figure 4.45). All sodium silicate priming treatments reduce the MDA content under both non-stress and osmotic stress conditions. Seed priming with 40 and 60 mM sodium silicate more prominently reduced the level of MDA in leaves under non stress condition as compared to 20 mM priming concentration. Under osmotic stress all concentrations of sodium silicate were effective to reduce the MDA content with most prominent decrease in case of 20 mM sodium silicate priming. Hydropriming of seeds raised the MDA level in leaves under osmotic stress while it remained at the level of non-primed non-stress under non-stress condition.

The level of reducing sugars increased significantly under osmotic stress in the leaves of seedling grown from non-primed seeds (Figure 4.46). Seed priming with 40 and 60 mM concentrations of sodium silicate significantly increased the level of reducing sugars in leaves under non stress and osmotic stress conditions with maximum increase was
observed by 40 mM sodium silicate priming under stress. Hydropriming of seeds raised the reducing sugars level in leaves under non stress whereas it remained at the level of non-primed non-stress under osmotic stress condition. On the other hand, level of non reducing sugars dropped significantly under osmotic stress in the leaves of seedling grown from non-primed seeds (Figure 4.46). Seed priming with 40 and 60 mM concentrations of sodium silicate significantly reduced the level of non reducing sugars in leaves under non stress condition. While 20 mM sodium silicate priming more prominently reduce the non-reducing sugars under osmotic stress. Hydropriming of seeds further decreased the non reducing sugars level in leaves under osmotic stress as well as non-stress condition.

Total sugars content in the leaves significantly increased under osmotic stress in non-primed non-stress seedlings (Figure 4.46). Seed priming with all tested concentrations of sodium silicate significantly lower down the level of total sugars in leaves under non stress as well as osmotic stress conditions. Seed priming with 40 mM sodium silicate induced more decrease in total sugars as compared all other treatments in leaves under both stressed and non-stress conditions. Hydropriming did not alter the total sugars contents; it remained same as that of non-primed under non stress as well as osmotic stress conditions.

Leaf relative water content significantly dropped under osmotic stress in seedlings grown from non-primed seeds (Figure 4.47). Seed priming with all sodium silicate concentrations significantly improved the leaf relative water content under non stress and osmotic stress conditions. Sodium silicate priming with increasing concentrations more significantly improved the leaf relative water content under non stress as well as osmotic stress conditions. Hydropriming of seeds improved the leaf relative water content under osmotic stress but could not affect it under non-stress condition. Leaf cell membrane stability was increased by all sodium silicate seed priming treatments providing evidence for lesser osmotic stress induced injury to membrane in seedlings grown from primed seeds (Figure 4.48). Hydropriming of seeds also improve the cell membrane stability under osmotic stress.
Figure 4.42: Peroxidase and catalase activities in leaves of wheat seedlings grown after sodium silicate (SS) seed priming under non-stress and osmotic stress conditions.
Figure 4.43: Superoxide dismutase activity and total phenolics in leaves of wheat seedlings grown after sodium silicate (SS) seed priming under non-stress and osmotic stress conditions.
Figure 4.44: Total soluble proteins and protease activity in leaves of wheat seedlings grown after sodium silicate (SS) seed priming under non-stress and osmotic stress conditions.
Figure 4.45: MDA content and α-amylase activity in leaves of wheat seedlings grown after sodium silicate (SS) seed priming under non-stress and osmotic stress conditions.
Figure 4.46: Sugar contents in leaves of wheat seedlings grown after sodium silicate (SS) seed priming under non-stress and osmotic stress conditions.
Figure 4.47: effect of sodium silicate (SS) seed priming on leaf relative water content of wheat seedlings grown after under non-stress and osmotic stress conditions.

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<td>40 mM SS</td>
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<td>60 mM SS</td>
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Figure 4.48: Effect of sodium silicate seed priming on cell membrane stability under osmotic stress.

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<th>CMS (%)</th>
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4.3 Experiment 3: Improvement of drought tolerance in wheat by seed priming

In these experiments, effects of seed priming with chitosan, sodium nitroprusside and sodium silicate on biochemical, physiological yield attributes of plants under normal (with out stress) and drought stress were investigated. The results from these experiments are as following

4.3.1 Seed priming with chitosan: Effects on biochemical, physiological and yield attributes.

Seed primed with three varying levels of chitosan (0.1, 0.25 and 0.5%) were germinated in pots under normal and drought stress conditions. Here the results of biochemical, physiological and yield responses of wheat plants are discussed.

4.3.1.1 Biochemical response in flag leaves

For non-primed seeds, leaf peroxidase activity increased under drought stress as compared to non-stress condition (Figure 4.49). Chitosan priming significantly increased the peroxidase activity in flag leaves under non-stress and drought stress conditions. Under non-stress condition, increase in peroxidase activity by chitosan priming increased with increasing concentration of treatment, being the highest by 0.5 % chitosan treatment. Chitosan priming induced increase in peroxidase activity was many folds as compared to non primed seeds.

Under drought stress, chitosan priming also gradually raised the peroxidase activity in flag leaves. The magnitude of increase in peroxidase activity by chitosan priming was comparatively high under stress as observed under non-stress condition with maximum increase was observed in case of 0.50 % chitosan priming. Drought stress showed no significant effect on catalase activity in the flag leaves of wheat plants grown from non-primed seeds (Figure 4.49). Chitosan priming significantly increased the catalase activity in leaves under non-stress as well as drought stress conditions.

Under non-stress condition, increase in catalase activity by chitosan priming was increased with increasing concentrations of chitosan treatments. Similarly, under drought stress, catalase activity was gradually increased by chitosan priming. However, increase
in the level of catalase activity was comparatively less under stress as observed under non-stress condition.

Superoxide dismutase activity was significantly decreased under drought stress in flag leaves of wheat plants grown from non-primed seeds (Figure 4.50). All tested chitosan priming treatments reduced the SOD activity under non-stress and drought stress conditions. Under both non stress and drought stress conditions, the SOD activity was gradually decreased with increasing concentrations of chitosan priming.

Drought stress significantly enhanced the level of total phenolics in the leaves of wheat plants grown from non-primed seeds (Figure 4.50). Chitosan priming significantly decreased the total phenolic contents of leaves under both non-stress and drought stress conditions. Similar trend was seen in level of total phenolics as that of superoxide dismutase activity. Most prominent effect was observed after 0.5% chitosan treatment under both non-stress and drought stress conditions.

Drought stress significantly elevated the total soluble proteins in the leaves of wheat plants grown from non-primed seeds (Figure 4.51). Protein content was significantly increased by chitosan priming under non-stress and drought stress with more prominent increase under drought stress conditions. Maximum increase in total soluble proteins was observed after 0.50% chitosan priming under drought stress.

The level of leaf protease activity in wheat plants from non-primed seeds was significantly decreased by drought stress (Figure 4.51). Seed priming with varying levels of chitosan significantly decreased the protease activity in leaves under non-stress and drought stress conditions. Decrease in protease activity by chitosan priming increased with increasing concentration of treatments under drought stress. Thus, the highest decrease in leaf protease activity was induced by 0.5% chitosan treatment.

For non-primed seeds, leaf α-amylase activity increased under drought stress as compared to non-stress condition (Figure 4.52). Chitosan priming significantly decreased the leaf α-amylase activity under non-stress and drought stress conditions. Under non-stress as well as drought stress conditions, drop in flag leaf α-amylase activity by chitosan priming increased with increasing concentration of treatment, being the highest by 0.5% chitosan treatment. MDA content the product of lipid peroxidation was not significantly affected by Drought stress in the leaves of wheat plants grown from non-primed seeds (Figure 4.52).
Seed priming with 0.10 % and 0.25 % chitosan equally reduced the level of MDA in flag leaves and 0.50 % chitosan priming further reduced the MDA level in flag leaves under non stress conditions. Under drought stress conditions, chitosan priming gradually decreased the MDA level in flag leaves. Decrease in lipid peroxidation product the MDA contents was more prominent under drought stress condition as compared to non- stress condition.

The level of reducing sugars increased significantly under drought stress in the flag leaves of wheat plants grown from non-primed seeds (Figure 4.53). Seed priming with chitosan gradually decreased the level of reducing sugars in flag leaves under non stress and drought stress conditions. On the other hand, level of non reducing sugars dropped significantly under drought stress in the leaves of wheat plants grown from non-primed seeds (Figure 4.53). Seed priming with 0.50 % chitosan significantly raised the level of non reducing sugars in flag leaves under non stress condition.

Under drought stress conditions, non reducing sugars were gradually increased after chitosan priming. Total sugars content in the flag leaves were not significantly changed under drought stress in non-primed non-stress wheat plants (Figure 4.53). Seed priming with chitosan was not resulted in any change in the level of total sugars in flag leaves under both non stress and drought stress conditions.

Drought stress significantly elevated the proline contents in the flag leaves of wheat plants grown from non-primed seeds (Figure 4.54). Proline content was significantly decreased by chitosan priming under non-stress and drought stress with more prominent decrease under drought stress conditions. Maximum decrease in proline content was observed after 0.50 % chitosan priming under non stress and drought stress conditions.

The level of flag leaf glycine betaine content in wheat plants from non-primed seeds was significantly decreased by drought stress (Figure 4.54).

Seed priming with varying levels of chitosan significantly increased the glycine betaine content in leaves under non-stress and drought stress conditions. Increase in glycine betaine content by chitosan priming increased with increasing concentration of treatments under drought stress. Thus, the highest increase in glycine betaine content was induced by 0.5 % chitosan treatment.
The level of chlorophyll a increased significantly under drought stress in the flag leaves of wheat plants grown from non-primed seeds (Figure 4.55). Under non-stress condition, seed priming with 0.50 % chitosan only increased the chlorophyll a content. While, all other chitosan priming treatments reduced the leaf chlorophyll a contents. Only 0.10 % chitosan priming decreased the leaf chlorophyll a contents in flag leaves under drought stress conditions. All other treatments show no significant effect on leaf chlorophyll a contents under drought stress.

On the other hand, level of chlorophyll b contents dropped significantly under drought stress in the leaves of wheat plants grown from non-primed seeds(Figure 4.55). Seed priming with 0.25 % chitosan significantly raised the level of chlorophyll b contents in flag leaves under non-stress condition. Under drought stress conditions, chlorophyll b contents were significantly decreased after chitosan priming.

Total chlorophyll contents in the flag leaves were significantly decreased under drought stress in non-primed non-stress wheat plants (Figure 4.55). Seed priming with 0.25 % chitosan was significantly increased the total chlorophyll contents in flag leaves under non-stress conditions as compared to all other treatments that decreased the total chlorophyll contents. Under drought stress conditions, total chlorophyll contents were significantly decreased after chitosan priming.

Drought stress significantly reduced the total carotenoids in the flag leaves of wheat plants grown from non-primed seeds (Figure 4.55). Priming with 0.25 and 0.50 % chitosan priming significantly increased the total carotenoids in flag leaves under non-stress and drought stress conditions with more prominent increase by 0.50 % chitosan priming under drought stress.

**4.3.1.2 Physiological response in flag leaves**

Leaf water potential was significantly increased under drought stress in wheat plants grown from non-primed seeds (Figure 4.56). Seed priming with all concentrations of chitosan gradually decreased the leaf water potential under non-stress and drought stress conditions. Leaf osmotic potential was significantly increased under drought stress in wheat plants grown from non-primed seeds (Figure 4.56).
Under non-stress conditions, chitosan priming not show any significant affect on leaf osmotic potential. However, it was gradually decreased after chitosan priming under drought stress. Leaf turgor potential was significantly increased under drought stress in wheat plants grown from non-primed seeds (Figure 4.56). Chitosan priming significantly improved the leaf turgor potential under both non stress and drought stress conditions. Leaf cell membrane stability was gradually increased by chitosan seed priming treatments providing evidence for lesser drought stress induced injury to membrane in wheat plants grown from primed seeds (Figure 4.57).

4.3.1.3 Yield attributes

The grain yield was not significantly affected under drought stress in wheat plants grown from non-primed seeds (Figure 4.58). Seed priming with chitosan gradually increased the grain yield under non stress and drought stress conditions. 0.25 % and 0.50 % chitosan seed priming equally enhanced the grain yield of wheat grown under normal and drought stress conditions. On the other hand, 100 grains weight was significantly decreased under drought stress in wheat plants grown from non-primed seeds (Figure 4.58).

Under non stress conditions, 100 grains weight was only increased by 0.50 % chitosan treated wheat plants. Any other chitosan priming treatment show no significant effect on 100 grains weight. Under drought stress conditions, 100 grains weight of wheat plants were equally increased after all chitosan priming treatments. Plant biomass was not significantly affected under drought stress in wheat plants grown from non-primed seeds (Figure 4.58). All chitosan priming treatments abruptly increased the plant biomass under both non-stress and drought stress conditions.
Figure 4.49: Peroxidase and catalase activities in flag leaf of wheat plants grown after chitosan seed priming under non-stress and drought stress conditions.
Figure 4.50: Superoxide dismutase activity and total phenolics in flag leaf of wheat plants grown after chitosan seed priming under non-stress and drought stress conditions.
Figure 4.51: Total soluble proteins and protease activity in flag leaf of wheat plants grown after chitosan seed priming under non-stress and drought stress conditions.
Figure 4.52: MDA content and α-amylase activity in flag leaf of wheat plants grown after chitosan seed priming under non-stress and drought stress conditions.
Figure 4.53: Sugar contents in flag leaf of wheat plants grown after chitosan seed priming under non-stress and drought stress conditions.
Figure 4.54: Proline and glycine betaine in flag leaf of wheat plants grown after chitosan seed priming under non-stress and drought stress conditions.
Figure 4.55: Chlorophyll contents and total carotenoids in flag leaf of wheat plants grown after chitosan seed priming under non-stress and drought stress conditions.
Figure 4.56: Water potential, osmotic potential and Turgor potential in flag leaf of wheat plants grown after chitosan seed priming under non-stress and drought stress conditions.
Figure 4.57: Effect of chitosan seed priming on cell membrane stability under drought stress.
Figure 4.58: Effects of chitosan seed priming on Yield, 100 grains weight and plant biomass of wheat plants grown under non-stress and drought stress conditions.
4.3.2 Seed priming with SNP: Effects on biochemical, physiological and yield attributes.

Seed primed with three varying levels of SNP (75, 100 and 125 µM) were germinated in pots under normal and drought stress conditions. Here the results of biochemical, physiological responses and yield attributes of wheat plants are discussed.

4.3.2.1 Biochemical response in flag leaves

For non-primed seeds, leaf peroxidase activity increased under drought stress as compared to non-stress condition (Figure 4.59). SNP priming significantly increased the peroxidase activity in leaves under non-stress as well as drought stress conditions. SNP priming induced increase in peroxidase activity was many folds as compared to non primed seeds. Increase in peroxidase activity by SNP priming increased with all concentration of SNP treatment, being the highest by 125 µM SNP priming treatment under non-stress and drought stress conditions. Drought stress significantly decreased the catalase activity in the leaves of wheat plants grown from non-primed seeds (Figure 4.59). Under non stress condition, SNP priming with 100 and 125 µM SNP significantly increased the catalase activity in leaves as compared to control and 75 µM SNP priming treatment. Maximum increase in catalase activity was observed after 125 µM SNP priming under non-stress condition. While under drought stress condition, 75 and 100 µM SNP were significantly and equally increased the catalase activity. 125 µM SNP priming treatment further significantly enhanced the catalase activity under stress. Superoxide dismutase activity was significantly increased as a result of drought stress in non primed seeds (Figure 4.60). Under non stress condition, 100 µM SNP priming treatment increase the SOD activity and all other tested SNP priming treatments significantly reduced the SOD activity. However under drought stress, SOD activity was significantly increased after all SNP priming treatments even more than the observed SOD level in leaves of wheat plants grown under non-stress condition. Drought stress significantly enhanced the level of total phenolics in the leaves of wheat plants grown from non-primed seeds (Figure 4.60). SNP priming with 75 µM concentration only significantly increased the total phenolic contents of leaves under non-stress condition. Under drought stress, 100 and 125 µM SNP treatment significantly increased the total
phenolics with highest increase in total phenolics was observed after 100 µM SNP priming treatment.

Drought stress significantly reduced the total soluble proteins in the leaves of wheat plants grown from non-primed seeds (Figure 4.61). Protein content was significantly increased by all SNP priming treatments under non-stress condition. Under drought stress, total soluble protein contents were gradually increased after SNP priming. The level of leaf protease activity in wheat plants from non-primed seeds was significantly decreased by drought stress (Figure 4.61). Seed priming with increasing levels of SNP significantly increased the protease activity in leaves under non-stress and drought stress conditions. The magnitude of increase in protease activity by SNP priming was more prominent under drought stress as compared to non-stress condition. Highest increase in leaf protease activity was induced by 125 µM SNP treatment in drought stress condition.

For non-primed seeds, leaf α-amylase activity was significantly decreased under drought stress as compared to non-stress condition (Figure 4.62). SNP priming significantly increased the leaf α-amylase activity under non-stress and drought stress conditions. Under non-stress as well as drought stress conditions, increase in leaf α-amylase activity by SNP priming increased with increasing concentration of treatment, being the highest by 125 µM SNP priming treatment.

Drought stress significantly raised the level of lipid peroxidation measured as MDA content in the leaves of wheat plants grown from non-primed seeds (Figure 4.62). Seed priming with all SNP priming treatments significantly reduced the level of MDA in leaves under non stress and drought stress conditions. Under non-stress condition, seed priming with 125 µM SNP more prominently reduced the leaf MDA content. Under drought stress, MDA content the product of lipid peroxidation many fold decreased after all SNP priming treatments.

The level of reducing sugars was significantly raised under drought stress in the leaves of wheat plants grown from non-primed seeds (Figure 4.63). Seed priming with all concentrations of SNP significantly increased the level of reducing sugars in leaves under non stress and drought stress conditions with maximum increase was observed after 125 µM SNP priming treatment under drought stress. On the other hand, level of non reducing sugars increased significantly under drought stress in the leaves of wheat plants.
grown from non-primed seeds (Figure 4.63). Seed priming with increasing concentrations of SNP significantly decreased the level of non reducing sugars in leaves under non stress as well as drought stress conditions. SNP priming abruptly decreased the level of non- reducing sugars under non-stress condition more prominently as under drought stress. Total sugars content in the leaves significantly increased under drought stress in non-primed non-stress wheat plants (Figure 4.63). Seed priming with SNP significantly enhanced the level of total sugars in leaves under non stress as well as osmotic stress conditions. Seed priming with 100 µM SNP induced more prominent enhancement in total sugars as compared to all other treatments under non-stress and drought stress conditions.

Drought stress significantly elevated the proline contents in the flag leaves of wheat plants grown from non-primed seeds (Figure 4.64). Proline content was gradually decreased by SNP priming under non-stress and drought stress conditions. Maximum decrease in proline content was observed after 125 µM SNP priming under non stress and drought stress conditions. The level of flag leaf glycine betaine content in wheat plants from non-primed seeds was significantly increased by drought stress (Figure 4.64). Seed priming with varying levels of SNP significantly increased the glycine betaine content in leaves under non-stress and drought stress conditions. Increase in glycine betaine content by SNP priming increased with increasing concentration of treatments under non-stress and drought stress condition. Thus, the highest increase in glycine betaine content was induced by 125 µM SNP treatment.

The level of chlorophyll a decreased significantly under drought stress in the flag leaves of wheat plants grown from non-primed seeds (Figure 4.65). Under non-stress condition, seed priming with 125 µM SNP only increased the chlorophyll a contents under non-stress and drought stress conditions. On the other hand, level of chlorophyll b contents not altered under drought stress in the leaves of wheat plants grown from non-primed seeds (Figure 4.65). Seed priming with SNP significantly lower down the level of chlorophyll b contents in flag leaves under non-stress and drought stress conditions. Total chlorophyll contents in the flag leaves were significantly decreased under drought stress in non-primed non-stress wheat plants (Figure 4.65). Seed priming with varying levels of SNP significantly reduced the total chlorophyll contents in flag leaves under non-stress as
well as drought stress conditions. Drought stress significantly reduced the total carotenoids in the flag leaves of wheat plants grown from non-primed seeds (Figure 4.65). Priming with 100 µM SNP only improved the total carotenoids in flag leaves under drought stress.

4.3.2.2 Physiological response in flag leaves

Leaf water potential was significantly increased under drought stress in wheat plants grown from non-primed seeds (Figure 4.66). Seed priming with all concentrations of SNP significantly decreased the leaf water potential under non-stress and drought stress conditions with highest decrease in case of 125 µM SNP priming. Leaf osmotic potential was significantly increased under drought stress in wheat plants grown from non-primed seeds (Figure 4.66). Under non-stress conditions, SNP priming significantly increased the leaf osmotic potential. However, increased osmotic potential under drought stress was gradually decreased after SNP priming. Leaf turgor potential was significantly decreased under drought stress in wheat plants grown from non-primed seeds (Figure 4.66). SNP priming significantly improved the leaf turgor potential under both non-stress and drought stress conditions. Increase was more prominent under non-stress than drought stress conditions. Leaf cell membrane stability was significantly increased by SNP seed priming treatments with highest increase was observed after 75 µM SNP priming providing evidence for lesser drought stress induced injury to membrane in wheat plants grown from primed seeds (Figure 4.67).

4.3.2.3 Yield attributes

The grain yield was significantly decreased under drought stress in wheat plants grown from non-primed seeds (Figure 4.68). Seed priming with all SNP concentrations equally increased the grain yield under non-stress and drought stress conditions. SNP priming many fold enhanced the level of grain yield in wheat plants as compared to non-primed non stressed wheat plants. Similarly, 100 grains weight was significantly decreased under drought stress in wheat plants grown from non-primed seeds (Figure 4.68). 100 grains weight was gradually increased by all tested SNP concentrations under non-stress as well as drought stress conditions, being highest increase was observed after 125 µM SNP priming. Plant biomass was significantly decreased under drought stress in wheat plants
grown from non-primed seeds (Figure 4.68). All SNP priming treatments significantly increased the plant biomass under both non-stress and drought stress conditions. Under non stress condition, effect of SNP priming with varying concentrations was same, so all these treatments equally enhanced the plant biomass. SNP priming significantly increased the plant biomass with increasing concentrations and 125 µM SNP priming show highest increase in plant biomass.

Figure 4.59: Peroxidase and catalase activities in flag leaf of wheat plants grown after SNP seed priming under non-stress and drought stress conditions.
Figure 4.60: Superoxide dismutase activity and total phenolics in flag leaf of wheat plants grown after SNP seed priming under non-stress and drought stress conditions.
Figure 4.61: Total soluble proteins and protease activity in flag leaf of wheat plants grown after SNP seed priming under non-stress and drought stress conditions.
Figure 4.62: MDA content and α-amylase activity in flag leaf of wheat plants grown after SNP seed priming under non-stress and drought stress conditions.
Figure 4.63: Sugar contents in flag leaf of wheat plants grown after SNP seed priming under non-stress and drought stress conditions.
Figure 4.64: Proline and glycine betaine in flag leaf of wheat plants grown after SNP seed priming under non-stress and drought stress conditions.
Figure 4.65: Chlorophyll contents and total carotenoids in flag leaf of wheat plants grown after SNP seed priming under non-stress and drought stress conditions.
Figure 4.66: Water potential, osmotic potential and Turgor potential in flag leaf of wheat plants grown after SNP seed priming under non-stress and drought stress conditions.
Figure 4.67: Effect of SNP seed priming on cell membrane stability under drought stress.
Figure 4.68: Effects of sodium nitroprusside (SNP) seed priming on Yield, 100 grains weight and plant biomass of wheat plants grown under non-stress and drought stress conditions.
4.3.3 Seed priming with Sodium Silicate: Effects on biochemical, physiological and yield attributes.

Seed primed with three varying levels of sodium silicate (20, 40 and 60 mM) were germinated in pots under normal and drought stress conditions. Here the results of biochemical, physiological responses and yield attributes of wheat plants are discussed.

4.3.3.1 Biochemical response in flag leaves

For non-primed seeds, leaf peroxidase activity decreased under drought stress as compared to non-stress condition (Figure 4.69). Sodium silicate priming gradually increased the peroxidase activity in leaves under non-stress and drought stress conditions. 60 mM sodium silicate priming maximally increased the leaf peroxidase activity under non-stress and drought stress conditions.

Drought stress showed no profound effect on catalase activity in the leaves of wheat plants grown from non-primed seeds (Figure 4.69). Sodium silicate priming significantly increased the catalase activity with increasing concentrations in leaves under non-stress as well as drought stress conditions. The level of increase in catalase activity by sodium silicate priming was comparatively high under non-stress condition as observed under drought stress condition.

Superoxide dismutase activity was significantly decreased as a result of drought stress (Figure 4.70). Only 40 mM sodium silicate priming under non-stress condition increased the SOD activity. Under drought stress, 20 mM sodium silicate priming significantly enhanced the SOD activity. SOD activity significantly dropped by all other sodium silicate priming treatments under both non-stress and drought stress conditions. Drought stress significantly enhanced the level of total phenolics in the leaves of wheat plants grown from non-primed seeds (Figure 4.70). Sodium silicate priming significantly increased the total phenolic contents of leaves under non-stress condition by all priming concentrations and by 20 and 60 mM sodium silicate priming under drought stress condition. Under non-stress condition, most prominent effect was observed after 60 mM sodium silicate treatment while under drought stress, highest increase in total phenolics was induced by 20 mM sodium silicate priming treatment.
Drought stress significantly declined the total soluble proteins in the leaves of wheat plants grown from non-primed seeds (Figure 4.71). Protein content was gradually increased by all sodium silicate priming treatments under non-stress and drought stress conditions with maximum increase by 60 mM sodium silicate. The level of leaf protease activity in wheat plants from non-primed seeds was not significantly altered by drought stress (Figure 4.71). Seed priming with varying levels of sodium silicate significantly increased the protease activity in leaves under non-stress and drought stress conditions. Under non-stress condition, sodium silicate priming resulted in gradual increase in protease activity with maximum increase after 60 mM sodium silicate treatment. Under drought stress, 20 and 60 mM showed comparatively more increase in protease activity as that of 40 mM sodium silicate priming increase in leaf protease activity.

For non-primed seeds, leaf α-amylase activity decreased under drought stress as compared to non-stress condition (Figure 4.72). Sodium silicate priming significantly increased the leaf α-amylase activity under non-stress and drought stress conditions. Under non-stress as well as drought stress conditions, increase in leaf α-amylase activity by sodium silicate priming increased with increasing concentration of treatment, being the highest by 60 mM sodium silicate treatment. Drought stress significantly raised the MDA content the product of lipid peroxidation measured in the leaves of wheat plants grown from non-primed seeds to many fold (Figure 4.72). All sodium silicate priming treatments reduce the MDA content under both non-stress and drought stress conditions. Seed priming with increasing concentrations of sodium silicate more prominently reduced the level of MDA in leaves under non-stress condition. Under drought stress all concentrations of sodium silicate were effective to reduce the MDA content with most prominent decrease in case of 60 mM sodium silicate priming. Decrease in MDA content by sodium silicate priming was more prominent under drought stress as compared to non-stress conditions.

The level of reducing sugars many fold decreased under drought stress in the leaves of wheat plants grown from non-primed seeds (Figure 4.73). Seed priming with 20 and 60 mM concentrations of sodium silicate significantly increased the level of reducing sugars in leaves under non-stress conditions. 40 mM sodium silicate priming even showed further increase in level of reducing sugars contents. Under drought stress, level of
reducing sugars was gradually increased with varying concentrations of sodium silicate priming. On the other hand, level of non reducing sugars many fold increased under drought stress in the leaves of wheat plants grown from non-primed seeds (Figure 4.73). Seed priming with 20 and 60 mM concentrations of sodium silicate with equal effect significantly reduced the level of non reducing sugars in leaves under non stress condition and 40 mM sodium silicate priming further decrease the level of non-reducing sugars. Under drought stress, increased level of non reducing sugars in non-primed seeds was significantly reduced by all sodium silicate priming treatments.

Total sugars content in the leaves significantly increased under drought stress in non-primed non-stress wheat plants (Figure 4.73). Seed priming with 60 mM sodium silicate priming increased the total sugars level, all other treatments showed no significant effect on total sugars level under non-stress conditions. Under drought stress 40 and 60 mM sodium silicate treatment concentrations induced the significant increase in the level of total sugars. Seed priming with 60 mM sodium silicate induced maximum increase in total sugars as compared all other treatments in leaves under both stressed and non-stress conditions.

Drought stress significantly elevated the proline contents in the flag leaves of wheat plants grown from non-primed seeds (Figure 4.74). Proline content was gradually decreased by sodium silicate priming under non-stress and drought stress conditions. Maximum decrease in proline content was observed after 60 mM sodium silicate priming under non stress and drought stress conditions. The level of glycine betaine content in flag leaves of wheat plants grown from non-primed seeds was significantly decreased under drought stress (Figure 4.74). Seed priming with varying levels of sodium silicate significantly increased the glycine betaine content in leaves under non-stress and drought stress conditions. Increase in glycine betaine content by sodium silicate priming increased with increasing concentration of treatments under both non-stress and drought stress conditions. Thus, the highest increase in glycine betaine content was induced by 60 mM sodium silicate treatment. The level of chlorophyll a remained unchanged under drought stress in the flag leaves of wheat plants grown from non-primed seeds (Figure 4.75). Under non-stress condition, seed priming with 40 mM sodium silicate only increased the chlorophyll a contents. Under drought stress conditions, 60 mM sodium silicate priming
only increased the chlorophyll a contents. On the other hand, level of chlorophyll b contents significantly increased under drought stress in the leaves of wheat plants grown from non-primed seeds (Figure 4.75). Seed priming with sodium silicate significantly lower down the level of chlorophyll b contents in leaves under non-stress and drought stress conditions. Total chlorophyll contents in the flag leaves were significantly increased under drought stress in non-primed non-stress wheat plants (Figure 4.75). Seed priming with varying levels of sodium silicate significantly reduced the total chlorophyll contents in flag leaves under non-stress condition and only 60 mM sodium silicate significantly increased the total chlorophyll contents under drought stress conditions. Drought stress significantly enhanced the total carotenoids in the flag leaves of wheat plants grown from non-primed seeds (Figure 4.75). Priming with 60 mM sodium silicate priming only improved the total carotenoids in flag leaves under drought stress.

4.3.3.2 Physiological response in flag leaves

Leaf water potential was significantly increased under drought stress in wheat plants grown from non-primed seeds (Figure 4.76). Seed priming with all concentrations of sodium silicate significantly decreased the leaf water potential under non-stress and drought stress conditions with highest decrease in case of 60 mM sodium silicate priming. Leaf osmotic potential was significantly increased under drought stress in wheat plants grown from non-primed seeds (Figure 4.76). Under non-stress conditions, 40 and 60 mM sodium silicate priming significantly increased the leaf osmotic potential. However, increased osmotic potential under drought stress was gradually decreased after sodium silicate priming. Leaf turgor potential was significantly decreased under drought stress in wheat plants grown from non-primed seeds (Figure 4.76). Sodium silicate priming significantly improved the leaf turgor potential under both non-stress and drought stress conditions. Increase was more prominent under non-stress than drought stress conditions. Leaf cell membrane stability was significantly increased by sodium silicate seed priming treatments with highest increase was observed after 60 mM sodium silicate priming providing evidence for lesser drought stress induced injury to membrane in wheat plants grown from primed seeds (Figure 4.77).
4.3.3.3 Yield attributes

The grain yield remained unchanged under drought stress in wheat plants grown from non-primed seeds (Figure 4.78). Seed priming with all sodium silicate concentrations increased the grain yield under non stress and drought stress conditions. 60 mM sodium silicate priming maximally enhanced the level of grain yield in wheat plants as compared to non-primed non stressed wheat plants. Similarly, 100 grains weight was significantly decreased under drought stress in wheat plants grown from non-primed seeds (Figure 4.78). 100 grains weight was only increased by 20 mM sodium silicate priming under non-stress as well as drought stress conditions, Plant biomass was significantly decreased under drought stress in wheat plants grown from non-primed seeds (Figure 4.78). All sodium silicate priming treatments significantly increased the plant biomass under both non-stress and drought stress conditions. Under non stress condition, effect of 60 mM sodium silicate priming was more profound as compared to any other treatments for enhancement of plant biomass. Under drought stress, sodium silicate priming significantly increased the plant biomass with 60 mM sodium silicate priming show highest increase in plant biomass.
Figure 4.69: Peroxidase and catalase activities in flag leaf of wheat plants grown after sodium silicate seed priming under non-stress and drought stress conditions.
Figure 4.70: Superoxide dismutase activity and total phenolics in flag leaf of wheat plants grown after sodium silicate seed priming under non-stress and drought stress conditions.
Figure 4.71: Total soluble proteins and protease activity in flag leaf of wheat plants grown after sodium silicate seed priming under non-stress and drought stress conditions.
Figure 4.72: MDA content and α-amylase activity in flag leaf of wheat plants grown after sodium silicate seed priming under non-stress and drought stress conditions.
Figure 4.73: Sugar contents in flag leaf of wheat plants grown after sodium silicate seed priming under non-stress and drought stress conditions.
Figure 4.74: Proline and glycine betaine in flag leaf of wheat plants grown after sodium silicate seed priming under non-stress and drought stress conditions.
Figure 4.75: Chlorophyll contents and total carotenoids in flag leaf of wheat plants grown after sodium silicate seed priming under non-stress and drought stress conditions.
Figure 4.76: Water potential, osmotic potential and Turgor potential in flag leaf of wheat plants grown after chitosan seed priming under non-stress and drought stress conditions.
Figure 4.77: Effect of sodium silicate seed priming on cell membrane stability under drought stress.
Figure 4.78: Effects of sodium silicate (SS) seed priming on Yield, 100 grains weight and plant biomass of wheat plants grown under non-stress and drought stress conditions.
5 DISCUSSION

Among various strategies that scientists have developed to cope with abiotic stresses, most commonly used is seed priming (Basra et al., 2006; Iqbal and Ashraf, 2006). Seed priming with different chemicals, ions, organic compounds, hormones and antioxidants has been reported to have profound effects on seed germination, growth and yield (Ashraf and Foolad, 2005). However, biochemical changes that take place in the primed seeds have not been revealed out. This research aspect has not been given much consideration. To our knowledge, changes that are induced in different biochemical processes in the seeds by priming with chitosan, SNP and sodium silicate have not been investigated so far. Results being discussed here are therefore first reports on these aspects.

Seed germination brings out the production or activation of enzymes that are required for the degradation and mobilization of seed reserves (Subramani et al., 2011). In germinating seeds, a series of actions including respiratory enzymes activation, breakdown of food molecules and mobilization of seed nutritional reserves can take place (Bewley 1997). Among these events, starch digestion is primarily controlled by α-amylase, protein digestion by different proteases (Bishnoi et al., 1993) and a group of enzymes esterases catalyzed the hydrolysis of different types of esters (Subramani et al., 2011). During germination of seed of cereals, the aleurone layer is the site of production of these hydrolases (Palmiano and Juliano 1973). In our study, seed priming with all levels of chitosan, promoted the activities of proteases in the seeds to many folds along with increased level of soluble proteins. This may be due to the reason that the activities of proteases maintain the respiratory requirements of the vigorously growing root as well as shoot apices (Maheshwari and Dubey 2008). Moreover, the parallel increase in the protease activity and soluble proteins in the seeds as a result of chitosan seeds priming appeared to be an indication of improvement in nitrogen metabolism in the primed seeds. Protein breakdown and recycling, which depend on the levels of proteolytic enzymes, are essential parts of the plant response to environmental stress (Hieng et al., 2004). Proteases are involved in protein maturation, degradation, and protein rebuilding in
response to different external stimuli and to removal of abnormal, misfolded proteins (Grudkowska and Zagdanska 2004).

Among other hydrolases, α-amylase and α- naphthyl acetate esterase activities increased in the seeds by 0.25 % and 0.50% chitosan treatments while β-naphthyl acetate esterase activity increased only after 0.50 % chitosan priming. Esterases are supposed to involve in the metabolic process of seed germination and even in maturation of plants. During germination esterases are expressed in seeds to discharge the stored food materials for the growing embryo (Subramani et al., 2011). Moreover, the increased level of α-amylase activity in the seeds seems to be responsible for parallel increase in the level of non-reducing sugars in the seeds by chitosan priming. Previously, Farooq et al., (2006) have reported a direct relationship between increased α-amylase activity and levels of soluble sugars in primed rice kernels. Present and previous findings thus support the view that seed priming either induces the de novo synthesis or increases the activities of existing enzymes (Sung and Chang 1993; Lee and Kim 2000).

During optimal growth conditions, balance between ROS formation and consumption is tightly controlled by plant antioxidant defense system (Hameed et al., 2011). Chitosan has some sort of antioxidative properties in it and can act as scavengers of ROS by enhancing the antioxidative pool of plant cells (Guan et al., 2009). Chitosan is an exogenous elicitor of defense responses in plants, as its application has been reported to induce an increase in peroxidase activity in date palm roots (El-Hassni et al., 2004). A similar defense response induced by chitosan was observed in the present study as catalase (CAT) superoxide dismutase (SOD) and peroxidase activities (POD) increased sharply in the primed seeds. This observation is indirectly supported by a previous report in which increased CAT, POD, SOD enzyme activities were reported in low temperature stressed maize plants after chitosan treatments, either as priming agent or in growing media (Guan et al., 2009). Present study thus provide evidence that seed priming with chitosan also induce enhancement in antioxidant enzymes in the wheat seeds that possibly results in better seed performance in terms of germination and vigor.

A decrease in the level of total phenolics in seeds as a result of priming with chitosan was observed in present study that coincided with increased peroxidase activity in the seeds. Recently, the increase of POX activity coincided with the decrease of total phenolic
compounds was reported in the embryonic axes of pedunculate oak (*Quercus robur*) seeds during rapid desiccation (Pukacka *et al.*, 2011). On the bases of this observation, they suggested that in embryonic axes cells, POX utilizes phenolic compounds as the electron donors to scavenge of H$_2$O$_2$. Similar mechanism has also been shown in *Castanea sativa* embryonic axes during wounding and desiccation (Roach *et al.*, 2010). Similarly, the process of utilization of phenolic compounds as the electron donors to remove the hydrogen peroxide by peroxidase may occur in the wheat seeds as a result of chitosan priming.

Generation and reactions of ROS, that is, singlet oxygen, superoxide radical (O$_2^-$), hydrogen peroxide (H$_2$O$_2$), and hydroxyl radical (•OH), are common events during stressed and normal cellular metabolic process (Hameed *et al.*, 2012). Monitoring the NO and ROS production in *Pisum sativum* guard cells by fluorescent probes (DAF-2DA and H$_2$DCFDA) indicated that on exposure to chitosan, the levels of NO rose after only 10 min, while those of ROS increased already by 5 min. (Srivastava *et al.*, 2009). Autocatalytic peroxidation of membrane lipids by ROS leads to loss of membrane semipermeability (Xu *et al.*, 2006) and increase in MDA content (Hameed *et al.*, 2012). As in the present study, seed priming with chitosan increased the MDA contents in the seeds it seems logical to infer that ROS level may has increased in the seeds by chitosan that resulted in enhanced MDA content. Previously, increase in MDA content in wheat leaves after mannose treatment has been indirectly related to excessive ROS production (Hameed *et al.*, 2009). An increase in the MDA content of sunflower seeds following 3 days of drying after osmo-priming of seeds with PEG(~2.0 MPa) has been reported (Bailly *et al.*, 2000). Moreover, as MDA content was lower down by hydropriming of seeds, the observed effect on MDA seems to be because of seed priming with chitosan treatment. An increase in membrane lipid peroxidation due to abiotic stresses is a common response (Jiang and Huang 2001; Hameed *et al.*, 2012).

**Seed priming with sodium nitroprusside (SNP)**

Sodium nitroprusside (SNP), a NO donor can extensively improve the cellular as well as metabolic function of plants (Tian and Lei, 2006). Similarly, in present study antioxidants like SOD and POD activities were up regulated in the wheat seeds by SNP priming treatments. This observation is indirectly supported by the previous findings in which key
antioxidant enzymes i.e. CAT, POD and SOD increased after SNP application in growing medium to cope with drought stress conditions in wheat (Tian and Lei, 2006), rice (Shehab et al., 2010) and pea plants (Moussa and Mohamed, 2011). As NO acts as a second messenger in plants, it is believed that in low concentration, NO might be a signal molecule that stabilize the expression of many antioxidative enzymes including SOD and CAT (Frank et al., 2000; Tian and Lei, 2006).

Decrease in the level of total phenolics in seeds as a result of priming with SNP in present study coincided with increased POD activity in the seeds. Recently, the increase of POD activity coincided with the decrease of total phenolic compounds was reported in the embryonic axes of pedunculate oak (Quercus robur) seeds during rapid desiccation (Pukacka et al., 2011). On the bases of this observation, they suggested that in embryonic axes cells, POD utilizes phenolic compounds as electron donors to scavenge of H$_2$O$_2$. Similar mechanism has also been shown in Castanea sativa embryonic axes during wounding and desiccation (Roach et al., 2010). Similarly, the process of utilization of phenolic compounds as the electron donors to remove the hydrogen peroxide by peroxidase may occur in the wheat seeds as a result of SNP priming. Hydropriming also decreased the phenolic contents of the seeds; however, intensity of this decrease was more in case of SNP priming. There is possibility that similar mechanism with less intensity may be induced in the seeds by hydropriming.

In present study seed priming with SNP increased the MDA concentration in the seeds as compared to non-primed non-stress. In fact, MDA can be produced during arachidonic acid metabolism that is produced during lipid mobilization. Therefore, increased level of MDA in the seeds as a result of SNP priming may be due to production of arachidonic acid and its metabolism after stored lipids mobilization. On the other hand, MDA level was lowered down by hydropriming as compared to non-primed seeds indicating lesser damage to the membranes.

In germinating seeds, a series of actions including respiratory enzymes activation, breakdown of food molecules and mobilization of seed nutritional reserves can take place (Bewley, 1997; Gallardo et al., 2001). During germination of seed of cereals, the aleurone layer is the site of production of these hydrolases (Palmiano and Juliano, 1973). In our study, seed priming with SNP raised the activities of proteases and soluble proteins
in the seeds. The parallel increase in the protease activity and soluble proteins in the seeds as a result of SNP seed priming appeared to be an indication of improvement in nitrogen metabolism in the primed seeds. As the proteases maintain the respiratory requirements of the vigorously growing root as well as shoot apices (Maheshwari and Dubey, 2008), this may be the other reason for increased proteases in the SNP primed seeds. Hydropriming of the seeds also increased these two parameters however; the magnitude was comparatively low as induced by SNP priming. This means seed priming generally increase the process of proteolysis in the seeds while SNP priming further speed up or enhanced this process.

Seed germination brings out the production or activation of enzymes that are required for the degradation and mobilization of seeds reserves (Subramani et al., 2011). Among these events, starch digestion is primarily controlled by α-amylase, protein digestion by different proteases (Bishnoi et al., 1993) and a group of enzymes esterases catalyzed the hydrolysis of different types of esters (Subramani et al., 2011). In our study, all concentrations of SNP as seed priming significantly elevated the α-amylase activity in the seeds. This increased level of α-amylase activity in the seeds seems to be responsible for parallel increase in the level of non-reducing sugars in the seeds by SNP priming. Among esterases, 125 µM SNP priming treatment increased the level of both α- naphthyl and β-naphthyl acetate esterase activities in the seeds. Esterases are actually supposed to involve in the metabolic process of seed germination and even in maturation of plants. During germination esterases are expressed in seeds to discharge the stored food materials for the growing embryo (Subramani et al., 2011). Similar mechanisms seem to be induced in the SNP primed seeds. Because hydropriming reduced the level of α-naphthyl acetate esterase while did not influenced the β-naphthyl acetate esterase and α-amylase activities in seeds it is obvious that observed uplifts in these hydrolyzing enzymes was primarily because of SNP seed priming.

**Seed priming with sodium silicate**

Silicon is most abundant and essential element of crop plants that gives strength, mineral nutrition; resistance to stresses and improves early seedling growth (Gong et al., 2003). Silicone as sodium silicate has been reported to improve the germination, growth, antioxidant enzymes activities and lipid peroxidation status during drought stress in
wheat (Pei et al., 2010). In present study, different concentrations of sodium silicate as seed priming considerably altered the most of analyzed biochemical constituents in the wheat seeds. Among antioxidant enzymes, SOD activity was significantly increased by all sodium silicate priming treatments. POD activity was enhanced by only 20mM sodium silicate treatment while higher concentrations did not change the level in the seeds. There is no previous report regarding antioxidant changes in the seeds as a result of SNP seed priming. However, present results indicate that SNP treatment may have raised the superoxide level in the seeds that was scavenged by increasing the SOD activity. However, CAT activity was significantly decreased by all sodium silicate seed priming treatments. On the other hand, SOD activity significantly decreased while POD and CAT activities increased by hydropriming. This clearly indicates that hydropriming target the hydrogen peroxide scavenging enzyme system that is quite different from the effects induced by SNP priming. Moreover in present study, the level of total phenolics after sodium silicate seed priming was almost half as that of non-primed non-stressed seeds. Hydropriming also lowered down the phenolic contents in the seeds however, degree of reduction was much lesser as compared to that by sodium silicate priming. This observation also strengthen this view that SNP induced changes in the seeds differ from that induced by simple socking of seeds in water. Seed priming with sodium silicate increased the MDA concentration in the seeds as compared to non-primed non-stressed seeds. Actually MDA can be produced during arachidonic acid metabolism that is produced during lipid mobilization. Therefore increased level of MDA in the seeds as a result of sodium silicate priming may be due to production of arachidonic acid and its metabolism after stored lipids mobilization. On the other hand, MDA level was lowered down by hydropriming as compared to non-primed seeds indicating lesser damage to the membranes. Different set of processes including respiratory enzymes activation, breakdown of food molecules and mobilization of seed nutritional reserves can take place in germinating seeds, (Bewley, 1997; Gallardo et al., 2001). During germination of seed of cereals, the aleurone layer is the site of production of these hydrolases (Palmiano and Juliano, 1973). In present study, seed priming with all levels of sodium silicate raised the activities of proteases in the seeds. In parallel an increased level of soluble proteins was also
observed. The parallel increase in the protease activity and soluble proteins in the seeds as a result of sodium silicate seed priming appeared to be an indication of improvement in nitrogen metabolism in the primed seeds. It also point towards the priming induced mobilization of seed reserve proteins by proteases. The other reason for this increase in protein and proteases may be that the activities of proteases maintain the respiratory requirements of the vigorously growing root as well as shoot apices (Maheshwari and Dubey 2008). Hydropriming of the seeds also increased these two parameters however; the magnitude was comparatively low as induced by sodium silicate priming. This shows that seed priming increases the process of proteolysis in the seeds while sodium silicate priming further speed up or enhanced this process. This may be the reason for comparative better performance of sodium silicate priming as compare to simple water soaking of seeds.

Seed germination brings out the production or activation of enzymes that are required for the degradation and mobilization of seeds reserves (Subramani et al., 2011). Among these events, starch digestion is primarily controlled by α-amylase, protein digestion by different proteases (Bishnoi et al., 1993) and a group of enzymes esterases catalyzed the hydrolysis of different types of esters (Subramani et al., 2011). In present study, all concentrations of sodium silicate as seed priming significantly elevated the α-amylase activity in the seeds. This increased level of α-amylase activity in the seeds seems to be responsible for parallel increase in the level of non-reducing sugars in the seeds by sodium silicate priming. Among esterases, 40 mM and 60 mM sodium silicate treatments increased the level of β-naphthyl acetate esterase activity in the seeds. Esterases are actually supposed to be involved in the metabolic process of seed germination and even in maturation of plants. During germination esterases are expressed in seeds to discharge the stored food materials for the growing embryo (Subramani et al., 2011). Similar processes seem to be enhanced in the seeds by sodium silicate priming. As in present study hydropriming reduced the level of α-naphthyl acetate esterase in the seeds while β-naphthyl acetate esterase and α-amylase activity levels were not influenced by this priming treatment. It clearly indicates that observed uplifts in these hydrolyzing enzymes by sodium silicate treatment was primarily because of sodium silicate seed priming.
**Improvement of osmotic stress tolerance in wheat by seed priming**

Osmotic stress is the major consequence of drought stress that eventually reduced the water status and in turn changes the whole biochemistry of plant cells. Poly ethylene glycol (PEG) is most commonly used to create osmotic stress in plants because it is not naturally produced in the plant tissue neither penetrate into it from the media. PEG eventually destroys the normal emergence, growth, biochemical attributes and even yield in crop plants (Pei et al., 2010). A decrease in plant water status under stress conditions has been reported in wheat (Triticum durum) (Siddique et al., 2000), in sorghum (Gill et al., 2001) and in Chenopodium (Chenopodium quinoa) (Prado et al., 2000). We also observed that osmotic stress dropped the relative water content and in turn changes the whole biochemistry of the leaves of wheat seedlings. Previously, Gong et al., (2005) reported the increased activities of APX, POD, SOD and CAT in leaves of wheat seedlings under drought stress. In present study, osmotic stress also increased the peroxidase, protease, α-amylase activities, and total soluble proteins, MDA content, reducing and total sugars while dropped the relative water content in the leaves of seedlings grown from non-primed seeds. Previously, Pie et al., 2010 reported that osmotic stress enhanced the accumulation of H₂O₂ and MDA contents with parallel increase of electrolyte leakage in wheat seedlings. Similarly, MDA contents also increased in leaves of wheat seedlings under osmotic stress in present work. Moreover, the present observation of increased soluble sugar levels under osmotic stress are in agreement with a previous study in which increased soluble sugar levels were found in wheat by progressive field water stress (Zhu et al., 2005). In present study, non-reducing sugars were decreased by osmotic stress. This clearly put forward that in osmotic stress condition used in present study, the catabolism of soluble non-reducing sugars was enhanced. This observation is in agreement with the previous report in which Pie et al., (2010) reported the reduced level of leaf soluble sugars in wheat seedlings due to enhanced catabolism of sugars under osmotic stress.

**Seed priming with chitosan**

Chitosan application highly improves the germination of plants under drought stress. Chitosan priming has been reported to reduce the mean germination time and enhance the
germination index resulting in promotion of early seedling establishment and synchronized growth in rice (Suchada et al., 2007) and maize (Guan et al., 2009). Similar was true for present findings as final germination percentage, germination energy (%), vigor index, germination rate and germination index were improved while mean germination time was reduced by chitosan priming under osmotic stress in wheat. Chitosan has been proved to work as a positive factor in enhancing shoot and root length, fresh and dry weights of shoots and roots and leaves area in bean plants watered with the chitosan solution ((Sheikh and Malki, 2011). Similarly, we also observed that seed priming with 0.50 % chitosan increased the shoot and root length of seedlings under non-stress and osmotic stress conditions. From above said finding supported by the previous reports it can be deduced that seed priming with chitosan improved the germination attributes that in turn resulted in better early seedling growth. Chitosan having exceptional bioactive properties is actually a cationic marine polysaccharide that also an excellent scavenger of ROS. Drought stress induce oxidative stress has been reduced by chitosan application to crop plants (Yang et al., 2009). Similar was true for the present findings as chitosan used as seed priming significantly increased the peroxidase activity in seedling leaves under non-stress and osmotic stress conditions. However, catalase activity and total phenolics decreased while SOD activity remained unchanged after chitosan seed priming. Recently, the increase of POD activity coincided with the decrease of total phenolic compounds was reported in the embryonic axes of pedunculate oak (Quercus robur) seeds during rapid desiccation (Pukacka et al., 2011). On the bases of this observation, they suggested that in embryonic axes cells, POD utilizes phenolic compounds as electron donors to scavenge of H₂O₂. Similar mechanism has also been shown in Castanea sativa embryonic axes during wounding and desiccation (Roach et al., 2010). Similar process of utilization of phenolic compounds as the electron donors to remove the hydrogen peroxide by peroxidase may occur in the wheat seedlings as a result of chitosan priming. Chitosan and SOD have similar antioxidant abilities to scavenge superoxide anion (Yin et al., 2002; Sun et al., 2004). As SOD remained unchanged in the present study, there is possibility that chitosan scavenged the excessive superoxide radical produced due to osmotic stress. The superoxide anion scavenging mechanism of chitosan related to its
structure that has many hydroxyl and amino groups available to react with ROS (Xie et al., 2001; Li et al., 2002; Sun et al., 2004).

Chitosan priming to wheat seeds decreased the MDA contents and increased the POD antioxidant activity in leaves under osmotic stress. These present findings are in line with those reported by Guan et al., (2009), where the application of chitosan significantly decreased lipid peroxidation by stimulating antioxidant enzymes, leading to decreased membrane permeability and improved function. Other researchers have also reported the similar effects (Ghoname et al., 2010; Farouk et al., 2008, 2011).

In present study, seed priming with varying levels of chitosan significantly increased the protease activity and total soluble proteins in leaves under non-stress and osmotic stress conditions. Chitosan priming significantly decreased the leaf α-amylase activity under non-stress and osmotic stress conditions. This clearly indicates that chitosan priming enhanced the proteolysis process while inhibits or lower down the starch hydrolysis. Under osmotic stress seed priming with 0.25 % chitosan was successful in undoing the stress induced increase in MDA content and bring the level back to that in non-stress condition. This means seed priming with chitosan improved the osmotic stress tolerance in wheat seedlings and was helpful in mitigating the stress effects.

**Seed priming with sodium nitroprusside:**

Sodium nitroprusside (SNP) has been reported as a NO donor (Bethke et al., 2004). Nitric oxide (NO) has been proved as an important signaling molecule that regulates a series of physiological processes in both animals and plants (Crawford and Guo, 2005; Besson-Bard et al., 2008). Moreover, it has been reported to promote seed germination of various crops under abiotic stresses (Libourel et al., 2006; Bethke et al., 2007; Noman et al., 2010) and can enhance germination rate in wheat seedlings under salt stress (Zheng et al., 2009). SNP has also been reported as germination promoter in wheat that activated the germination responsive genes and resulted in enhanced seed vigor index under abiotic stresses (Duan et al., 2007; Sen, 2010). Present observations are in accordance with these previous reports as the final germination percentage, germination energy (%), vigor index, germination rate and germination index were improved while mean germination time was reduced by SNP priming under osmotic stress.
Previous reports on antioxidant responses under osmotic stress after SNP application are contrasting. Zhao et al., 2008 reported the stimulation of CAT activity by NO under osmotic stress while a decrease in CAT activity under severe water stress has been also reported in some studies (Baisak et al., 1994; Huang and Guo, 2005). Also SNP application has been reported to enhance the SOD activity under salt stress in *Lupinus Luteus* and cucumber (Kopyra and Gwozdz, 2003; Shi et al., 2007). Moreover, SNP has been reported to enhance the SOD, CAT, APX and GR in wheat under osmotic stress (Zhang et al., 2003). In present study, SNP priming significantly increased the POD, CAT and SOD activities in leaves under non-stress condition while lowered down the activities to the control level or even below under osmotic stress. This clearly depicted that SNP seed priming undo the osmotic stress induced effects on antioxidant defense system bring it back to the non-stressed condition.

Previously, it has been reported that exogenous application of SNP to wheat and chickpea is responsible for the enhanced antioxidant enzymes activities and reduced lipid peroxidation under drought and salt stress (Sheokand et al., 2010; Wang et al., 2011a). Actually, NO react with lipid alcoxyl (LO⁻) and peroxyl (LOO⁻) radicals and break the chain reaction of lipid peroxidation (Lamotte et al., 2004). In present study, SNP (NO donor) priming also reduced the leaf MDA content under osmotic stress condition that indicates the stabilization of membrane structure and reduction in the membrane damage due to osmotic stress. Previously, a protective effect of NO on relative membrane injury has been reported under drought stress (Garcia-Mata and Lamattina 2001; Zhao et al., 2008). Moreover, Garcia-Mata and Lamattina (2001) reported that SNP treated water stressed wheat seedlings tended to retain more water content. Similar changes in were also observed in present study as leaf cell membrane stability and relative water content significantly improved in wheat seedlings by SNP priming that provide evidence for lesser osmotic stress induced injury to membranes.

Previously, it has been found that total phenolic contents increased in rice plants after SNP pre-treatment in growing media (Shehab et al., 2010). SNP treatment has also been reported to increased soluble sugars in wheat seeds under salt stress (Zheng et al., 2009). Soluble sugars in fact play an essential role in plant metabolism not only by acting as osmoprotectants that stabilize cellular membranes and maintain turgor (Couee et al.,
2006) but also as signal molecules (Gibson, 2005; Chen et al., 2009). Furthermore, soluble sugars were also found to involve in ROS balance and responded to oxidative stress in plants (Couee et al., 2006). Application of sodium nitroprusside in growing media has been reported as not only to improve relative water contents but also its application to wheat seedlings under osmotic stress, enhanced the antioxidants, osmolytes concentration and stomatal conductance in leaves (Jinfang et al., 2008). We also observed that SNP priming of wheat seeds resulted in enhanced leaf total phenolics, protein contents and reducing sugars under osmotic stress. All these observations supported by previous reports provide evidence for improvement of osmotic stress tolerance in wheat by seed priming with SNP.

**Seed priming with sodium silicate:**
Silicone can promote the growth and development of plants under water stress and potassium deficient medium (Hattori et al., 2005; Gong et al., 2005, 2008; Miao et al., 2010). Evidence has been provided that sodium silicate application resulted in higher germination percentage and index of wheat seedlings which ultimately leads to improved yield (Abro et al., 2009). We also observed that final germination percentage, germination energy (%), vigor index, germination rate and germination index were improved while mean germination time was reduced by sodium silicate priming in wheat under osmotic stress. Thus present observations strengthen this view that sodium silicate improves the osmotic stress tolerance in plants in general and in wheat in particular.

Previously, Pie et al., (2010) have suggested that sodium silicate application enhanced the antioxidant defense system more as compared to osmotic adjustment to improve the wheat growth under osmotic stress conditions. In potted wheat experiment Gong et al., 2005 has been reported that Si alleviated the oxidative stress by regulating the activities of antioxidant enzymes under drought. Also in salt stressed barley and cucumber, increased antioxidant defense activity and decreased oxidative damage has been reported after silicone application (Zhu et al., 2004; Liang et al., 2003, 2005, 2006). A similar phenomenon has been observed in freeze-stressed wheat plants (Liang et al., 2008). This was further supported by a complete transcriptome analysis of powdery mildew-stressed Arabidopsis (Fauteux et al., 2006; Liang et al., 2008). Ashraf and Foolad (2007) and Liang et al., (2008) have also suggested that Si enhanced antioxidant defense activity
might be a universal mechanism for Si-enhanced tolerance to various abiotic and biotic stresses in plants. In our study, seed priming with sodium silicate (60mM) enhanced the POD activity, total phenolics, protein contents and reducing sugars in leaves under osmotic stress while all concentrations improved these parameters under non-stress condition. Sodium silicate priming also significantly decreased MDA content in leaves under non-stress and osmotic stress conditions. This indicates lesser stress induced injury to membranes as a result of silicate priming, possibly due to better scavenging of ROS by improved antioxidant status.

In addition to antioxidant defense, plants can also adapt to water stress by changing solute levels so that turgor and hence physiological activity are maintained at low leaf water potentials. As earlier, it has been reported that the addition of Si increased water use efficiency by reducing leaf transpiration and the water flow rate (Zhu et al., 2005; Gao et al., 2004, 2006). Hattori et al., (2005, 2007) also suggested that Si could facilitate water uptake and transport in sorghum under drought conditions. Similarly in our study, the leaf relative water content and cell membrane stability significantly improved after sodium silicate priming showing lesser osmotic stress induced injury to membrane in seedlings grown from primed seeds. The ameliorative effects of Si on osmotic stress observed in this study are consistent with the results of pot experiments under drought conditions in wheat (Gong et al., 2005) and sorghum (Hattori et al., 2005).

It is interesting to note that with all three priming agents, some parameters such as final germination (Figure 4.16, 4.27 and 4.38) were higher with priming under stress than with priming without stress. Better performance of primed seeds under osmotic medium may be due to the reason that applied PEG concentration acted as osmotic treatment to seeds that further enhanced their germination and multiply the seed priming effects. Low osmotic stress seems to serve as osmopriming treatment to primed seeds. Previously, several researchers have used PEG treatment as osmopriming of seeds for improvement of stress tolerance in different crops including wheat. For example, Al-Karaki, (1998) has reported that seeds osmopriming increased the germination percentage and decreased the time to 50% germination in wheat seeds under low osmotic stress (-0.4 MPa) induced by PEG-8000. Al-Karaki, (1998) further added that fresh weight and length of shoots
(plumules) and roots (radicles) were enhanced in osmoprimed seeds in comparison to untreated seeds of wheat and barley. Moreover, polyethylene glycol osmopriming has been reported to cause early seed emergence, early maturity and enhanced biomass yield of rainfed wheat (Shams-ur-Rehman et al., 2010). Furthermore, osmopriming has been reported to significantly improve the final germination percentage, energy of germination, germination index, shoot length, root length, vigor index and reduce the mean germination time in lettuce achene seeds subjected to supra-optimal germination environments (Jahangir et al., 2009). All these previous reports strengthen the view that low osmotic stress used in present study served as osmopriming treatment to seeds that resulted in better effects of priming treatments under stress than non-stressed condition.

**Improvement of drought tolerance in wheat by seed priming**

Several earlier reports of increased antioxidant enzymes i.e. POD, SOD, CAT under drought stress reflects that it is a common adaptive mechanism in plants to reduce or conquer the adverse stress effects (Zhang and Kirham, 1994; Chen and Gallie, 2004; Dhanda et al., 2004). Gupta and Gupta, (2005) and Sairam et al., (2000) also reported that perhaps, the higher activities of CAT and POD are responsible for the removal of the $O_2^-$ radicals and its product $H_2O_2$ over produced by drought stress. Some other reports also provided evidence that the amounts and activities of enzymes involved in scavenging ROS were altered under drought and salt stress (Dalmia and Sawhney, 2004; Sairam et al., 2002, 2005). In our experiment peroxidase and total phenolic contents increased in the leaves of wheat plants under drought stress as compared to non-stressed control. The increased activities of antioxidant enzymes also act as damage control system and thus provide protection from oxidative stress, which otherwise could cause lipid peroxidation (Sairam and Saxena, 2000).

A decline in membrane stability index (MSI) due to different abiotic stresses has been reported earlier (Bhattacharjee and Mukherjee, 1996; Sairam et al., 2005). The drought stress induced decrease in MSI indicates the extent of lipid peroxidation caused by ROS (Pastori and Trippi 1992; Baisak et al., 1994; Menconi et al., 1995). Quan et al., (2004) also lower MSI in drought stressed maize (*Zea mays* L.). Similar was true for our
experiment as drought stress significantly increased the lipid peroxidation (MDA contents) and resulted in declined cell membrane stability in the wheat leaves.

Increased proline accumulation has been reported in water-stressed wheat (Hamada, 2000), *Gossypium hirsutum* (Ronde *et al*., 1999), sorghum (Yadav *et al*., 2005) and bell pepper (Nath *et al*., 2005). We also observed that proline contents of wheat leaves increased under drought stressed plants as compared to non-stressed control. Actually, accumulation of proline in plants under stress may be an adaptive process to overcome the stress conditions. This high amount of proline accumulated under stress provides energy for growth and survival that helps the plant to tolerate stress and maintain plant water status (Chandrashekar and Sandhyarani, 1996; Sankar *et al*., 2007).

Glycine betaine is known to accumulate under stresses in several crop plants, including spinach (*Spinacia oleracea*), sugar beet (*Beta vulgaris*), barley (*Hordeum vulgare*), sorghum (*Sorghum bicolor*) and wheat (*Triticum aestivum*) (Weimberg *et al*., 1984; Fallon and Phillips, 1989; McCue and Hanson, 1990; Rhodes and Hanson, 1993; Yang *et al*., 2003). In present study, glycine betaine also accumulated in the leaves of drought stressed plants as the level was higher as compared to control plants. Glycine betaine, has received attention as a compatible solute that may aid in water stress tolerance by allowing maintenance of turgor pressure (Agboma *et al*., 1997; Makela *et al*., 1997; Nepomuceno *et al*., 1998; Makela *et al*., 1999). Glycine betaine not only acts as an osmoregulator, but also stabilizes the structures and activities of enzymes and protein complexes, and maintains the integrity of membranes against the damaging effects of different abiotic stresses (Sakamoto and Murata, 2002). Observed higher concentration of glycine betaine in the drought stressed wheat plants seems to be for osmoregulation.

Previously, it has been reported that drought stress leads to reduction in total chlorophyll contents due to reduced chlorophyll formation (Mondal and Paul, 1992; Begum and Paul, 1993; Allakhverdiev *et al*., 2003; Nikolaeva *et al*., 2010; Kumar *et al*., 2011;) and increased chlorophyll breakdown (Bano and Aziz, 2003) under drought stress. Drought stress significantly decreased the chlorophyll b, total chlorophyll contents and total carotenoids in the leaves of wheat seedlings in present study. Oxidation of photosynthetic pigments i.e. carotenoids due to high amount of ROS formation under environmental stresses has been reported (Knox and Dodge, 1985; Alscher *et al*., 1997; Lai *et al*., 2007).
According to Majeed and Bano, (2008), yield is directly varied under drought stress by affecting the physiological processes involved in plant growth and grain production. In present study, drought stress also adversely affected the yield and yield components in wheat plants. The decrease in grain weight may be due to disturbed nutrient uptake efficiency and photosynthetic activity within the plants (Iqbal et al., 1999) or due to the effects of drought stress on the process of cell division (Ahmadi and Baker, 2001).

**Seed priming with chitosan**

Seed priming has proved to be the easiest approach to overcome drought stress effects (Hao et al., 2008; Farooq et al., 2009a; Tanou et al., 2009; Siddiqui et al., 2011). Chitosan has been reported to reduce transpiration and maintain plant water status under drought stress by closing of stomata of plants (Farouk et al., 2011; Farouk and Amany, 2012). In present study, wheat plants grown from seeds primed with chitosan maintained the improved water status by enhancing leaf turgor potential and reduced leaf osmotic and water potential under non-stress and drought stress conditions.

In our experiment, chitosan seed priming resulted in enhanced POD, CAT, protein content, non-reducing sugars, glycine betaine and cell membrane stability index in wheat flag leaves under both normal and drought stress conditions. Moreover, SOD, α-amylase, protease, total phenolics, MDA, reducing sugars and proline decreased in flag leaves as a result of chitosan priming under normal and drought stress conditions. These observations are in line with the results of Guan et al., (2009), where the application of chitosan significantly decreased the lipid peroxidation by stimulating antioxidant enzymes, leading to decreased membrane permeability and improved function. Our results are also consistent with earlier reports that chitosan could promote antioxidant enzyme activity and decrease MDA concentration in plants (Xu et al., 2007, Gao et al., 2008). Some other reports also described similar changes (Farouk et al., 2008, 2011; Ghoname et al., 2010).

Previously, it has been reported that chlorophyll contents of bean plants and photosynthetic pigments of tomato plants improved after chitosan application (El-Tantawy, 2009; Sheikh and Malki, 2011). Similarly, in present study chitosan seed priming enhanced the level of chlorophyll contents and carotenoids pigments in the wheat leaves under drought stress conditions. The effects of chitosan, in increasing chlorophylls
and total carbohydrate contents have also been confirmed in cucumber (Farouk et al., 2008). Moreover, Farouk et al., (2011) also reported that chitosan application to radish plant resulted in increased contents of chlorophyll a, chlorophyll b and total chlorophylls after cadmium stress development. It seems logical to infer that chitosan application has the potential to improve the pigments in the leaves of stressed plants that may help in better photosynthetic activity and growth.

Chitosan treatments may enhance survival and growth under water stress conditions by improving water relations and providing higher protection for the inner tissues and hence increasing yield (Bacelar et al., 2006). In present study, chitosan priming improved the grain yield, 100 grain weight and plant biomass grown under drought stress. These results are consistent with El-Tantawy (2009), who reported that plant growth and development was enhanced by the application of chitosan in tomato. Moreover, Mahmood et al., (2012) also reported that total grain yield and yield components of wheat plants were severely affected by drought stress, however, these effects of drought stress were reversed after chitosan treatment. Increased yield and related parameters has also been reported after chitosan priming in rice seedlings under drought stress (Suchada et al., 2007). Furthermore, chitosan has been shown to increase the plant dry weight in rice (Chibu and Shibayama 1999), sweet pepper (Ghoname et al., 2010), cucumber and radish plants (Farouk et al., 2008, 2011) under different abiotic stresses. The present observations supported by the previous ones proved that chitosan can enhance the growth and yield in wheat and also in the other crops under stressful conditions. This stimulating effect of chitosan on plant growth may be attributed to an increase in the availability and uptake of water and essential nutrients through adjusting cell osmotic pressure, and reducing the accumulation of harmful free radicals by increasing antioxidants and enzyme activities (Guan et al., 2009).

**Seed priming with SNP**

Many earlier reports, have demonstrated that nitric oxide (NO) could counteract oxidative damage and had protective effect against various stressful conditions (Song et al., 2006; Arasimowicz and Floryszak-Wieczorek, 2007; Shi et al., 2007; Sun et al., 2007; Vital et al., 2008; Zhao et al., 2008; Singh et al., 2009). In present work, wheat plants grown
from seeds primed with SNP, resulted in improved leaf POD, CAT, SOD, protease and alpha amylase activities under drought and non-stress conditions. Therefore, the present results are in agreement with Moussa and Mohamed, (2011); Wang et al., (2005); Tian and Lei (2006) and Yang et al., (2008) who found that, NO increase the activities of SOD, CAT, APX, and GR under drought stress to counteract oxidative injury. Tanou et al., (2009) also showed that SNP alone or SNP with NaCl treatment increased the APX, CAT, POD and GR activities. Sheokand et al., (2010) have reported that exogenous application of SNP to chickpea plants enhanced the antioxidants level. Antioxidant genes and antioxidant enzymes including APX and CAT have been found to induce by NO in maize leaves Zhang et al., (2007).

In previous studies, two mechanisms which may explain NO protective action against oxidative stress have been proposed. Firstly, NO might detoxify ROS directly, such as superoxide radicals, to form peroxynitrite, which is less toxic and thus limit cellular damage (Martinez et al., 2000). Secondly, NO could function as a signaling molecule, which activates cellular antioxidant system (Huang et al., 2002; Shi et al., 2005, 2007). In order to detoxify ROS and prevent cellular damage, a balance is required between the antioxidant defense system, which includes antioxidant compounds and antioxidant enzymes (Athar et al., 2008).

Total soluble sugars have been reported to increase after SNP application to rice plants under drought stress (Shehab et al., 2010) and in wheat plants under salt stress (Zheng et al., 2009). We also observed that wheat plants grown from seeds primed with SNP, maintained higher reducing & total sugars, total phenolics, protein contents, glycine betaine in leaves under drought and non-stress conditions. Soluble sugars in fact play an essential role in plant metabolism not only by acting as osmoprotectants that stabilize cellular membranes and maintain turgor (Couee et al., 2006) but also as signal molecules (Gibson, 2005; Chen et al., 2009). Furthermore, soluble sugars were also found to involve in ROS balance and responded to oxidative stress in plants (Couee et al., 2006).

NO produced by SNP alleviate the toxic effects of lipid peroxidation with reduction of ROS produced under nickel stress in wheat seedlings (Wang et al., 2011a). In our study, SNP priming to wheat seeds reduced the level of leaf MDA content i.e. an indicator of lipid peroxidation under drought and non-stress conditions.
In many previous reports, SNP treatment in growing media has showed significant effect on leaf proline content under osmotic stress in wheat seedlings (Jinfang et al., 2008; Khan et al., 2009; Simaei et al., 2011). Chlorophyll the photosynthetic pigments of plants has been reported to decrease during drought stress while SNP treatment highly improved the chlorophyll content under drought stress (Wang et al., 2011a) and both chlorophyll and total carotinoids increased in pea plants under drought stress (Moussa and Mohamed, 2011; Simaei et al., 2011). In present study, chlorophyll a and carotenoids were increased in wheat plants grown from seeds primed with SNP. Previously, due to improvement in photosynthetic pigments, the photosynthetic activity of wheat seedling was highly enhanced after exogenous application of SNP (Jinfang et al., 2008). SNP application to wheat seedlings has been reported to result in highly improved cell membrane stability under drought stress (Hao et al., 2008). SNP pre treatment to of wheat leaves could enhance the stomatal closure that leads to increased relative water contents. Cell injury index also decreased after SNP treatment under drought stress (Gracia-Mata and Lamattina, 2001). Our results are in accordance with these previous reports as leaf cell membrane stability and water relations were improved by SNP seed priming indicating lesser drought induced injury.

Previously, Zheng et al., (2006) has reported that NO can increase the dry matter accumulation in maize seedlings under salt stress. Moreover, increased grain yield and plant biomass after SNP application has been reported in chick pea under cadmium stress and in pea seedlings under drought stress (Kumari et al., 2010; Moussa and Mohamed, 2011). Similar was true for present study, because SNP priming greatly improved the grain yield, 100 grain weight and plant biomass under drought stress.

**Seed priming with sodium silicate**

Silicone as sodium silicate has been proved to be responsible for the improvement of germination, growth, antioxidant enzymes activities and lipid peroxidation status during drought stress in wheat (Pei et al., 2010). Activities of antioxidants (SOD, POD and CAT) have been stimulated after sodium silicate treatment in wheat under stress (Ali et al., 2012). Moreover, sodium silicate treatment lowered down the oxidative stress caused by drought by enhancement of antioxidant production (glutathione reductase, catalase,
peroxidase, and superoxide dismutase) in wheat, barley and soy bean plants (Liang et al., 2003; Gong et al., 2005; Miao et al., 2010; Wang et al., 2011b). We also observed that in wheat plants grown from seeds primed with silicone as sodium silicate, the peroxidase, catalase, protease, α-amylase activities, protein contents, total and reducing sugars were increased in leaves under drought and non-stress conditions. All these observations showed that sodium silicate priming can enhance the antioxidant defense enzymes and improve the level of osmoprotectants like sugars contents in wheat plants that results in improvement of drought tolerance.

Phenolic compounds are diverse groups of plant secondary metabolites and possess various health benefits (Liu et al., 2007b). Phenolics can protect plants from physiological stresses such as oxidative stress by preventing breakdown of macromolecules and cellular structures (Toor and Savage, 2006). In present study, wheat plants grown from seeds primed with sodium silicate maintained the increased level of phenolics in leaves under drought and non-stress conditions. This clearly indicates that the sodium silicate seed priming improves the level of phenolics in wheat leaves that helps in preventing the damage to macromolecules by ROS produced during normal metabolic process or as a result of drought stress.

There are reports that sodium silicate treatment may result in accumulation of proline and glycine betaine under drought stress in potato and wheat plants (Carlos et al., 2009; Ahmad and haddad, 2011). Similarly in present study, the glycine betaine, chlorophyll contents and carotenoids were improved by sodium silicate priming drought stress. Previously, increased chlorophyll contents after sodium silicate treatment has also been observed in wheat leaves under osmotic stress (Pei et al., 2010).

Sodium silicate not only deposited in the plant cells but also actively reduced the MDA contents and enhanced the activities of antioxidants in higher plants (Liang et al., 2003; Miao et al., 2010). We also observed that MDA content declined under both drought and non-stress conditions after sodium silicate priming. Moreover, sodium silicate seed priming reduced the drought stress induced injury to membranes as depicted from improved cell membrane stability under drought. Previously, due to reduction in lipid peroxidation as significant improvement in the cell membrane stability has been reported under drought stress and salt stress (Liang et al., 2007; Pei et al., 2010; Wang et al.,
Moreover, sodium silicate application has been shown to result in better cell membrane stability under drought or osmotic stress earlier (Pei et al., 2010).

Sodium silicate, a derivative of silicone, can improve the water balance by increasing root endodermal silification (Faroq et al., 2009a). Sodium silicate application has been reported to improve the decreased water potential and RWC under drought stress. This increased water status of plant resulted in better plant growth under drought stress (Gong et al., 2003). In present study, seed priming with sodium silicate significantly decreased the leaf water and osmotic potential while improved the leaf turgor potential under non-stress and drought stress conditions. In this connection, Murillo-Amador, (2007) has been reported that the ameliorative effect of Si to drought is due to its hydrophilic nature that maintains plant water status. Moreover, shoot water uptake has been reported to increase in sorghum plants after sodium silicate application that leads to higher relative water content, photosynthetic activity, better stomatal conductance and increased dry matter accumulation (Hattori et al., 2005). Gong et al., (2003) observed that silicon increased plant height, leaf area and dry mass of wheat even under drought.

Sodium silicate priming significantly improved the grain yield, 100 grain weight and plant biomass of wheat seedlings grown under drought stress in present study. Previously, application of sodium silicate in growing media of wheat has shown a highly positive effect on the yield and yield parameters. There are other reports that yield of plants was effectively improved under drought stress after sodium silicate application (Abro et al., 2009; Carlos et al., 2009; Pei et al., 2010). Mukkram et al., (2006) also found that silicon increased the growth and yield in wheat under salt stress that was due to decreased Na+ uptake.

5.1 Conclusions

1. Seed priming with chitosan, SNP and sodium silicate brings out the production or activation of enzymes in the seeds that are required for the degradation and mobilization of seeds reserves and defense response.

2. Osmotic stress induced by PEG adversely affected the seed germination attributes while chitosan SNP and sodium silicate seed priming improved the germination...
(FGM, GE, VI, GR, MGT and GI) and promoted the early seedling growth under non-stress and osmotic stress conditions.

3. Chitosan, SNP and sodium silicate priming generally resulted in promotion of early seedling establishment and synchronized growth along with better biochemical and physiological attributes.

4. Osmotic stress increased the peroxidase, protease, α-amylase activities, total soluble proteins, malondialdehyde (MDA) contents, reducing and total sugars while dropped the relative water content in the leaves.

5. Chitosan seed priming improved the osmotic stress tolerance of seedlings evident from adjusted antioxidants activities (POD, CAT, SOD) soluble sugars, improved CMS and leaf RWC and reduced lipid peroxidation.

6. Seed priming with SNP significantly improved the CMS, RWC, TPC, proteins and reducing sugars while reduced the hydrolases (protease, α-amylase) activities and lipid peroxidation in seedlings providing evidence for lesser osmotic stress induced injury and improved stress tolerance.

7. Sodium silicate (60mM) priming enhanced the POD, TPC, CMS, RWC and reducing sugars while decreased the CAT, protease, α-amylase and SOD activities and MDA content in leaves under osmotic stress indicating improvement in stress tolerance.

8. Drought stress adversely affected the biochemical, physiological processes, and yield in wheat plants grown from non-primed seeds.

9. In wheat plants grown from seeds primed with chitosan, SNP or sodium silicate antioxidants (CAT, POD, SOD and TPC), osmoprotectants (GB, proline and sugars), CMS, leaf water relations (WP, OP, TP and RWC) and pigments generally improved while hydrolyzing enzymes and lipid peroxidation decreased under drought stress. Chitosan SNP and sodium silicate priming improved the grain yield, 100 grain weight and plant biomass grown under drought stress.

10. In general, tested seed priming treatments successfully improved the seed germination and performance and alleviated the adverse effects of osmotic and drought stress in wheat seedling and plants respectively.
6 SUMMARY

The experiments were planned to investigate the use of chitosan, sodium nitroprusside and sodium silicate as seed priming agents and to analyze different biochemical events or changes that take place in the primed seeds, in seedlings grown from primed seeds and in wheat plants. Furthermore, the possible use of these priming treatments for improvement of osmotic stress (PEG) and drought tolerance in wheat was also tested. Seed priming with chitosan, SNP and sodium silicate promoted the activities of proteases, soluble proteins, hydrolases, α-amylase, α- naphthyl acetate esterase activities increased in the seeds. Moreover, tested seed priming treatments induced enhancement in antioxidant enzymes and decreased the level of total phenolics in the wheat seeds. Hydropriming induced modulations in seed biochemical processes were generally different and less prominent as observed by other tested priming treatments. In general tested seed priming treatments brings out the production or activation of enzymes in the seeds that are required for the degradation and mobilization of seeds reserves and defense response.

Osmotic stress is the major consequence of drought stress that eventually reduced the water status and in turn changes the whole biochemistry of plant cells. Osmotic stress induced by PEG adversely affected the germination attributes of the seeds. Final germination percentage, germination energy (%), vigor index, germination rate and germination index were improved while mean germination time was reduced by chitosan SNP and sodium silicate priming under osmotic stress in wheat. All these changes resulted in promotion of early seedling establishment and synchronized growth. Osmotic stress increased the peroxidase, protease, α-amylase activities, total soluble proteins, MDA content, reducing and total sugars while dropped the relative water content in the leaves of seedlings grown from non-primed seeds. Non-reducing sugars were decreased by osmotic stress.

Chitosan is actually a cationic marine polysaccharide with exceptional bioactive properties that make it an excellent scavenger of reactive oxygen species. Chitosan used as seed priming significantly increased the peroxidase, protease, non-reducing sugars,
CMS and relative water content in seedling leaves under non-stress and osmotic stress conditions. However, catalase, α-amylase and total phenolics, reducing and total sugars decreased while SOD activity remained unchanged after chitosan seed priming. Under osmotic stress seed priming with 0.25 % chitosan was successful in undoing the stress induced increase in MDA content and bring the level back to that in non-stress condition. SNP priming significantly increased the peroxidase, catalase and SOD activities in leaves under non-stress condition but under osmotic stress they decreased after SNP priming. SNP priming significantly increased the total phenolics protein contents and reducing sugars while decreased the protease, α-amylase activities; MDA contents, total sugars and non-reducing sugars in leaves under both non-stress and osmotic stress conditions. Leaf cell membrane stability and relative water content were significantly improved by SNP seed priming treatments providing evidence for lesser osmotic stress induced injury to membrane in seedlings grown from primed seeds.

Seed priming with sodium silicate (60mM) enhanced the peroxidase activity, total phenolics, protein contents and reducing sugars in leaves under osmotic stress while all concentrations improved these parameters under non-stress condition. Sodium silicate priming significantly decreased the catalase, protease, α-amylase and SOD activities; MDA contents, non reducing sugars and total sugars in leaves under non-stress and osmotic stress conditions. Leaf relative water content and cell membrane stability significantly improved after sodium silicate priming showing lesser osmotic stress induced injury to membrane in seedlings grown from primed seeds.

In pot experiment, drought stress significantly decreased the protease and superoxide dismutase activities; non reducing sugars, glycine betaine contents, chlorophyll b and total chlorophyll contents and total carotenoids in the leaves of seedlings grown from non-primed seeds. Moreover, peroxidase, alpha amylase activities, total phenolic contents, total soluble proteins, reducing sugars, chlorophyll a and proline contents increased under drought stress as compared to non-stress condition. Leaf water and osmotic potential was significantly increased while turgor potential decreased under drought stress in seedlings grown from non-primed seeds. Drought stress also adversely affected the yield and yield components in wheat plants grown from non-primed seeds.
In wheat plants grown from seeds primed with varying concentrations of chitosan, peroxidase, catalase, protein content, non-reducing sugars and glycine betaine contents enhanced while SOD, \( \alpha \)-amylase and protease activities; total phenolic contents, level of MDA contents, reducing sugars and proline content diminished in flag leaves under normal and drought stress conditions. Chitosan seed priming enhanced the level of chlorophyll, carotenoids pigments, leaf turgor potential and CMS under drought stress while decreased the leaf water and osmotic potential under non-stress and drought stress conditions. Chitosan priming improved the grain yield, 100 grain weight and plant biomass grown under drought stress.

In wheat plants grown from seeds primed with SNP, peroxidase, catalase, SOD, protease, \( \alpha \)-amylase activity; total phenolics, protein contents, reducing & total sugars glycine betain, turgor potential and chlorophyll a were improved in leaves under drought and non-stress conditions. On the other hand, SNP priming reduced the level of MDA, non-reducing sugars; proline, water potential, osmotic potential and chlorophyll contents in leaves under drought and non stress conditions. Leaf cell membrane stability was also enhanced by SNP seed priming treatments indicating lesser drought induced injury. SNP priming highly improved the grain yield, 100 grain weight and plant biomass grown under drought stress.

In wheat plants grown from seeds primed with silicone as sodium silicate the peroxidase, catalase, protease \( \alpha \)-amylase activities; TPC, protein contents, total and reducing sugars, glycine betain contents, total chlorophyll and carotenoids increased in leaves under drought and non-stress conditions. Sodium silicate priming reduced the MDA content under both non-stress and drought stress conditions. Moreover, seed priming with sodium silicate significantly decreased the leaf water and osmotic potential while improved the leaf turgor potential under non-stress and drought stress conditions. Sodium silicate seed priming reduced the drought stress induced injury to membranes as depicted from improved cell membrane stability under drought. Sodium silicate priming greatly improved the grain yield, 100 grain weight and plant biomass grown under drought stress.
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