EXPLORING THE POTENTIAL OF MORINGA
(Moringa oleifera) LEAF EXTRACT AS
NATURAL PLANT GROWTH ENHANCER

AZRA YASMEEN

DOCTOR OF PHILOSOPHY
IN
AGRONOMY

DEPARTMENT OF AGRONOMY
FACULTY OF AGRICULTURE,
UNIVERSITY OF AGRICULTURE,
FAISALABAD, PAKISTAN.

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AZRA YASMEEN

M.Sc. (Hons.) Agriculture
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A thesis submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY
IN
AGRONOMY

DEPARTMENT OF AGRONOMY
FACULTY OF AGRICULTURE,
UNIVERSITY OF AGRICULTURE,
FAISALABAD, PAKISTAN.

2011
DEDICATED
TO
MY LOVING AND CARING,
HUSBAND,
MY SWEET SISTERS IN LAW,
MY INNOCENT CHILDREN,
MY RESPECTED MOTHER IN LAW,
AND
MY DEAR PARENTS,
So much I have become is because of you and
I want to tell you
That I appreciate you thank you and love you
ACKNOWLEDGEMENTS

Saying of Prophet Muhammad (PBUH) `A person who is not thankful to his benefactor is not thankful to ALLAH'. All and every kind of praises is upon ALLAH ALMIGHTY, the strength of universe, who ever helps in darkness & difficulties. All and every kind of respect to His Holy Prophet Muhammad (PBUH) for unique comprehensive and everlasting source of guidance and knowledge for humanity.

I would like to extend my heartiest gratitude to Dr. Shahzad Maqsood Ahmad Basra, Professor, Department of Crop Physiology, University of Agriculture, Faisalabad, under whose supervision, scholastic guidance, consulting behavior, this work was planned, executed and completed. I appreciate and thank to members of my supervisory committee, Dr. Rashid Ahmad, Professor, Department of Crop Physiology, University of Agriculture, Faisalabad for his helping attitude and Dr. Abdul Wahid, Professor and Chairman, Department of Botany, University of Agriculture, Faisalabad, for their valuable and continuous guidelines during the preparation of this manuscript and kind hearted behavior throughout my doctoral studies. I am also highly indebted to Higher Education Commission (HEC), Government of Pakistan for granting me Indigenous fellowship throughout my doctoral study.

Azra Yasmeen
The Controller of Examination,
University of Agriculture,
Faisalabad,

We, the supervisory committee, certify that the contents and form of thesis submitted by Ms. Azra Yasmeen, Regd. No. 96-ag-662 have been found satisfactory and recommend that it be processed for evaluation by the External Examiner(s) for award of Degree.

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I hereby declare that contents of the thesis, “Exploring the potential of moringa (Moringa oleifera) leaf extract as natural plant growth enhancer” are product of my own research and no part has been copied from any published source (except the references, standard mathematical or genetic models/equations/formulate/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. (In case of any default, the scholar will be proceeded against as per HEC plagiarism policy).

Azra Yasmeen
96-ag-662
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<td>%</td>
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<tr>
<td>AA</td>
<td>Ascorbic acid</td>
</tr>
<tr>
<td>APX</td>
<td>Ascorbate peroxidase</td>
</tr>
<tr>
<td>cm</td>
<td>centimeter (s)</td>
</tr>
<tr>
<td>CAT</td>
<td>catalase</td>
</tr>
<tr>
<td>CGR</td>
<td>Crop growth rate</td>
</tr>
<tr>
<td>d</td>
<td>day (s)</td>
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<td>DAS</td>
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<td>E50</td>
<td>time to 50% emergence</td>
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<td>GR</td>
<td>Glutathione reductase</td>
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<td>ha</td>
<td>hectare</td>
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<td>per hectare</td>
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<tr>
<td>H(_2)O(_2)</td>
<td>Hydrogen peroxide</td>
</tr>
<tr>
<td>K</td>
<td>Potassium</td>
</tr>
<tr>
<td>kg</td>
<td>kilogram</td>
</tr>
<tr>
<td>kg ha(^{-1})</td>
<td>kilogram per hectare</td>
</tr>
<tr>
<td>LSD</td>
<td>Least Significant Difference</td>
</tr>
<tr>
<td>m</td>
<td>meter</td>
</tr>
<tr>
<td>m(^{-2})</td>
<td>per square meter</td>
</tr>
<tr>
<td>MDAR</td>
<td>Monodehydroascorbate reductase</td>
</tr>
<tr>
<td>MET</td>
<td>mean emergence time</td>
</tr>
<tr>
<td>MGT</td>
<td>mean germination time</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
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</tr>
<tr>
<td>ml</td>
<td>milliliter</td>
</tr>
<tr>
<td>MLE</td>
<td>Moringa leaf extract</td>
</tr>
<tr>
<td>mm</td>
<td>millimeter</td>
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<tr>
<td>mM</td>
<td>milli Molar</td>
</tr>
<tr>
<td>MPa</td>
<td>Mega Pascal</td>
</tr>
<tr>
<td>N</td>
<td>Nitrogen</td>
</tr>
<tr>
<td>NAR</td>
<td>Net assimilation rate</td>
</tr>
<tr>
<td>NS</td>
<td>non-significant</td>
</tr>
<tr>
<td>O²⁻</td>
<td>Superoxide radical</td>
</tr>
<tr>
<td>OH⁻</td>
<td>Hydroxyl radical</td>
</tr>
<tr>
<td>P</td>
<td>Phosphorus</td>
</tr>
<tr>
<td>POD</td>
<td>Peroxidase</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>RO</td>
<td>Alkoxy radical</td>
</tr>
<tr>
<td>Rs.</td>
<td>Rupees</td>
</tr>
<tr>
<td>SLAD</td>
<td>Seasonal leaf area duration</td>
</tr>
<tr>
<td>SOD</td>
<td>Superoxide dismutase</td>
</tr>
<tr>
<td>T₅₀</td>
<td>time to 50% germination</td>
</tr>
<tr>
<td>TPC</td>
<td>total phenolic contents</td>
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Abstract

Among naturally occurring plant growth stimulants, *Moringa oleifera* has attained enormous attention because of having cytokinin in addition to other growth enhancing compounds like ascorbates, phenolics, other antioxidants along with macro- micro nutrients in its leaves. With these properties, exogenous application of Moringa leaf extract (MLE) was done in wheat, pea and tomato to evaluate its efficacy as crop growth enhancer. The objective was to optimize dose, method of exogenous application, enhancement of growth, yield and antioxidant level under normal and abiotic stresses (late sowing, salinity, and drought). The results showed that 30 times diluted MLE priming in wheat under normal conditions was found to be effective. MLE priming improved the seedling emergence rate, speed and early growth and increased level of antioxidants, leaf total soluble protein and chlorophyll contents as compared to MLE10, Hydro priming, On-farm priming and CaCl₂ priming. Large number and heavier grains were obtained in plants raised from MLE30 primed seed which resulted in highest grain yield per plant. All the priming treatments gave more yield than non primed control. Foliar spray of MLE caused an increase of 10.73, 6.00, 10.70 and 4.00% in 1000 grain weight, biological yield, grain yield and harvest index respectively, with MLE spray at tillering + jointing + booting + heading. MLE spray only at heading gave 6.84, 3.17, 6.80 and 3.51% more 1000-grain weight, biological yield, grain yield, and harvest index respectively, as compared to control. MLE extended the seasonal leaf area duration (Seasonal LAD) by 9.22 and 6.45 d over control when applied at all growth stages and single spray at heading, respectively. MLE foliar improved salinity tolerance in wheat by improving wheat seedlings vigour, more chlorophyll *a* and chlorophyll *b* contents, exclusion of Na⁺ and Cl⁻ along with accumulation of K⁺ and larger contents of enzymatic antioxidants (catalase, superoxide dismutase and peroxidase) and non enzymatic antioxidants (ascorbic acid and total phenolic contents) leading to more 100 grain weight as compared to benzylaminpurine (BAP), hydrogen peroxide (H₂O₂) and control (water spray) under salinity. The water sprayed plants under highest salinity showed maximum accumulation of Na⁺ and Cl⁻ while largest K⁺ contents were observed in case of MLE spray under moderate salinity. MLE and BAP application improved leaf total soluble protein and superoxide dismutase (SOD). For non-enzymatic antioxidants (total phenolics and ascorbic acid), MLE was ranked first under moderate salinity. BAP improved number of grains spike⁻¹ but heavier grains were observed under MLE application in moderately saline conditions. These effects of MLE were more apparent under moderate salinity (8dS m⁻¹) as compared to higher salinity (12dS m⁻¹). The water stress caused reduction in growth and grain yield of wheat due to decreased leaf area and reduced chlorophyll *a* and chlorophyll *b* contents. However, foliar application of MLE and BAP minimized these effects of drought. MLE application produced more grain yield under moderate and severe water stress as compared to BAP, K⁺ and control. Moringa leaves being a rich source of β-carotene, protein, vitamin C, calcium and potassium and act as a good source of natural antioxidants such as ascorbic acid, flavonoid, phenolics and carotenoid, so the exogenous applications of MLE improve the antioxidant status and yield of wheat under drought stress. In case of tomato crop foliar application of MLE30 exhibited large number of flowers, more number of fruits as well as heaviest fruits. The foliar application of BAP produced same number of flowers but lighter weight fruits as compared to MLE30. The minimum fruit weight was recorded in case of foliar applied MLE10, MLE0 and control. The effectiveness of MLE as crop growth enhancer might be due to the presence of growth promoting substances. MLE proved a potential growth enhancer in mitigation of abiotic stresses.
Chapter 1

Introduction

Agriculture is facing the dual challenges of increasing crop production and climate change. Rising temperature, drought, salinity, floods, desertification and weather extreme are adversely affecting agriculture especially in developing world (IPCC, 2007). Most of the predicted population growth to 2030 will be in developing countries (Population Reference Bureau, 2011) and more than half of the work force engaged in agriculture in the third world countries is prone to more damage by climate change. Thus, there is need to improve crop productivity under changed climate, abiotic stresses and to meet the needs of increasing world populations.

Of various abiotic stresses, high temperature, salt stress and drought alone or in combination are major threats to crop productivity. Rising temperatures may lead to altered geographical distribution and growing season of agricultural crops by allowing the threshold temperature for the start of the season and earlier crop maturity (Porter, 2005). An extreme temperature shortens the growing period and adversely effects all phases of growth such as tillering, flowering and grain filling in late sown wheat. The early senescence of leaves results in too low photosynthetic rate to contribute in fixing carbon to rest of the plant (Hensel et al., 1993; Sharma-Natu et al., 2006) leading to poor quantity and quality of the harvest (Hussain et al., 2008). A series of morphological, physiological, biochemical and molecular changes may reduce expression of full yield potential of crop plants under these climatic stress conditions (Wang et al., 2001).

The decreased soil water potential causes much reduction in leaf expansion as compared to root expansion rate under drought or salinity (Kaminek et al., 1997). The water scarcity disturbs plant metabolic activities by upsetting the membrane structure, altered mineral uptake (Pospíšilová et al., 2000), reduction in the chlorophyll contents, relative water contents and membrane stability index (Tas and Tas, 2007). The limited availability of water not only reduces number of grains per spike but also decreases the grain weight in wheat (Li et al., 2000) and quality like low proteins (Garg et al., 2004).
The increased accumulation of Na$^+$ and Cl$^-$ under saline conditions leads to the reduced growth of vascular plants (Munns, 2002). Salt stress causes less germination and poor seedlings establishment in most of the crops such as wheat (Afzal et al., 2006b), maize, barley (El-Tayeb, 2005), sugar beet (Ghoulam et al., 2001), sunflower (Ashraf and Tufail, 1995), canola (Athar et al., 2009), cotton and rice (Sattar et al., 2010). Reduction in growth and yield under salinity is mainly due to salt-induced osmotic stress and specific ion toxicities (Munns and Tester, 2008). The depressed level of natural osmoprotectants and endogenous hormones are observed in several plants growing in saline soils (Debez et al., 2001).

The combined effect of extreme temperature, drought and salinity includes osmotic damages (Xiong et al., 2002), oxidative stress like increased generation of reactive oxygen species (ROS) (Mittler, 2002, Gill and Tuteja, 2010) and protein denaturation (Zhu, 2002). Biomolecules such as proteins, DNA and lipids are badly injured by reactive oxygen species (ROS) resulting in denaturation, mutation and peroxidation (Quiles and Lopez, 2004). The peroxidation of membrane lipids of plasmalemma and other cellular organelles (Candan and Tarhan, 2003) leads to cell death as a consequence of ROS toxicity.

It has been identified that plants develop many adaptations to cope with stress conditions i.e. osmotic adjustment, compartmentalization of compatible solutes, alterations in nutrients ratios specially K$^+$/Na$^+$, evapotranspiration modifications by reducing leaf size, changes in photosynthetic pigments, stimulation of plant hormones and better antioxidant scavengers (Sairam and Tyagi, 2004). The breeding programmes to develop crop plants with aforementioned traits are laborious and time consuming (Javid et al., 2011). The alternative approaches are management practices including exogenous application of various antioxidants, mineral elements and plant growth regulators (PGRs). The antioxidants increase the scavenging capacity against ROS (Mano, 2002). The antioxidants involved in detoxification of ROS exist in all plants under stress and are categorized as enzymatic such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidase (POD), glutathione reductase (GR) and monodehydroascorbate reductase (MDAR) and non-enzymatic i.e. total phenolics (TPC) and ascorbic acid (AA) (Foyer, 2002). The degree to which the amount and activities of antioxidant enzymes increase under abiotic stress is extremely variable among several plant species and even between two cultivars of the same species (Chaitanya et al., 2002). The level of response depends on the species, the
development and the metabolic state of the plant, as well as the duration and intensity of the stress.

Many plants produce significant amount of antioxidants to prevent the oxidative stress caused by photons and oxygen, they represent a potential source of compounds with antioxidant activity such as ascorbic acid, total phenols, and vitamins in addition to mineral elements K⁺, Ca²⁺ and PGRs.

Ascorbic acid is an important antioxidant, which reacts not only with H₂O₂ but also with O₂, OH and lipid hydroperoxidases. In addition to the well established ascorbic acid in animals against a wide range of ailments and diseases it has been implicated in several types of biological activities in plants such as an enzyme co-factor, as an antioxidant and as a donor/acceptor in electron transport at the plasma membrane or in the chloroplasts, all of which are related to oxidative stress resistance (Conklin, 2001). In chloroplasts ascorbate peroxidase uses ascorbic acid thereby minimizes the risk of escape and reaction of ROS with each other near PSI (Foyer and Noctor, 2000). Ascorbate also protects or regenerates oxidized carotenoids or tocopherol (Imai et al., 1999).

Plant phenolic compounds are also known for their function as antioxidants due to their free radical-scavenging capabilities (Wattenberg et al. 1980; Barclay et al. 1990; Fauconneau et al. 1997).

In addition to antioxidants plants also contain a wide range of vitamins that are essential not only for human metabolism but also for plants, because of their redox chemistry and role as cofactors, some of them also have strong antioxidant potential. The antioxidant vitamins that have been the focus of most attention in plants are carotenoids (pro-vitamin A), ascorbate (vitamin C) and tocochromanols (vitamin E, including both tocopherol and tocotrienols) (Demmig-Adams and Adams, 2002; DellaPenna and Pogson, 2006; Linster and Clarke, 2008; Foyer and Noctor, 2009: Cazzonelli and Pogson, 2010; Falk and Munne-Bosch 2010; Me’ne-Saffrane´ and DellaPenna, 2010).

To combat abiotic stresses and enhance crop yields, application of mineral nutrients is widely used. Among mineral nutrients calcium is a very important not only for cell wall and membrane stabilisation, but has also been found to be involved in the regulation of specific plant responses to environmental stresses (Braam et al., 1996). Cramer et al. (1988) depicted
that Ca\textsuperscript{2+} has alleviating effect on Na\textsuperscript{+}-induced root growth inhibition. Similarly, shortening of root growth zones was prevented by Ca\textsuperscript{2+} under saline condition (Bernstein et al., 1993). Perera et al. (1995) reported that Ca\textsuperscript{2+} increased the stomatal conductance and restores the photosynthesis. Transport of water in the root and leaf growing zones was affected with Na\textsuperscript{+}/Ca\textsuperscript{2+} ratio (Cramer, 2002). Tyerman et al. (1997) showed that salinity changed the ionic content and transport within the plants. They concluded that calcium has many regulatory functions over membrane characteristics and ionic transport in glycophytes and halophytes. For example, increasing the external Ca\textsuperscript{2+} concentration decreased the transport of Na\textsuperscript{+}, thereby reducing Na\textsuperscript{+} influx in root cells. The cross talk between Ca\textsuperscript{2+} and ROS has been studied during defense and growth responses and stomatal closure (Foreman et al., 2003; Hu et al., 2007; Mori and Schroeder, 2004). Other studies also implicated Ca\textsuperscript{2+} function both upstream and downstream of ROS production (Jiang and Zhang, 2003; Hu et al., 2007).

Potassium (K) is the most abundant cation in higher plants. K\textsuperscript{+} has been the target of some researchers mainly because it is essential for enzyme activation, protein synthesis and photosynthesis (Marschner, 1995; Silva, 2004), and it mediates osmoregulation during cell expansion, stomatal movements, tropisms, phloem solute transport and the maintenance of cation: anion balance in the cytosol as well as in the vacuole. K\textsuperscript{+} supply from soil can be rate limiting for agricultural production under conditions of osmotic and ionic stress.

Proper exogenous application of PGRs along with certain nutrients, antioxidants, organic and inorganic chemicals has been used to promote plant growth and development for inducing abiotic and biotic stress tolerance that results in higher economic return (Ashraf and Foolad, 2007; Farooq et al., 2009). Exogenous use of cytokinins improves the crop growth and yield under normal or stressful environments in a number of crop species (Zahir et al., 2001). Cytokinin retards the leaf senescence and increased the photosynthetic pigments (Galuszka et al., 2001). In a field trial with the application of a commercial cytokinin containing product, cytogen, increase in yields of corn (26.3%), rice (45.8%), pepper (24.4%), cucumber (62.9%) and cantaloupe (36.8%) has been reported (Mayeux et al., 1983). Priming with cytokinins like kinetin or benzyl amino purine (BAP) induces physiological adaptation in wheat by hormonal balance under stress conditions (Iqbal and Ashraf, 2006). BAP delayed the ROS-induced senescence of wheat leaves owing to increased activities of catalase and ascorbate peroxidase in addition to preventing chlorophyll degradation under oxidative stress (Zavaleta
et al., 2007). Under drought less reduction in total soluble protein, chlorophyll and carotenoids contents were observed by exogenous application of cytokinin like products such as thidiazuran, BAP, Kartolin 4 and Kartolin 2 (Chernyad’ev and Monakhova, 2003).

Ashraf and Foolad (2005; 2007) suggested that exogenous application of these compounds as seed priming or foliar spray enhanced endogenous level and abiotic stress tolerance. These biologically active substances can modulate plant responses to stress factors. But continuous use of synthetic chemicals and use of commercially available plant hormones as osmoprotectants and stimulators of antioxidants to quench ROS is usually not cost effective and environmentally friendly. Alternatively, toxicological effects of synthetic antioxidants and consumer preference for natural products have resulted in increased interest in the application of natural antioxidants (Castenmiller et al., 2002; Kaur and Kapoor, 2001; Koleva et al., 2002; Pizzale et al., 2002). The search for safe and effective naturally occurring antioxidants is now focused on edible plants, especially spices and herbs (Nakatani, 1997). A large number of plants have been screened as a viable source of natural antioxidants including tocopherols, vitamin C, carotenoids and phenolic compounds which are responsible for maintenance of health and protection from coronary heart diseases and cancer (Castenmiller et al., 2002; Kaur and Kapoor, 2001). Such as seaweed extract which is a natural products possess cytokinin and auxin like properties and can stimulate endogenous cytokinin activities of plants (Crouch et al., 1990). Another example is humic acid, which has auxin-like activity, not only enhances plant growth and nutrient uptake but also improves stress resistance (Zhang and Ervin, 2004). Among different natural sources used to extract PGRs and antioxidants moringa (Moringa oleifera) is gaining a lot of attention these days (Foidle et al., 2001).

Moringa is one of the 13 species of genus Moringa and family Moringnance. It is well known vegetable in Africa, Arabia, India, Southeast Asia, America and Pakistan (Sengupta and Gupta, 1970). Its roots, fruits, leaves and flowers been used as vegetables (Siddharaju and Becker, 2003). Moringa leaves are potential source of vitamin A and C, iron, calcium, riboflavin, beta-carotene and phenolic acid (Nambiar et al., 2005). Its leaves and oil are a powerful natural antioxidant (Njoku and Adikwu, 1997). Siddharaju and Becker (2003) observed antioxidant properties in the solvent extract of moringa leaves. On the basis of their results they reported that, moringa leaves are a potential source of natural antioxidants.
According to Arabshahi et al. (2007) the extracts from drumstick and carrot had a higher antioxidant activity (83% and 80%) than α-tocopherol (72%). Jongrunruangchok et al. (2010) compared the composition and mineral contents of moringa leaf obtained from different regions of Thailand and reported 19.1-28.8, 2.1-2.5, 16.3- 3.9 and 8.5-13.5 percent of protein, fat, fiber and moisture. The potassium, calcium and iron contents were in range of 1504.2 - 2054.0, 1510.4 - 2951.1 and 20.3 - 37.6 mg / 100 g dry weight basis. Moreover, moringa leaf extract (MLE) is enriched with zeatin, a purine adenine derivative of plant hormone group cytokinin (Barciszewski et al., 2000) known for stay green and stress tolerance capabilities.

It is evident from earlier mentioned reports that MLE possess antioxidants in considerable amounts but very little published literature is available that explains MLE regulated metabolic/physiological processes of wheat and other crops subjected to abiotic stress.

In view of all these reports, it is hypothesized that leaf extract from moringa, having a number of plant growth promoters, mineral nutrients and vitamins in a naturally balanced composition, may be beneficial for plant growth and development. This study was conducted to evaluate whether the adverse effects of stress on wheat plants could be mitigated by exogenous application of osmoprotectants i.e. K\(^+\), H\(_2\)O\(_2\), synthetic cytokinin benzyl amino purine (BAP) and MLE especially focusing the plant antioxidant enzyme system. The objectives of study were the optimization of MLE dose as natural plant growth enhancer in comparison to synthetic ones in different crops under normal and abiotic stresses.
Chapter 2

Review of Literature

Plant growth and productivity is regulated by a variety of external and endogenous factors. Optimum growth and development is ensured when external and internal climatic factors are within the optimum range. The external factors include different biotic and abiotic stresses. Among abiotic stresses extreme temperatures (Ferris et al., 1998), drought (Bosch and Alegre, 2004), salinity (Munns and Tester, 2008), waterlogging (Olgun et al., 2008), low or high solar radiation (Alexieva et al., 2001), phototoxic compounds (Jamal et al., 2006) and inadequate availability of mineral nutrients in soil (Fageria et al., 2010) are important. The endogenous factors comprised of mineral nutrients (Azeem and Ahmad, 2011), plant growth regulators (Pospíšilová et al., 2000), antioxidants (Shoresh et al., 2011) and plant water status (Athar and Ashraf, 2005). It is believed that biotic and abiotic stresses cause a major reduction in crop yields (Athar and Ashraf, 2009).

Plants responses to these stresses are not simple pathways, but are integrated complex circuits comprised of multiple pathways and specific cellular compartments, tissues, and the interaction of additional cofactors and/or signaling molecules to coordinate against an external stress or stimuli to show a specified response (Dombrowski, 2003).

Therefore a brief review for external stresses like extreme temperature, drought and salinity along with endogenous factors effecting plant growth and their mitigation is mentioned below:

2.1. Abiotic stresses

2.1.1. High temperature stress

In many areas of the world heat stress induced by increased temperature is one of major agricultural problems. The economic yield reduced drastically as a result of morpho physiological and biochemical changes caused by transient or constant high temperature which also badly affect plant growth and development.

2.1.1.1. Heat stress

Heat stress is often defined as the rise in temperature beyond a threshold level for a period of time sufficient to cause irreversible damage in plant growth and development (Wahid et al., 2007). In general, a transient elevation in temperature, usually 10–15°C above ambient, is
considered heat shock or heat stress. However, heat stress is a complex function of intensity (temperature in degrees), duration and rate of increase in temperature. The extent to which it occurs in specific climatic zones depends on the probability and period of high temperatures occurring during the day and/or the night. The heat stress as a result of high ambient temperatures is becoming a serious threat to crop production worldwide (Hall, 2001). A short exposure to very high temperatures, caused severe cellular injury and even cell death within minutes (Schöffl et al., 1999) whereas moderately high temperature may take long time to cause injuries or cell death. The denaturation and aggregation of protein with increased fluidity of membrane lipids were direct injuries due to high temperatures. Indirect or slower heat injuries were comprised of enzymes inactivation in mitochondria and chloroplast, retardation of protein synthesis, protein degradation and loss of membrane integrity (Howarth, 2005). These injuries eventually lead to starvation, growth inhibition, reduced ion flux, generation of toxic compounds and reactive oxygen species (ROS) (Schöffl et al., 1999; Howarth, 2005).

2.1.1.2. Heat stress threshold

The threshold temperature is important in physiological research as well as for crop production. The value of daily mean temperature at which a detectable reduction in growth begins referred as threshold temperature. The knowledge of lower and upper developmental threshold temperatures below and above which growth stops is necessary in physiological research as well as for crop production. For many plant species upper and lower developmental threshold temperatures have been determined through experimentation. The different plant species have different base or lower and upper threshold temperatures but 0°C is often predicted as best base temperature for cool season crops (Miller et al., 2001). The determination of a consistent upper threshold temperature is difficult due to variation in plant behavior depending on other environmental conditions (Miller et al., 2001). The threshold temperature values for tropical crops often higher than temperate or cool season crops. Threshold temperatures for wheat is 26°C at post-anthesis (Stone and Nicol´as, 1994), rice 34°C at grain yield (Morita et al., 2005), pearl millet 35°C at seedling (Ashraf and Hafeez, 2004), corn 38°C at grain filling (Thompson, 1986), brassica 29°C at flowering (Morrison and Stewart, 2002), cotton 45°C at reproductive (Rehman et al., 2004), tomato 30°C at emergence (Camejo et al., 2005), cool season pulses 25°C at flowering (Siddique et al.,
groundnut 34°C at pollen production (Vara et al., 2000), and cowpea 41°C at flowering (Patel and Hall, 1990).

2.1.1.2. Heat stress sensitive growth stages

The developmental stage at which the plant is exposed to the stress may determine the severity of possible damages experienced by the crop. It is, however, unknown whether damaging effects of heat episodes occurring at different developmental stages are cumulative (Wollenweber et al., 2003). Vulnerability of species and cultivars to high temperatures may vary with the stage of plant development, but all vegetative and reproductive stages are affected by heat stress to some extent. During vegetative stage, for example, high day temperature can damage leaf gas exchange properties. High temperature induced reduction in relative growth rate and net assimilation rate along with minimum effects on leaf expansion were observed in sugar cane (Wahid, 2007), pearl millet and maize (Ashraf and Hafeez, 2004). Moreover, yield of a crop species depends on amount of photosynthetic tissue, number of leaves, size of leaf or total leaf area. For example, in wheat, yield can be forecasted upon leaf area index (LAI) at flowering stage as well as crop growth rate (CGR) (Islam, 1992). The leaf area index (LAI) and crop growth rate (CGR) are determined by temperature prevails during vegetative growth period (Zhang et al., 1999). While assessing the effect of late sowing on wheat yield, Farooq et al. (2008) found that prevailing high temperature reduced LAI and CGR, which resulted in reduced yield.

From these reports mentioned above, it is suggested that increase in ambient temperature during early or late vegetative growth stage reduced yield indirectly by reducing growth. Of various plant developmental stages, reproductive stage is most sensitive to temperature stress. During reproductive stage, flowering, fertilization, and post fertilization processes markedly affected by high temperatures in most plants, which resulted in reduced crop yield. For example, a short period of heat stress can cause significant increases in floral buds and opened flowers abortion (Guilioni et al., 1997; Young et al., 2004). Similarly, impairment of pollen and anther development by elevated temperatures is another important factor contributing to decreased fruit set in many crops at moderate to high temperatures (Peet et al., 1998; Sato et al., 2006). Under high temperature conditions, earlier heading is advantageous in the retention of more green leaves at anthesis, leading to a smaller reduction in yield (Tewolde et al., 2006). Heat stress during anthesis induced sterility in many plant
species. It was concluded from controlled conditions experiments that 10-15 days after flower visibility, 8-9 days prior to anthesis and fertilization were most sensitive for high temperature stress in various crop plants (Foolad, 2005). The heat stress associated infertility and yield decline have been observed in many crops such as wheat (Ferris et al., 1998) cotton, rice (Hall, 1992), pea (Guilioni et al., 1997) and peanut (Vara Parsad et al., 1999). At the mid-anthesis stage high temperature is also injurious particularly for grain set and grain fertilization affecting number of grains per spike and grain weight, thereby, leading to low yield (Ferris et al., 1998; Siddique et al., 1999). This is because under such conditions plants tend to divert resources to cope with the heat stress and thus limited photosynthates would be available for reproductive development (Paulsen, 1994; Gifford and Thorne, 1984; Blum et al., 1994). The reduced fruit set at high temperature stress can be explained in view of the arguments of Kinet and Peet (1997) who suggested that high temperature caused reduction in carbohydrates and growth regulators biosynthesis and their translocation in plant sink tissues. In dwarf wheat variety, high temperature induced decrease in cytokinin content was found to be responsible for reduced kernel filling and its dry weight (Banowetz et al., 1999). The improvement in grain yield by BA supply was found with enhanced number of grains (Trckova et al., 1992) whereas due to increased grain weight rather than grain number (Warrier et al., 1987; Gupta et al., 2003) under normal as well as late sown conditions. The heavier grains reflected the more accumulation and partitioning of dry matter towards sinks (grains) resulting in higher harvest index under BA application during high temperature stress in late sown wheat (Gupta et al., 2003).

Thus, for successful crop production under high temperatures, it is important to know the threshold temperature, developmental stages and plant processes that are most sensitive to heat stress.

### 2.2.1. Salinity stress

Among abiotic stresses, salinity stress is becoming a major limitation to crop productivity (Munns, 2011). According to estimates, about 900 Mha land is affected with salt stress (Flowers, 2004). The presence and accumulation of salts approximately affected 30% of cultivated soils (Zhu et al., 1997). Excessive amounts of neutral soluble salts such as sodium chloride (NaCl), sodium carbonate (Na₂CO₃) and partially calcium chloride (CaCl₂) results in salty soils.
2.2.1. Adverse effects of salt stress on plant growth and yield

Salt stress causes adverse effects on plant growth and development by altering physiological and cellular processes (Greenway and Munns, 1980; Ashraf, 1994; Munns, 2002; 2011; Munns and Tester, 2008). The reduction of plant growth and dry matter accumulation under saline conditions has been reported in several important grain legumes (Tejera et al., 2006; Munns et al., 2006). It is believed that salt stress greatly reduced wheat growth at all developmental growth stages, particularly at the germination and seedling stage (Munns et al., 2006). Salt stress causes less germination and poor seedlings establishment in most of the crops such as wheat, barley, rice, alfalfa (Munns and Tester, 2008).

2.2.1.1. Bases of adverse effects of salinity

The toxic effects of salinity can be visualized from impairment of physiological and biochemical processes i.e. photosynthesis, protein synthesis and lipid metabolism thereby reducing plant growth. The salt induced growth reduction is generally attributable to water deficit or osmotic stress, specific ion toxicities, nutritional imbalances and oxidative stress (Greenway and Munns, 1980; Munns, 1993; Afzal et al., 2006a; Munns and Tester, 2008).

2.2.1.1.1. Osmotic stress

Reduction in growth and yield under salt stress is mainly due to salt induced osmotic stress (Munns and Tester, 2008). Excessive amount of soluble salts in the root environment causes osmotic stress, which may result in the disturbance of the plant water relations in the uptake and utilization of essential nutrients and also in toxic ion accumulation. As a result of these changes, the activities of various enzymes and the plant metabolism are affected (Munns, 2002; Lacerda et al., 2003). Reduction in the rate of leaf and root growth is probably due to factors associated with water stress rather than a salt specific effect (Munns, 2002). The reduced leaf area observed under highest salinity level may be due to reduction in water uptake and the nutritional imbalance causing toxicities or deficiencies of ions, so resulting in leaf injuries (Munns and Tester, 2008). The degree of growth inhibition due to osmotic stress depends on the time scale of the response, for the particular tissue and species in question, and whether the stress treatments are imposed abruptly or slowly (Ashraf, 1994; Munns et al., 2000). Mild osmotic stress leads rapidly to growth inhibition of leaves and stems, whereas roots may continue to grow and elongate (Hsiao and Xu, 2000).
2.2.1.1.2. Specific ion toxicities

Besides salt-induced osmotic stress, accumulation of toxic salts also causes growth suppression in plants. Dominant salts found in salt-affected soils are generally Na\(^+\), Cl\(^-\), SO\(_4\)\(^{2-}\), and HCO\(_3\) which result in severe toxicity. However, the crops show variations in their sensitivities to different toxic ions. It is generally believed that excessive accumulation of Na\(^+\) causes nutrient imbalance, thereby resulting in specific ion toxicity (Greenway and Munns, 1980; Grattan and Grieve, 1999). In salt-sensitive species or at higher salinity levels, plant loses its ability to control Na\(^+\) transport as a result salt-induced ionic effect dominates the osmotic effect (Munns and Tester, 2008). In most species, accumulation of Na\(^+\) to a toxic level appears to occur more rapidly than Cl\(^-\), therefore most studies focused on Na\(^+\) exclusion and that of Na\(^+\) transport control within the plant (Munns and Tester, 2008). For example, more accumulation of Na\(^+\) and Cl\(^-\) was observed in all parts of guava, especially in the leaves thereby resulting in reduced growth (Ferreira et al., 2001). In addition to Na\(^+\) being a toxic ion, Cl\(^-\) is considered to be the more toxic ion in some species such as soybean, citrus, and grapevine (Lauchli, 1984; Grattan and Grieve, 1999; Storey and Walker, 1999). Thus, the accumulation of any cation or anion in excessive amounts in growth medium can cause toxicity and growth reduction depending upon species or cultivar.

2.2.1.1.3. Nutritional imbalances

The interactions of salts with mineral nutrients may result in considerable nutrient imbalances and deficiencies (McCue and Hanson, 1990). Ionic imbalance occurs in the cells due to excessive accumulation of Na\(^+\) and reduces uptake of other mineral nutrients such as K\(^+\), Ca\(^{2+}\), and Mn\(^{2+}\) (Karimi et al., 2005). High sodium to potassium ratio due to accumulation of high amounts of sodium ions inactivates enzymes and affects metabolic processes in plants (Booth and Beardall, 1991). Excess Na\(^+\) and Cl\(^-\) inhibits the uptake of K\(^+\) and leads to the appearance of symptoms like those in K\(^+\) deficiency. The deficiency of K\(^+\) initially leads to chlorosis and then necrosis (Gopal and Dube, 2003). The role of K\(^+\) is necessary for osmoregulation and protein synthesis, maintaining cell turgor and stimulating photosynthesis (Freitas et al., 2001). Both K\(^+\) and Ca\(^{2+}\) are required to maintain the integrity and functioning of cell membranes (Wenxue et al., 2003). Maintenance of adequate K\(^+\) in plant tissue under salt stress seems to be dependent upon selective K\(^+\) uptake and selective cellular K\(^+\) and Na\(^+\) compartmentation and distribution in the shoots (Munns et al., 2000;
Carden et al., 2003). The maintenance of calcium acquisition and transport under salt stress is an important determinant of salinity tolerance (Soussi et al., 2001; Unno et al., 2002). Salt stress decreases the Ca\textsuperscript{2+}/Na\textsuperscript{+} ratio in the root zone, which affects membrane properties due to displacement of membrane associated Ca\textsuperscript{2+} by Na\textsuperscript{+}, leading to dissolution of membrane integrity and selectivity (Kinraide, 1998). The increased levels of Na\textsuperscript{+} inside the cells change enzyme activity resulting in cell metabolic alteration. Disturbance in K\textsuperscript{+} uptake and partitioning in the cells and throughout the plant may even affect stomatal opening thus diminishing the ability of the plant to grow. Externally supplied Ca\textsuperscript{2+} has been shown to ameliorate the adverse effects of salinity on plants, presumably by facilitating higher K\textsuperscript{+}/Na\textsuperscript{+} selectivity (Hasegawa et al., 2000). Another key role attributed to supplemental Ca\textsuperscript{2+} addition is its help in osmotic adjustment and growth via the enhancement of compatible organic solutes accumulation (Girija et al., 2002). From above discussion it is clearly evident that reduced accumulation of Ca\textsuperscript{2+}, K\textsuperscript{+} and Mg\textsuperscript{2+} with increased uptake and accumulation of Na\textsuperscript{+} greatly reduced crop growth and yield under salt stress.

2.2.1.4. Oxidative stress

Salt stress causes oxidative stress in plants i.e., the production of reactive oxygen species (ROS) such as H\textsubscript{2}O\textsubscript{2} (hydrogen peroxide), 1\textsuperscript{O} (singlet oxygen) and OH\textsuperscript{-} (hydroxyl radical). Many biomolecules such as proteins, DNA and lipids are badly injured by ROS resulting in cell death (Apel and Hirt, 2004). The decrease in the content and the activity of various antioxidants in response to salt stress has been reported in several species (Shalata and Neumann, 2001; Al-Hakimi and Hamada, 2001; Athar et al., 2008) and is regarded as one of the mechanism explaining, at least in part, the deleterious effects of salinity on crops. Plants use two systems to defend against and repair damage caused by oxidizing agents. First, the enzymatic antioxidant system which is mainly represented by superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and ascorbate peroxidase (ASPX) (Harinasut et al., 1996), then, the non-enzymatic antioxidant system, consisting of molecules involved in ROS scavenging such as ascorbic acid (vitamin C), alpha-tocopherol (vitamin B), β-carotene, glutathione (tripeptide) (Piotr and Klobus, 2005). Several studies have demonstrated the existence of a close positive correlation between the rate and extent of the increase in antioxidant activity and plant salt tolerance (Sairam et al., 2005). The significant decrease in cytokinin concentration was observed in roots and shoots of barley cultivars under salinity
(Goicoechea et al., 1995). According to Zhang and Ervin (2008) the improvement in stress tolerance of creeping bent grass with increased SOD level has been observed by the application of cytokinin containing sea weed extract (SWE). It becomes clearly evident that antioxidant status of plant for scavenging of ROS is an important salt tolerant trait.

2.2.1.2. Crop growth stage and salt stress

The plants differ in their extent of salt tolerance at different stages of vegetative and reproductive growth. Germination is the most crucial stage in this regard (Ahmad and Jabeen, 2005). Seed often fails to germinate in salt affected soils. The reduction in rate of seed germination and final germination percentage were observed under high salt contents (Fowler, 1991). Almost all stages of growth and development, flowering and fruit set were adversely affected by salinity, thereby, causing low economic yield and poor quality of production (Ashraf and Harris, 2004).

2.2.1.3. Salinity and photosynthetic capacity

The yield of a crop is mostly affected by its photosynthetic ability (Akram et al., 2002) which is one of the main contributing factors in salt induced reduction in plant growth and yield. Tolerance of the photosynthetic system to salinity depends on how effectively a plant excludes or compartmentalizes the toxic ions. Plant characteristics such as leaf surface area, CO$_2$ assimilation and yield negatively correlated with salinity. According to Hanaa et al. (2008) considerable decease in chlorophyll $a$ and $b$ contents were observed in wheat plants irrigated with sea water as compared to normal water irrigated plants. It may be attributed that more Na$^+$ at higher salinity enhanced degradation of chlorophyll or decreased its biosyntheses by lowering Mg$^{2+}$, an important constituent of chlorophyll (Rubio et al., 1995). Salt stress enhances the accumulation of NaCl in chloroplasts and is often associated with decrease in photosynthetic electron transport activities (Kirst, 1989). In higher plants, salt stress inhibits photosystem (PS-II) activity (Kao et al., 2003), although some studies showed contrary results (Brugnoli and Björkman, 1992).

From these reports it is summarized that salt induced growth inhibition may be resulted from reduction in photosynthetic capacity. A number of factors such as photosynthetic pigments, amount of photosynthesizing tissue or leaf area, gas exchange and metabolism etc. affect the
photosynthetic capacity. Plant’s tolerance to salinity depends on how the photosynthetic machinery may be protected from osmotic and toxic effects of salt stress.

2.3.1. Drought stress

Global climate change makes drought a serious threat to food security world wide (Elisabeth et al., 2009). Crop productivity is determined by the total amount of precipitation and also by its distribution during the growing season (Loss and Siddique, 1994; Slafer, 2003). It has been estimated that the countries that generate two-third of the world’s agricultural product experience water deficit conditions on a regular basis (Revenga et al., 2000). For instance, China, Australia, many African and South American countries, the Middle East, Central Asia, and many states of the United States often experience severe drought conditions (http://www.globalresearch.ca/index.phpcontext¼ava&aid¼12252), resulting in significant decline in food grain production. The projected changes in climate in the coming years may exacerbate the adverse effects of drought not only in food crops but also in other economically important crops. The severity of drought is unpredictable as it depends on many factors such as occurrence and distribution of rainfall, evaporative demands and moisture storing capacity of soils (Wery et al., 1994).

A brief review of drought induced changes in crop plants is mentioned below:

2.3.1.1. Osmotic stress

Exposure of plants to drought stress substantially decreases the leaf water potential, relative water content and transpiration rate, with a concomitant increase in leaf temperature (Siddique et al., 2001). The ratio between dry matter produced and water consumed is termed as water use efficiency at the whole-plant level (Monclus et al., 2005). Abbate et al. (2004) concluded that under limited supply, water use efficiency of wheat was greater than in well-watered conditions. They correlated this higher water-use efficiency with stomatal closure to reduce the transpiration. Drought causes a significant reduction in plant water content and cell turgor potential, which in most cases results in reduced growth rate and final crop yield.

2.3.1.2. Nutrient stress

An important effect of water deficit is on the acquisition of nutrients by the root and their transport to shoots. Lowered absorption of the inorganic nutrients can result from interference in nutrient uptake and the unloading mechanism, and reduced transpirational flow (Garg, 2003; McWilliams, 2003). The nutrients uptake by the plants were reduced under
drought stress conditions, the nitrogen and phosphorus being stored in grains while K accumulation sites were stem and leaves. 67 and 82 % reduction was observed in K uptake under mild and severe water stress conditions (Baque et al., 2006). The insufficient K supply induced reduction of photosynthesis under drought stress (Cakmak, 2005). As K played a role in CO2 fixation during photosynthesis so the plants suffering from drought exhibit more K requirements (Cakmak and Engels, 1999). If K become deficient under drought stress additional disturbances were observed in water relations, stomatal movements and photosynthesis (Marschner, 1995).

2.3.1.3. Oxidative stress

Similar to many other stresses, drought can cause oxidative stress in plants, under which reactive oxygen species (ROS) such as superoxide radical (O²⁻), hydroxyl radical (OH⁻), hydrogen peroxide (H₂O₂), and alkoxy radical (RO) are produced (Munne-Bosch and Penuelas, 2003). ROS can damage cell membranes, nucleic acids, and proteins (Ashraf, 2009; Mittler, 2002), causing metabolic imbalances in plants. A linear relationship exists between generation of reactive oxygen species and severity of drought, which enhanced membrane lipids peroxidation, disintegration of nucleic acid and degradation of proteins (Farooq et al., 2009). Simultaneously, plants produce a variety of antioxidants that counteract the generation of ROS in response to drought stress (Munne-Bosch and Penuelas, 2003; Wang et al., 2009). A reduction in the activities of superoxide dismutase, peroxidase, catalase and protein contents in leaves of maize crop towards the physiological maturity were observed under water stress (Ti-da et al., 2006). The enhanced activities of SOD, CAT and POD in maize leaves were observed during light water stress and then reduced under severe stress but their levels were even better than fully irrigated plants (Ti-da et al., 2006).

2.3.1.4. Hormonal imbalance

Drought stress often leads to hormonal imbalances (Bajguz and Hayat, 2009; Farooq et al., 2009), changes in activities of enzymes responsible for regulation of key metabolic processes (Tu’rkan et al., 2005) and modulation of signal transduction (Chaves et al., 2003), gene expression (Denby and Gehring, 2005), respiration (Ribas-Carbo et al., 2005), and photosynthesis (Flexas et al., 2004). The hormonal status of plant cells might be balanced by addition of cytokinin during water stress (Banowetz, 1998). The chlorophyll contents were significantly improved by exogenous application of growth regulators under water scarcity
conditions (Zhang et al., 2004). In the presence of cytokinin like activity compounds e.g. Kartolin less reduction was observed in protein contents (Chernyad’ev and Monakhova, 2003). The positive effects of BAP and TDZ on protein pool were also pronounced under drought stress. The phytoregulators exhibiting cytokinin activity show protective effects in water deficit and prevent reduction in chlorophyll and protein contents. The dehydration stress drastically decreases the cytokinin level. The decreased contents under drought stress may be due to reduced cytokinin biosyntheses or its enhanced degradation (Pospíšilová et al., 2000). The lesser quantities of cytokinin was also observed in xylem sap under drought stress (Dodd, 2003) emphasized the need for exogenous application of cytokinin.

2.3.1.5. Crop growth stage and drought stress

The reduced germination and seedling stand is the first and foremost effect of drought (Kaya et al., 2006). In a study on pea, drought stress impaired the germination and early seedling growth of five cultivars tested (Okcu et al., 2005). Moreover, in alfalfa (Medicago sativa), germination potential, hypocotyl length and shoot and root fresh and dry weights were reduced by polyethylene glycol induced water deficit, while the root length was increased (Zeid and Shedeed, 2006). Water deficit during pollination increased the frequency of kernel abortion in maize (Zea mays). In pigeon pea, drought stress coinciding with the flowering stage reduced seed yield by 40–55% (Nam et al., 2001) Post-anthesis drought stress was detrimental to grain yield regardless of the stress severity (Samarah, 2005). Following heading, drought had little effect on the rate of kernel filling in wheat but crop duration (time from fertilization to maturity) was shortened and caused dry weight reduction at maturity (Wardlaw and Willenbrink, 2000). This yield reduction was due to reduced grain filling duration rather than grain filling rate (Wardlaw and Willenbrink, 2000). Water stress applied at pre-anthesis reduced time to anthesis while at post anthesis it shortened the grain filling period in triticale genotypes (Estrada-Campuzano et al., 2008) and badly effected grain yield irrespective of its severity (Samarah, 2005).

2.3.1.6. Photosynthesis and drought stress

Drought stress produced changes in photosynthetic pigments and components (Anjum et al., 2003), damaged photosynthetic apparatus (Fu and Huang, 2001) and diminished activities of calvin cycle enzymes which are important causes of reduced crop yield (Monakhova and Chernyadèv, 2002). The leaf senescence visualized initially by leaf yellowing due to
chlorophyll degradation and decrease in the ratio of chlorophyll to carotenoids has been observed during drought induced leaf senescence in many field crops (Yang et al., 2002). A 13-15 % reduction in chlorophyll content was observed in wheat seedlings on 7th day followed by drought exposure (Nikolaeva et al., 2010). The chlorophyll a, b and leaf soluble protein contents were decreased under osmotic stress conditions (Singh et al., 1998). Water deficiency caused a 30-40% reduction in soluble protein contents of adult leaves (Chernyad’ev and Monakhova, 2003). Another important effect that inhibits the growth and photosynthetic abilities of plants is the loss of balance between the production of reactive oxygen species and the antioxidant defense (Fu and Huang, 2001; Reddy et al., 2004).

2.3.1.7. Crop yield and drought stress

Drought stress adversely affects a variety of vital physiological and biochemical processes in plants, leading to reduced growth and final crop yield. Reduction in yield depends on water stress severity, duration and plant developmental stage at which plant experience water stress (Plaut, 2003). In barley, drought stress reduced grain yield by reducing the number of tillers, spikes, grains per plant and individual grain weight. In maize, water stress reduced yield by delaying silking thus increasing the anthesis to silking interval. This trait was highly correlated with grain yield, specifically ear and kernel number per plant (Cattivelli et al., 2008). Drought stress in soybean reduced total seed yield and the branch seed yield (Frederick et al., 2001). In pearl millet (Pennisetum glaucum), co-mapping of the harvest index and panicle harvest index with grain yield revealed that greater drought tolerance was achieved by greater partitioning of dry matter from stover to grains (Yadav et al., 2004). Moisture deficit reduced cotton (Gossypium hirsutum) lint yield, due to reduced boll production as a result of fewer flowers and greater boll abortions (Pettigrew, 2004). In rice, on the other hand, water stress imposed during the grain filling period enhanced remobilization of pre-stored carbon reserves to grains and accelerated grain filling (Yang et al., 2001). The prevailing drought reduces plant growth and development, leading to hampered flower production and grain filling and thus smaller and fewer grains. A reduction in grain filling occurs due to a reduction in the assimilate partitioning and activities of sucrose and starch synthesis enzymes.
2.2. Mitigation of abiotic stresses

Abiotic stresses may cause yield reduction as much as 50% in most major crop plants (Bray et al., 2000). Extreme temperatures, drought, salinity and oxidative stress are often interrelated and may cause cellular damage (Wang et al., 2003). Abiotic stresses altered levels of plant growth regulators (PGRs) resulted in decreased plant growth (Morgan, 1990). Plants acclimatized to these abiotic stresses by certain physiological and biochemical modifications. Abiotic stress tolerance in crop plants is associated with osmotic adjustment, ion exclusion, maintenance of water status, higher nutrient acquisition ability, higher plant hormone concentrations, higher photosynthetic capacity or antioxidant capacity (Athar and Ashraf, 2009). Based on this information, various scientists suggest a number of strategies to manage or improve abiotic stress tolerance in plants. Of various cultural practices or management practices, early sowing to avoid high temperature, shortage of water at reproductive stage is most important. Water stress at later phases of grain filling were due to shortage of assimilate but if vegetative phase of plants extended and thus increased the duration of photosynthesis, it provides more photoassimilates to grain that resulted in improved the grain weight. Duration of vegetative phase or active photosynthetic period can be managed by adjusting time of planting.

Similarly, if considerable amount of genetic variability exists in a crop species, some abiotic stress tolerant individuals can be selected. Moreover, through conventional breeding, abiotic stress tolerant traits can be introgressed in stress sensitive cultivars. In addition, with the advancement of molecular biology techniques, genes for specific traits can also be transformed in crops to improve stress tolerance (Wang et al., 2003; Ashraf et al., 2008; Athar and Ashraf, 2009). The serious complexities in screening and conventional breeding result in slow pace in development of stress resistance methods and pose a major limitation in use of theses methodologies for induction of stress tolerance.

Since stress tolerant plants had higher compatible solutes, nutrients, plant hormones, antioxidant compounds than those of stress sensitive cultivars, Ashraf and Foolad (2005; 2007) suggested that exogenous application of these compounds as seed priming or foliar spray enhanced endogenous level and abiotic stress tolerance. These biologically active substances can modulate plant responses to stress factors. These strategies are discussed below:
2.2.1 Seed priming

Seed priming is an effective tool to minimize time between germination and emergence which resulted in synchronized emergence (Harris et al., 2002). In some earlier studies it has been observed that seed priming with plant growth regulators, inorganic salts, compatible solutes or sugar beet extract caused improvement in seed germination by providing physiological and biochemical adaptations (Pill and Savage, 2008; Afzal et al., 2006a). It is largely known that higher or enhanced mobilization of metabolites/inorganic solutes to germinating plumule/embryo result in enhanced growth (Taiz and Zeiger, 2002). An improvement in seedling vigor was observed with CaCl\(_2\) and CaCl\(_2\) + NaCl priming in greenhouse conditions (Ruan et al., 2002). Moreover the higher accumulation of Na\(^+\) and Cl\(^-\) were also observed in seedlings raised from CaCl\(_2\) or NaCl primed seeds. The salinity decreased the protein contents but remarkable increase in protein content was obtained from CaCl\(_2\) seed priming (Afzal et al., 2006b) which may be due to better defense of membrane and membrane bound enzymes. Moreover the highest value of 1000 kernel weight in rice was produced by KCl followed by CaCl\(_2\) seed priming (Farooq et al., 2006). Similarly, priming with cytokinins like kinetin or benzyl amino purine (BAP) increased salt tolerance in wheat at seedling stage (Iqbal and Ashraf, 2006). In another study with pigeon pea, the adverse effects of salinity on germination were mitigated by seed priming with kinetin and ascorbic acid (Jyotsna and Srivastava, 1998). Plant stress tolerance can be increased by seed priming with cytokinin (Iqbal et al., 2006). The negative effects of NaCl on wheat chlorophyll contents can be alleviated by application of benzyl amino purine (Mumtaz et al., 1997).

2.2.2 Foliar application

2.2.2.1 Exogenous foliar application of nutrients

In an extensive review on the role of mineral nutrients in improving stress tolerance, Grattan and Grieve (1999) stated that foliar applications of those nutrients which become deficient under stress conditions improved the nutrient status of plants thereby leading to increased stress tolerance. For example, potassium has been extensively used as a foliar spray to enhance crop salt tolerance (Ashraf et al., 2008). For increased active uptake of K\(^+\) by the guard cells exogenous application of K\(^+\) was required (Premachandra et al., 1993) under stress conditions. Ahmad and Jabeen (2005) found that foliar spray of plants with potassium
containing material minimized the antagonistic effects of soil salinity. The K⁺ uptake was improved by increasing application of K⁺ under water scarcity (Baque et al., 2006). In tomato, sunflower and lemongrass an increased vegetative growth, total nitrogen and total carbohydrate content were observed by foliar application of vitamin (thiamine) (Abdel-Halim, 1995 Tarraf et al., 1999 and Gamal El-Din, 2005). Akram et al. (2007) found that foliar spray of various inorganic salts of K⁺ in sunflower caused considerable improvement in the ion homeostatic conditions and plant photosynthetic activity through stomatal movement. Moreover, the extent of stress ameliorative effect of K⁺ salts thereby growth enhancement depends upon plant developmental stage of foliar application, salt concentration and accompanying anion in a specific salt. Thus, foliar application of mineral nutrients can be beneficial to improve crop salt tolerance.

2.2.1.2 Exogenous foliar application of PGRs

The other effective approach is the exogenous application of plant growth regulators (PGRs) involved in promoting plant growth and development under normal as well as stressful conditions (Brathe et al., 2002). Many attempts have been made previously for exogenous application of cytokinin (natural or synthetic) to enhance grain cytokinin content and the increment in number of grains and final grain weight was based upon growth stage i.e. time of application (Wang et al., 2001; Gupta et al., 2003; Yang et al., 2003). According to Gupta et al. (2003) during post anthesis temperature stress the exogenous application of benzyl adenine increased grain weight only in heat tolerant wheat genotypes under late sown but in all genotypes irrespective of their tolerance under normal sowing conditions. The significant increment in grain weight of wheat by attracting more assimilates towards the developing grain was observed with the application of benzyl adenine at anthesis (Warrier et al., 1987). Moreover more grain weight was observed when BAP was sprayed on ears i.e. at physiological maturity (Hosseini et al., 2008). In soybean not any modification in plant growth trait was observed by foliar application of cytokinin during vegetative growth period (Leite et al., 2003). The chlorophyll contents, total protein and tomato yield were increased under foliar spray of cytokinin (Nasr, 1993). Shahbaz et al. (2008) observed that foliar applied brassinosteroids counteracted the adverse effects of salt stress on growth of wheat but did not improve the grain yield. Similarly, foliar applied salicylic acid counteracted the NaCl deleterious effects on sunflower (Noreen and Ashraf, 2008).
2.2.1.3. Exogenous application of antioxidants

Ascorbic acid a potent antioxidant, the foliar application of ascorbic acid minimized the reduction of chlorophyll \( a \) by NaCl in wheat (Khan et al., 2006). Foliar application of ascorbic acid enhanced accumulation of macronutrient (N, P and K) in sweet pepper (Talaat, 2003). Emam and Helal (2008) found that foliar spray of ascorbic acid recovered the non-germinating flax seeds under saline conditions. The reduction in activities of antioxidants enzymes superoxide dismutase (14.82%), catalase (31.84%) and peroxidase (26.34%) were observed under abiotic stresses but the foliar spray of CaCl\(_2\) and salicylic acid not only improved protein content but also showed enhancements in antioxidant enzyme activities indicating positive regulatory effect of CaCl\(_2\) and salicylic acid on antioxidant defense system in tomato leaves (Li et al., 2009).

2.2.3. Sources of nutrients, PGRs and antioxidants

The exogenous use of commercially available mineral nutrients, antioxidants and cytokinin to alleviate the adverse effects of abiotic stresses on growth is expensive. So there is a need to explore the natural sources of PGRs for exogenous use such as algae extract (Hanaa et al., 2008), humic acid (HA), seaweed extract (SE) (Zhang and Ervin, 2008) and extract obtained from moringa (Moringa oleifera) leaves (Foidle et al., 2001).

According to Duval and Shetty (2001) the total phenolic contents in green pea shoots were enhanced under foliar spray of high cytokinin containing anise root extracts combined with yeast extract.

2.2.3.1. Microalgae

Microalgae constitute a potential source of antioxidant, vitamins, carotenoids, polysaccharides, phycobiliprotein and posses immune-modulating agent properties (El-Baz, et al., 2002; Abd El-Baky, et al., 2003 and 2004). It can be used widely in medicine, industry, marine culture and in combating pollution (Thajuddin and Subramanian, 2005). The presence of auxin, cytokinins, gibberellins and other growth regulating substances proved it a plant growth stimulating agent (Ördög et al., 2004). For instance, seaweed extracts (SE) have been used as a cytokinin-like growth regulator and exhibit multiple functions including stimulation of shoot growth and branching (Temple and Bomke, 1989), increase root growth and lateral root development (Metting et al., 1990), improve nutrient uptake (Yan, 1993), enhance resistance to diseases (Featoiby-Smith and Staden, 1983) and environmental
stresses such as drought and salinity (Nabati et al., 1994). Proper application of SE-based cytokinins may be an effective approach to improve summer performance of creeping bent grass. Application of SE enhanced SOD activity, photosynthetic capacity and chlorophyll content in tall fescue, especially at 4 weeks after treatment. The more increment in chlorophyll $a$ and $b$ were observed under foliar spray of algal extract as compared to foliar spray of bioregulator i.e. ascorbic acid and benzyl adenine in saline conditions (Hanaa et al., 2008). Moreover, the antioxidant status of wheat plant was positively correlated with antioxidant level of algal extract.

2.2.3.2. Humic acid

The foliar spray of humic acid not only improved growth and nutrients uptake of some crops but also enhanced their yields (Padem et al., 1999; Neri et al., 2002). Humic acid nutrient composition leads to a 25% reduction in soil applied NPK fertilizer requirement with increase in uptake of N, P, K, Ca, Mg, Fe, Mn, Zn and Cu nutrients by the crop resulting to enhanced yield (Shaaban et al., 2009). According to Neri et al., (2002) the wetting action and slow speed of droplet drying make the humic acid best suited for foliar spray. The significantly higher nutrient contents were observed in leaves as compared to control under foliar spray of humic acid (Guvenc et al., 1999). The significant positive effects of humic acid application were found on yield of faba bean and chlorophyll contents of soybean (Shuixiu and Ruizhen, 2001). 21-38% more root mass was observed in creeping bent grass by foliar application of cytokinin containing seaweed extract + humic acid under drought stress (Zhang and Ervin, 2004).

2.2.2.3. Moringa oleifera

The "Moringa" tree is grown mainly in semi-arid, tropical, and subtropical areas. It is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan (Fahey, 2005). It grows best in dry sandy soil; it tolerates poor soil including coastal areas. It is a fast growing, drought resistant tree. Today it is widely cultivated in Africa, Central and South America, Sri Lanka, India, Mexico, Malaysia, Indonesia and the Philippines. Moringa is a short, slender, deciduous, perennial tree about 10 m tall with drooping branches, brittle stems and branches, corky bark, feathery pale green 30–60 cm long compound leaves, with many small leaflets which are 1.3–2 cm long, 0.6–0.3 cm wide, fragrant white or creamy-white flowers having 2.5 cm in diameter and borne in sprays, pendulous brown triangular pods,
splitting lengthwise into 3 parts when dry, containing about 20 dark brown seeds embedded in the pith, pod tapering at both ends. Main root is thick (Foidle et al., 2001). It produces fruit between April to June in Pakistan. It is considered one of the world’s most useful tree, as almost every part of the Moringa tree can be used for food or has some other beneficial property. In the tropics, it is used as forage for livestock, and in many countries moringa micronutrient liquid, a natural anthelmintic (kills parasites) and adjuvant (to aid or enhance another drug) is used as a metabolic conditioner to aid against endemic diseases in developing countries (Foidle et al., 2001). Moringa oleifera is the most nutrient rich plant yet discovered. Moringa provides a rich and rare combination of nutrients, amino acids, antioxidants, antiaging and anti-inflammatory properties used for nutrition and healing.

*M. oleifera* is a miracle tree with a great indigenous source of highly digestible proteins, Ca, Fe and vitamin C (Fahey, 2005). Some articles and research studies have reported that the dry leaves of *M. oleifera* contain 7 times more vitamin C than orange, 10 times vitamin A than carrot, 17 times calcium than milk, 15 times potassium than bananas, 25 times iron than spinach and 9 times proteins than yogurt (Fuglie, 1999). In addition, it contains vitamin B-complex, chromium, copper, magnesium, manganese, phosphorus and zinc (Fuglie, 2000). Thurber and Fahey (2009) stated *M. oleifera* leaves as rich protein source, which can be used by doctors, nutritionists and community health conscious persons to solve worldwide malnutrition or under nutrition problems. According to researchers moringa has the potential to combat vitamin A and other micronutrient deficiencies (Nambiar, 2006). 40139 μg/100g total carotenoides on fresh weight basis in moringa leaves of which 47.8% or 19210 μg/100g was β-carotene. Ascorbic acid at 6.6 mg/g on dry weight basis, 0.26 mg/g Fe, 22.4 mg/g calcium, 6.3 mg/g P, 11.2 mg/g oxallic acid and 0.9 g/100 g fiber. Moringa has been in use since centuries for nutritional as well medicinal purposes. Another important point is that Moringa leaves contain all of the essential amino acids, which are the building blocks of proteins. It is very rare for a vegetable to contain all of these amino acids. Moringa contains these amino acids in a good proportion, so that they are very useful to our bodies. Given its nutritional value, it can be utilized in fortifying sauces, juices, spices, milk, bread, and most importantly, instant noodles. Many commercial products like Zija soft drink, tea, and nutroceuticals are available all over the globe.
2.2.2.3.1 Moringa leaf extract (MLE)

A plant growth spray made from moringa leaves increased crop production 20-35%. Spray affects the crops by longer life-span, heavier roots, stems and leaves, produce more fruit, larger fruit and increase in yield 20-35% (Foidle et al., 2001), highlighting its opportunity of use as a foliar spray to accelerate growth of young plants. MLE proved an ideal plant growth enhancer in many experiments (Makkar and Becker 1996; Nouman et al., 2011). Makkar et al. (2007) found the moringa leaves as a source of plant growth factors, antioxidants, β-carotene, vitamin C, and antioxidant agents. Sidduraju and Becker (2003) studied the antioxidants properties of moringa leaf extract and demonstrated that it: (1) reduced potassium ferricyanide, (2) scavenged superoxide radicals, (3) prevented the peroxidation of lipid membrane in liposomes, (4) could donate hydrogen and scavenge radicals. Cai et al. (2004) observed positive correlation between total phenolic contents and antioxidant activities of methanolic as well as aqueous extracts of Chinese medicinal plants. The aqueous extract obtained from *Andrographis paniculata* leaves showed more phenolic content and antioxidant potential as compared to extract from other parts such as stem and fruits (Arash et al., 2010). Makkar et al. (2007) found that MLE contains significant quantities of calcium, potassium, and cytokinin in the form of zeatin, antioxidants proteins, ascorbates and phenols. MLE priming in rangeland grass *Echinochloa crusgalli* showed encouraging results and significantly increased the shoot vigour along with improved number of leaves and fertile tillers (Nouman et al., 2011). MLE was used as priming agent in hybrid maize. Seed primed with moringa leaf extract (MLE) diluted to 30 times with tap water increased the germination speed and spread and seedling vigor under cool conditions (Noman, 2008). According to Mehboob (2011) high temperature at planting delayed the seedling emergence in control while seed priming treatments resulted in earlier and vigorous seedling stand. Among all the strategies, osmopriming with MLE diluted 30 times reduced mean emergence time (MET) (8.967 vs. 9.097 days) and increased final emergence (FEP) (83.33 vs. 86.333) under optimum as well late planted conditions as compared to control. Agronomic and yield related traits were significantly affected by seed priming at both sowing dates. Maximum number of grains rows per cob (34.933 vs. 31.500), total kernel rows per cob (14.30 vs 13.63) and higher number of grains per cob (1271.0 vs 1114.0) were recorded for MLE priming.
Similarly improved biological (66.75 vs 60.53 t ha\(^{-1}\)) and economical yield (6.97 vs 6.23 t ha\(^{-1}\)) were recorded for osmopriming with MLE under both optimum and delayed planted conditions. Increased yield by MLE priming was attributed to enhanced seedling emergence, chlorophyll contents and cell membrane permeability. The foliar application of MLE and BAP may also stimulate earlier cytokinin formation thus preventing premature leaf senescence and resulting in more leaf area with higher photosynthetic pigments (Hanaa et al., 2008; Rehman and Basra, 2010).

Abiotic stresses severely reduced the crop productivity world wide thus these becoming a major threat for food security. Based on physiological and molecular mechanisms of abiotic stress tolerance, various strategies have been advised to improve crop tolerance against abiotic stresses including screening, selection, breeding and genetic engineering etc. However, based on physiological and biochemical bases of stress tolerance in crops, scientists suggested exogenous use of compatible solutes, antioxidants compounds, mineral nutrients, and plant growth regulators as a shotgun approach. Since synthetic compatible osmolytes, antioxidants and plant growth regulators are costly, use of plant extracts having appreciable amount of these compounds could be an economically viable strategy. Among naturally occurring plant growth enhancers, Moringa oleifera has attained enormous attention because of having cytokinin, antioxidants, macro and micro nutrients in its leaves. Therefore, in the present study, moringa leaf extract was exogenously applied to assess up to what extent it can improve abiotic stress tolerance in some potential crops such as wheat, tomato and pea. MLE applied through seed priming and foliar application to identify the effective mode of application. Its secondary objective was to evaluate the dose of exogenous application of MLE under normal as well as stressful conditions especially focusing the plant antioxidant enzyme system.
CHAPTER 3

MATERIAL AND METHODS

The study was conducted to evaluate the efficacy of *Moringa oleifera* leaf extract (MLE) as a natural crop growth enhancer. The modes of application were seed treatment, soil and foliar application on wheat, peas and tomato crops under normal and abiotic stress conditions. The experiments were conducted under laboratory, wire house and field conditions. The details regarding experimental site and materials used during course of these studies are described here in general whereas the specific methodology is discussed under particular experiments.

3.1. Source of moringa leaves

Young leaves / branches of moringa were harvested from young full grown trees located at the experimental nursery area of Department of Forestry, Range Management and Wildlife, University of Agriculture, Faisalabad.

3.2. Analysis of moringa leaves

The oven dried moringa leaves were analysed for their nutrient contents i.e. total nitrogen (Ryan *et al*., 2001; Jackson, 1962), phosphorous, potassium, calcium, magnesium, copper, iron, manganese and zinc (Ryan *et al*., 2001) and boron (Gaines and Mitchell, 1979; Bingham, 1982). The fresh moringa leaves were used for determination of total soluble proteins (Bradford, 1976), enzymatic antioxidants catalase (CAT), peroxidase (POD) (Chance and Maehly, 1955), superoxide dismutase (SOD) (Giannopolitis and Ries, 1977) and nonenzymatic antioxidants such as total phenolics (Ainsworth and Gillespie, 2007) and ascorbic acid (Yin *et al*., 2008) were also determined (See details in section 3.6.3.3 and 3.6.3.4). The detailed analysis report is mentioned in Table 3.1.

3.3. Preparation of MLE

For preparation of MLE, leaf extraction was performed according to Price (2007), by grinding young shoots (leaves and tender branches) with a pinch of water (1 L / 10 kg fresh material) in a locally fabricated extraction machine (see Plate 1). After sieving through
Table 3.1 Bio-chemical composition of moringa leaf

<table>
<thead>
<tr>
<th>Name of nutrient element / enzymes</th>
<th>Moringa leaf (3replicates) ±S.E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total soluble protein (mg g⁻¹)</td>
<td>1.40 ± 0.003</td>
</tr>
<tr>
<td>Enzymatic antioxidants (IU min⁻¹ mg⁻¹ protein)</td>
<td></td>
</tr>
<tr>
<td>Super oxide dismutase (SOD) EC number (1.15.1.1)</td>
<td>191.86 ± 8.482</td>
</tr>
<tr>
<td>Peroxidase (POD) EC number (1.11.1.7)</td>
<td>21.99 ± 0.073</td>
</tr>
<tr>
<td>Catalase (CAT) EC number (1.11.1.6)</td>
<td>7.09 ± 0.045</td>
</tr>
<tr>
<td>Non-enzymatic antioxidants</td>
<td></td>
</tr>
<tr>
<td>Total phenolic contents (mg g⁻¹ GAE)</td>
<td>8.19 ± 0.007</td>
</tr>
<tr>
<td>Ascorbic acid (m mole g⁻¹)</td>
<td>0.36 ± 0.001</td>
</tr>
<tr>
<td>Nitrogen (%)</td>
<td>1.933 ± 0.145</td>
</tr>
<tr>
<td>Phosphorus (%)</td>
<td>0.180 ± 0.21</td>
</tr>
<tr>
<td>Potassium (%)</td>
<td>2.187 ± 0.104</td>
</tr>
<tr>
<td>Calcium (%)</td>
<td>2.433 ± 0.088</td>
</tr>
<tr>
<td>Magnesium (%)</td>
<td>0.012 ± 0.002</td>
</tr>
<tr>
<td>Zinc (mg kg⁻¹)</td>
<td>38.333 ± 0.667</td>
</tr>
<tr>
<td>Copper (mg kg⁻¹)</td>
<td>3.500 ± 0.289</td>
</tr>
<tr>
<td>Iron (mg kg⁻¹)</td>
<td>544.000 ± 2.082</td>
</tr>
<tr>
<td>Manganese (mg kg⁻¹)</td>
<td>49.667 ± 1.764</td>
</tr>
<tr>
<td>Boron (mg kg⁻¹)</td>
<td>21.333 ± 1.202</td>
</tr>
</tbody>
</table>
Plate 1: Locally fabricated moringa Leaf Juice extraction machine
cheese cloth the extract was centrifuged for 15 min at 8000 × g. Various dilutions MLE0, MLE10, MLE20, MLE30 and MLE40 (diluted to 0, 10, 20, 30 and 40 times with water respectively) of the extract were prepared with distilled water then used in experiments for priming as well as foliar spray.

3.4. Plant material

Seeds of wheat cv. Sehar-2006, tomato cv. Sahil and pea cv. Climax used as plant material were obtained from Vegetable Research Institute and Wheat Research Institute of Ayub Agriculture Research Institute, Faisalabad, Pakistan.

3.5. Crop season

The experiments were done during October-April 2008-2009 for pea and tomato and November- April 2008-2009 and 2009-2010 for wheat crop. Fig. 3.1 and 3.2 represented the meteorological data recorded during course of experiments.

3.6. Experimental sites

The analysis and optimization of MLE dilutions were done at laboratory, Dept. of Crop Physiology, Univ. of Agriculture, Faisalabad. The assessment of method for exogenous application of MLE was conducted in pea and tomato crop under green house and walk in plastic tunnel (high tunnel), respectively. The evaluation of MLE as priming agent in wheat was performed in pots under greenhouse conditions. The role of MLE in mitigating stress like salinity and drought in wheat was assessed in pots under wire-net house conditions of Department of Crop Physiology, University of Agriculture Faisalabad, Pakistan. The effect of MLE on wheat late sowing stress was evaluated under field conditions of the Experimental Farm of Department of Crop Physiology, University of Agriculture Faisalabad, Pakistan during 2008- 10.

3.7. Experiment I: Optimization of moringa oleifera leaf extract (MLE) dilutions

<table>
<thead>
<tr>
<th>Design</th>
<th>Completely Randomized Design (CRD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replications</td>
<td>3</td>
</tr>
<tr>
<td>Medium</td>
<td>Petriplates (5 seeds in each plate)</td>
</tr>
</tbody>
</table>

30
Fig. 3.1. Weather data for October 2008 to April 2009

Fig. 3.2. Weather data for October 2009 to April 2010

Source: Agricultural Meteorology Cell, Department of Crop Physiology, University of Agriculture, Faisalabad
The detail of treatments was as under

i. Control (Distilled water)
ii. MLE20 (20 times diluted MLE)
iii. MLE30 (30 times diluted MLE)
iv. MLE40 (40 times diluted MLE)

The treatments were applied to completely moisten the Whatman no.1 filter paper. Seven days after germination; seedlings were carefully harvested to record the data for the following parameters.

3.7.1. **Shoot and root lengths (cm)**
Shoot and root lengths in centimeters were measured with the help of scale at the time of harvest. The length was measured from the point where the root and shoot joins to the end of root for root length and to the top of shoot for shoot length.

3.7.2. **Shoot and root fresh and dry weights (g)**
After harvesting the seedling, the shoot was cut from root at the point where they joined together. The fresh weight was recorded for each part separately. The samples were dried in an oven at 70°C up to constant dry weight.

On the basis of higher germination and seedling vigor, 30 times diluted MLE was selected for further studies.

3.8. **Experiment II: Evaluation of MLE as priming agent**

<table>
<thead>
<tr>
<th>Design</th>
<th>Completely Randomized Design (CRD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replications</td>
<td>3</td>
</tr>
<tr>
<td>Medium</td>
<td>Earthen pots filled with 10 kg soil @ sand + silt + compost (1+1+1)</td>
</tr>
</tbody>
</table>

(see the soil analysis report presented in Table 3.2)

25 seeds per pot were sown. Three seedlings per pot were maintained at 10-days after sowing (DAS). Tap water was used for irrigation when required. Fertilizer application was done @ 120-100 kg NP ha\(^{-1}\).

**Treatments**

i. Control (unprimed)
ii. MLE10
iii. MLE30
iv. CaCl\(_2\) (-1.25 MPa) (Farooq *et al.*, 2006)
Table 3.2 Analysis report for soil filled in pots

<table>
<thead>
<tr>
<th>Soil Characteristics</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECe</td>
<td>4.03 dS m(^{-1})</td>
</tr>
<tr>
<td>pHs</td>
<td>7.53</td>
</tr>
<tr>
<td>SAR</td>
<td>12.65</td>
</tr>
<tr>
<td>TSS</td>
<td>45.06 m.mol L(^{-1})</td>
</tr>
<tr>
<td>Na(^+)</td>
<td>33.14 m.mol L(^{-1})</td>
</tr>
<tr>
<td>Cl(^-)</td>
<td>22.97 m.mol L(^{-1})</td>
</tr>
<tr>
<td>K(^+)</td>
<td>0.47 m.mol L(^{-1})</td>
</tr>
</tbody>
</table>

Ece= Electrical conductivity, TSS= Total soluble salts, SAR= Sodium adsorption ratio
v. HP (Hydro priming) (Harris et al., 2001)
vi. OFP (On-farm priming) (Harris et al., 2001)

The priming period was evaluated according to (Harris et al., 2001). For CaCl₂ priming, seeds were soaked in aerated 50 mmol solution of CaCl₂ for 12 h at 20°C in the dark and redried up to original weight with forced air under shade following Basra et al. (2005). In hydropriming, the seeds were soaked in distilled water (1:5 seed to solution volume used) and dried back near to original moisture with forced air under shade. 25 seeds were used in each pot. After emergence, three seedlings were maintained per pot. Data for the following parameters were collected.

3.8.1. Emergence parameters

3.8.1.1. Emergence index (EI)

EI was calculated according to the Association of Official Seed Analyst (1990):

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

3.8.1.2. Mean emergence time (MET)

MET was calculated as described by Ellis and Robert (1981) as:

\[ \text{MET} = \frac{\sum Dn}{\sum n} \]

Where \( n \) is the number of seeds which were germinating on day and \( D \) is the number of days counted from the beginning of emergence.

3.8.1.3. Time to 50% emergence (\( E_{50} \))

Time taken to 50 % emergence of seedlings was calculated according to Farooq et al. (2005):\n
\[ E_{50} = t_i + \left[ \frac{N/2 - n_i}{n_j - n_i} \right] \times (t_j - t_i) \]

Where \( N \) is the final number of germinated seeds; while \( n_i \) and \( n_j \) are the cumulative number of seeds emerged by adjacent counts at the times \( t_i \) and \( t_j \) where \( n_i < N/2 < n_j \).

After 14 days of emergence the seedlings will be evaluated for seedling vigor.

3.8.2. Seedling vigor:

3.8.2.1. Shoot and root lengths (cm)

Shoot and root lengths were measured as described in section 3.7.1.

3.8.2.2. Shoot and root fresh and dry weights (g)

Shoot and root fresh and dry weights were determined according to the section 3.7.2.
3.8.2.3. Leaf area plant$^{-1}$ (cm$^2$)

Leaf area was measured on leaf area meter (CI-203 Laser leaf area meter, CID Inc., USA) 75 days after sowing (DAS) when plants were fully grown.

3.8.3. Analytical parameters:

The leaves were analysed for following parameters.

3.8.3.1. Leaf chlorophyll contents

For determination of leaf chlorophyll contents, grinding of 0.5 g leaf sample was done in 80% acetone to extract chlorophyll. The absorbance of filtrate was determined at 663 and 645 nm and the chlorophyll contents were calculated with the following formula described by Arnon (1949).

\[
\text{Chlorophyll } a \ (\text{mg g}^{-1}) = \frac{(0.0127 \times A_{663} - 0.00269 \times A_{645}) \times 100}{0.5}
\]

\[
\text{Chlorophyll } b \ (\text{mg g}^{-1}) = \frac{(0.0229 \times A_{645} - 0.00468 \times A_{663}) \times 100}{0.5}
\]

3.8.3.2. Total soluble protein (mg g$^{-1}$)

The determination of total soluble protein was consist of following steps

3.8.3.2.1. Total soluble protein extraction / Sample preparation

For extraction of total soluble proteins, 0.5 g of fresh plant material (leaves) was ground in a pre-chilled pastor mortar by adding 1 mL extraction buffer with pH 7.2. Before extraction of proteins cocktail protease inhibitors in a concentration of 1 µM were added to the buffer. The buffer used was phosphate buffer saline (PBS) containing 10 mM Na$_2$HPO$_4$, 2 mM KH$_2$PO$_4$, 2.7 mM KCl and 1.37 mM NaCl dissolved in distilled water and made up to a volume 1 L. The pH 7.2 of PBS was adjusted with HCl and then autoclaved (Sambrook and Russell, 2001). The ground leaf material was centrifuged at 12000 x g for 5 min. Supernatant was preserved in a separate centrifuge tube for the analysis of soluble proteins, while the pellet was discarded.

3.8.3.2.2 Determination of total soluble protein

Total soluble proteins were determined followed by Bradford assay (Bradford, 1976). For construction of standard curve 10, 20, 30, 40 and 50 µg mL$^{-1}$ were prepared from bovine serum albumin (BSA) by adding 400 µL Dye stock (Biorad, USA) and distilled water followed by vortexed and incubation at room temperature for 30 min. The absorbance was recorded at 595 nm using spectrophotometer (UV 4000 UV-VIS spectrophotometer). The absorbance for the sample supernatant was also determined in a similar way. Concentration
(mg mL⁻¹) of total soluble or heat stable fractions of proteins was determined from standard curve (Fig. 3.3) prepared from absorbance of bovine serum albumin (BSA) and computed by applying the formula: slope x absorbance / mL of extract used.

### 3.8.3.3. Enzymatic antioxidants (IU min⁻¹ mg protein⁻¹)

For enzymatic antioxidants determination of leaf sample, extraction was done in 5 ml of 50 mM phosphate buffer (pH 7.8), after centrifugation at 15000 × g for 20 min, the supernatant was used in further assay for superoxide dismutase (SOD) activity (Giannopolitis and Ries, 1977), peroxidase and catalase activity (Chance and Maehly, 1955) by recoding absorbance at 560, 240 and 470 nm respectively.

#### 3.8.3.3.1. Superoxide dismutase EC number (1.15.1.1) (IU min⁻¹ mg protein⁻¹)

The SOD activity inhibits the photochemical reduction of nitroblue tetrazolium (NBT) at 560 nm. The monitoring of this inhibition is used to assay SOD activity. The reaction mixture was prepared by taking 50 µL enzyme extract and adding 1 mL NBT (50 μM), 500 µL methionine (13 mM), 1mL riboflavin (1.3 μM), 950 µL (50 mM) phosphate buffer and 500 µL EDTA (75 mM). This reaction was started by keeping reaction solution under 30 W fluorescent lamp illuminations and turning the fluorescent lamp on. The reaction stopped when the lamp turned off 5 min later. The NBT photo reduction produced blue formazane which was used to measure the increase in absorbance at 560 nm. The same reaction mixtures without enzyme extract in dark were used as blank. The SOD activity was determined and expressed as SOD IU min⁻¹ mg⁻¹ protein (Giannopolitis and Ries, 1977).

#### 3.8.3.3.2. Peroxidase (POD) EC number (1.11.1.7) (m.mol min⁻¹ mg protein⁻¹)

The POD activity assayed by guaiacol oxidation and defined as 0.01 absorbance change min⁻¹ mg⁻¹ protein. The reaction mixture was prepared by adding 400 µL guaiacol (20 mM), 500 µL H₂O₂ (40 mM) and 2 mL phosphate (50 mM) in 100 µL enzyme extract. The change in absorbance at 470 nm of the reaction mixture was observed every 20 s up to 5 min. The POD activity expressed as m.mol min⁻¹ mg protein⁻¹ (Chance and Maehly, 1955).
Fig. 3.3. Standard curve of protein estimation

Table 3.3 Dilutions of bovine serum albumin (BSA)

<table>
<thead>
<tr>
<th>BSA (µL)</th>
<th>Water (µL)</th>
<th>Dye stock (µL)</th>
<th>Total volume (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1600</td>
<td>400</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>1590</td>
<td>400</td>
<td>2</td>
</tr>
<tr>
<td>20</td>
<td>1580</td>
<td>400</td>
<td>2</td>
</tr>
<tr>
<td>30</td>
<td>1570</td>
<td>400</td>
<td>2</td>
</tr>
<tr>
<td>40</td>
<td>1560</td>
<td>400</td>
<td>2</td>
</tr>
<tr>
<td>50</td>
<td>1550</td>
<td>400</td>
<td>2</td>
</tr>
</tbody>
</table>

\[ y = 0.0043x + 0.0906 \]

\[ R^2 = 0.9924 \]
3.8.3.3 Catalase (CAT) EC number (1.11.1.6) ($\mu$ mol min$^{-1}$ mg protein$^{-1}$)

The CAT activity assayed by the decomposition of $H_2O_2$ and change in absorbance due to $H_2O_2$ was observed every 30 s for 5 min at 240 nm using a UV-visible spectrophotometer. Reaction mixture for CAT contained 900 $\mu$L $H_2O_2$ (5.9 mM) and 2 mL phosphate buffer (50 mM). Reaction was started by adding 100 $\mu$L enzyme extract to the reaction mixture. The Catalase activity was expressed as $\mu$mol of $H_2O_2$ min$^{-1}$ mg protein$^{-1}$ (Chance and Maehly, 1955).

3.8.3.4. Non-enzymatic antioxidants

3.8.3.4.1. Total phenolic content (mg g$^{-1}$)

Total phenols of leaf samples were determined at 760 nm using gallic acid as reference standard (Ainsworth and Gillespie, 2007). To create calibration curve (Fig. 3.4) the standard solutions were prepared in different concentrations i.e. 500, 250, 150 and 100 mg L$^{-1}$ gallic acid. For extraction and preparation of sample, 0.5 g leaf sample was homogenized in 5 mL acetone (80 %). Filtration followed by extraction and volume of filtrate was raised with acetone up to 10 mL. Then reaction mixture was prepared by taking 20 $\mu$L sample or standard and adding 1.58 mL water, 100 $\mu$L Folin-Ciocalteu reagents within 30 s up to 8 min. Reaction mixture was left at 40 °C for 30 min or at 25 °C for 120 minutes after adding and mixing 300 $\mu$L sodium carbonate. Absorbance of reaction mixture was read at 760 nm against the blank (80% acetone). Total phenol levels in samples were determined by plotting calibration curve from standards (Fig. 3.3) and reported as Gallic Acid Equivalent, GAE.

3.8.3.4.2 Ascorbic acid (m.mol g$^{-1}$)

Ascorbic acid of leaf samples was measured according to Yin et al. (2008). The protocol is given below:

3.8.3.4.2.1 Reagents

1. Trichloroacetic acid (TCA) 10%
2. Trichloroacetic acid (TCA) 5%
3. Phosphoric acid (44%)
4. 100 mM Phosphate Buffer Saline (BPS) PH 7.8
5. $FeCl_3$ (3%)
Table 3.4 Preparation of Gallic acid standards

<table>
<thead>
<tr>
<th>Stock solution</th>
<th>Gallic acid (g)</th>
<th>Ethanol (ml)</th>
<th>Water (ml)</th>
<th>Total volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5</td>
<td>10</td>
<td>90</td>
<td>100</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Standard (mg L⁻¹)</th>
<th>Stock (conc.) mg mL⁻¹</th>
<th>Stock (ml)</th>
<th>Water (ml)</th>
<th>Total volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>10</td>
<td>90</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>5</td>
<td>95</td>
<td>100</td>
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</tr>
<tr>
<td>150</td>
<td>3</td>
<td>97</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>2</td>
<td>98</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 3.4: Standard curve of total phenolic content estimation

\[ y = 0.001x - 0.0088 \]

\[ R^2 = 1 \]

Abs.  
Linear (Abs.)
6. 2,2- biphienyl (dissolved in 75% ethanol)

3.8.3.4.2.2. Standards
Standard curve was plotted using ascorbic acid as a reference standard. The stock solution of 100 µg mL\(^{-1}\) or 10 mg 100mL\(^{-1}\) was prepared in distilled water. From stock solution different dilutions i.e. 20, 40, 60, 80 and 100 µg /mL were prepared (Table 3.5).

3.8.3.4.2.3. Procedure
0.5 g of plant material (leaf) was ground in a pre-cold mortar on ice in presence of 2 ml TCA (5 %) with a little clean sand. After grinding, centrifuge at 12,000 x g for 20 min at 4°C. The supernatant was used for ascorbate determination whereas pellet was discarded. Sample mixtures was prepared by taking 200 µL of the supernatant + 1.4 mL PBS and mixed well. It was then incubated at room temperature for 1 min. After incubation, 0.4 mL TCA (10%), 44% phosphoric acid (0.4 mL), 0.2 mL 2, 2-biphienyl and 0.2 mL FeCl\(_3\) (3 %) were added and mixed. This mixture was again incubated at 35 °C for 60 min in water bath. The absorbance was measured at 525 nm using 100 mM PBS as a control. From this absorbance value, ascorbic acid of samples (m mol g\(^{-1}\) fresh weight) was determined on the base of standard curve plotted using absorbance value recorded for various standards (Fig.3.5).

3.8.4 Yield parameters
At maturity plants were harvested and threshed manually to record following yield related parameters.

3.8.4.1 Number of grains per spike
The grains threshed and counted separately from spikes of each pot and then averaged for number of grains per spike.

3.8.4.2 100 Grain weight (g)
From each pot, hundred grains were randomly separated and their weight was recorded.

3.8.4.3 Grain yield per plant (g)
Whole of the manually threshed produce were weighed to obtain grain yield per pot.

3.9. Experiment III: Evaluating the growth and development of Tomato to exogenous application of MLE (pot study)

**Design:** Factorial Completely Randomized Design (CRD Factorial arrangement)

**Medium** Earthen pots filled with 10 kg soil @ sand + silt + compost (1+1+1) (see the soil analysis in Table 3.1)
Table 3.5 Dilutions for ascorbic acid standard solutions

<table>
<thead>
<tr>
<th>Stock solution (100µg ml⁻¹)</th>
<th>water (ml)</th>
<th>Total volume (µg ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 ml</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td>4 ml</td>
<td>6</td>
<td>40</td>
</tr>
<tr>
<td>6 ml</td>
<td>4</td>
<td>60</td>
</tr>
<tr>
<td>8 ml</td>
<td>2</td>
<td>80</td>
</tr>
<tr>
<td>10 ml</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

\[ y = -0.0139x + 1.4641 \]
\[ R^2 = 0.9995 \]

Fig. 3.5 Standard curve of ascorbic acid estimation
**Factor A**  (Modes of application):

1. Soil application;
2. Foliar spray

**Factor B**  Treatments

i.  Control (distilled water)
ii.  MLE0
iii.  MLE10
iv.  MLE20
v.  MLE30
vi.  50 mg L$^{-1}$ BAP

MLE foliar or soil applications were started 30 days after sowing @ 25 mL/plant and repeated on weekly basis till plant maturity. Data for following parameters were recorded.

**3.9.1. Number of vegetative branches per plant**

Number of vegetative branches per plant was counted weekly and then was averaged.

**3.9.2. Number of reproductive branches per plant**

Number of reproductive branches per plant was counted weekly and then was averaged.

**3.9.3 Number of flowers per plant**

Number of flowers per plant were counted weekly and then averaged.

**3.9.4. Number of fruits per plant**

Number of fruits per plant were counted weekly and then averaged.

**3.9.5. Fruit yield per plant (g)**

The fruits harvested from individual plant and their weight was averaged.

**3.9.6. Leaf chlorophyll ‘a’ and ‘b’ contents (mg g$^{-1}$)**

Chlorophyll contents were calculated with the formula described by Arnon (1949) and mentioned in section 3.8.3.1.

**3.9.7. Total soluble proteins (mg g$^{-1}$)**

Total soluble proteins were determined by Bradford (1976) as described in section 3.8.3.2.

**3.9.8. Enzymatic antioxidants (IU min$^{-1}$ mg protein$^{-1}$)**

For determination of enzymatic antioxidants (IU min$^{-1}$ mg protein$^{-1}$) extraction and sample preparation was done as described in section 3.8.3.3.
3.9.8.1. Superoxide dismutase (SOD) (IU min\(^{-1}\) mg protein\(^{-1}\))
SOD activity was determined by Giannopolitis and Ries (1977) as described in section 3.8.3.3.1.

3.9.8.2. Peroxidase (POD) (m.mol min\(^{-1}\) mg protein\(^{-1}\))
POD activity was determined by following protocol of Chance and Maehly (1955) as explained in section 3.8.3.3.2.

3.9.8.3. Catalase (CAT) (µ mol min\(^{-1}\) mg protein\(^{-1}\))
Catalase activity was determined according to Chance and Maehly (1955) as given in section 3.8.3.3.3.

3.9.8.4. Leaf total phenolic contents (mg GAE g\(^{-1}\))
Total phenolic contents in leaf samples were determined as mentioned in section 3.8.3.4.1. (Ainsworth and Gillespie, 2007).

3.9.8.5. Fruit lycopene contents (µg g\(^{-1}\))
For determination of fruit lycopene contents, 1g fruit sample was ground / homogenized in acetone-hexane (4:6) according to the method described by Fish et al. (2002). The sample was shaked for 15 min on magnetic stirrer. Then 3 ml deionized water was added in each sample. The sample was again shaked on ice for 5 min. The separation of sample in two phases was achieved by leaving it at room temperature for 5 min. The absorbance of the hexane (upper) layer was observed at 503 nm and hexane alone used as a blank (Ravelo-Pérez et al., 2008). The lycopene contents were determined by using formula

\[
\text{Lycopene content (µg g}^{-1}\text{)} = (A_{503} – 0.0007) \times 30.3/\text{g tissue}
\]

3.10. Experiment IV: Evaluating the growth and development of pea to application of MLE (pot study)

**Design:** Factorial Completely Randomized Design (CRD Factorial arrangement)

**Medium:** Earthen pots filled with 10 kg soil @ sand + silt + compost (1+1+1) (see the soil analysis in Table 3.5)

**Factor A** (Modes of application):
1. Soil application;
2. Foliar spray

**Factor B** Treatments
i. Control (distilled water)
ii. MLE0 (100 % pure MLE)
MLE foliar or soil applications were started 30 days after sowing @ 25 ml/plant and repeated on weekly basis till maturity. Data for following parameters were collected.

3.10.1. Number of vegetative branches per plant
Same as given in section 3.9.1.

3.10.2. Number of reproductive branches per plant
Same as given in section 3.9.2.

3.10.3. Number of flowers per plant
Same as given in section 3.9.3.

3.10.4. Number of fruits per plant
Number of fruits per plant were counted weekly and then averaged.

3.10.5. Fruit yield per plant (g)
All the fruits harvested from an individual plant weighed and then averaged.

3.10.6. Leaf chlorophyll ‘a’ and ‘b’ contents (mg g⁻¹)
Chlorophyll contents were calculated with the formula described by Arnon (1949) and mentioned in section 3.8.3.1.

3.10.7. Leaf total phenolic contents (mg GAE g⁻¹)
Total phenolic contents in leaf samples were determined as mentioned in section 3.8.3.4.1. (Ainsworth and Gillespie, 2007).

3.11. Experiment V: Evaluating the response of late sown wheat to foliar application of MLE under field conditions

Design Randomized complete block design (RCBD)
Replications 3
Medium Field conditions (Soil was clayey loam in texture)
Net plot size 5.0 m × 2.0 m
Seed rate 110 kg ha⁻¹
Sowing date 16 December, 2008
For all experiments of stress conditions, 30 times diluted MLE foliar spray was selected, because it was found to be most effective in III and IV experiments conducted under normal conditions.

**Treatments:**

- \( T_1 \) = Foliar spray of water (control)
- \( T_2 \) = Foliar application of MLE at tillering stage (t)
- \( T_3 \) = Foliar application of MLE at t + jointing stage (j)
- \( T_4 \) = Foliar application of MLE at t + j + booting stage (b)
- \( T_5 \) = Foliar application of MLE at t + j + b + heading stage
- \( T_6 \) = Foliar application of MLE at heading stage

The quantity of MLE used in foliar spray was decided after calibration of hand sprayer on the basis of leaf saturation. All cultural practices were kept constant. Following parameters were studied during course of experiment.

### 3.11.1. Growth Traits

#### 3.11.1.1. Leaf area index (LAI)

Leaf area per plant in a unit area of 1 m\(^2\) was measured as explained in section 3.6.2.3. The ratio of leaf area to ground area was estimated to determine leaf area index. Data on LAI were started to record 50 DAS and continued on weekly bases up to 95 DAS.

#### 3.11.1.2. Seasonal leaf area duration (SLAD) days

\[
SLAD \ = \ (LAI_1 \ + \ LAI_2) \times (T_2 \ - \ T_1) \ / \ 2 \ \ (Reddy, 2004)
\]

Where \( LAI_1 \) was the leaf area index value recorded first time in the crop growing season and \( LAI_2 \) was the value of leaf area index calculated last time at crop maturity. \( T_2 \ - \ T_1 \) was the time interval of these two readings.

#### 3.11.1.3. Crop growth rate (CGR) (g m\(^{-2}\) day\(^{-1}\))

For estimation of crop growth rate plants were harvested in a unit area of one m\(^2\) and oven dried to obtain constant dry weight. This was started 50 DAS and continued up to 95 DAS on weekly bases. The 4 values of CGR were calculated during this period by using the formula mentioned below:

\[
CGR \ = \ (W_2 \ - \ W_1) \ / \ (T_2 \ - \ T_1) \ \ (Reddy, 2004)
\]

- \( W_1 \) = oven dried weight at first sampling
- \( W_2 \) = oven dried weight at second sampling
\[ T_1 = \text{time of first sampling} \]
\[ T_2 = \text{time of second sampling} \]

3.11.1.4 Net assimilation rate (NAR) (g m\(^{-2}\) day\(^{-1}\))

Net assimilation rate was started to estimate from 50 DAS and repeated on weekly bases up to 95 DAS. The 4 values of NAR were obtained during this period. Following formula was used to determine NAR.

\[ \text{NAR} = \frac{\text{TDM}}{\text{LAD}} \]

Where
\[ \text{TDM} = \text{Total dry matter accumulated} \ (W_2 - W_1) \]
\[ \text{LAD} = \frac{(\text{LAI}_1 + \text{LAI}_2) \times (T_2 - T_1)}{2} \]

3.11.2. Yield Traits

3.11.2.1. Number of fertile tillers (m\(^{-2}\))

At maturity, number of fertile tillers was counted manually in an area of 1 m\(^2\).

3.11.2.2. Number of grains per spike

At maturity, 10 spikes were selected randomly and their number of grains were counted and then averaged.

3.11.2.3. 1000 grain weight (g)

After threshing, 1000 grains were randomly counted and weighed from the produce of each plot.

3.11.2.4. Biological yield (t ha\(^{-1}\))

Whole above-ground plant biomass was harvested from each plot to measure biological yield in kg, which was converted in to biological yield t ha\(^{-1}\).

3.11.2.5. Grain yield (t ha\(^{-1}\))

The produce of each plot threshed separately to obtain grain yield and then converted in to t ha\(^{-1}\).

3.11.2.6. Harvest index (%)

The ratio of grain yield to biological yield was determined to obtain harvest index.
3.12. Experiment VI: Mitigating effects of salinity stress in wheat by MLE application

30 times diluted MLE used as priming or foliar application and was compared with control, a synthetic cytokinin (BAP) and \( \text{H}_2\text{O}_2 \) to evaluate the effectiveness of MLE in mitigating salinity stress (Wahid et al., 2007).

**Design**

- **CRD (Factorial arrangement)**

**Replications**

- 3

**Medium:**

- soil + compost + sand (1+1+1)

**Seed rate:**

- 25 seeds in each pot (At completion of emergence three seedlings were maintained per pot)

**Factor A** Salinity levels (4, 8 and 12 dS m\(^{-1}\)).

Prior to the development of salinity in soil medium used to fill the pots, analysis of soil was carried out to determine the soil nutrients status (For Soil analysis, see Table 3.5). \( \text{NaCl} \) was used to achieve the required level of salinity i.e. 4, 8 and 12 dS m\(^{-1}\) according to USDA Laboratory Staff (1954). The soil was analysed again to confirm the actual level of salinity developed.

**Factor B** Treatments

i. Control;

ii. MLE30 foliar application

iii. Benzyl amino purine foliar application (BAP, 50 mg L\(^{-1}\)) (Amin et al., 2007)

iv. \( \text{H}_2\text{O}_2 \) foliar application (120 \( \mu \)M) (Wahid et al., 2007)

Foliar application was started one week after completion of emergence and kept repeating on weekly basis @ 25 ml / plant up to maturity.

3.12.1 Seedling vigor

3.12.1.1. Shoot and root lengths (cm)

Shoot and root lengths were measured as described in section 3.7.1.

3.12.1.2. Shoot and root fresh and dry weights (g)

Shoot and root fresh and dry weights were determined as described in section 3.7.2.

3.12.1.3. Leaf area plant\(^{-1}\) (cm\(^2\))

Leaf area was measured as mentioned in section 3.8.2.3.
3.12.2. Analytical parameters
Leaves were randomly selected from each treatment after 75 days of sowing and analysed for following parameters.

3.12.2.1 Leaf chlorophyll ‘a’ and ‘b’ contents (mg g⁻¹)
Chlorophyll contents were calculated with the formula described by Arnon (1949) and mentioned in section 3.8.3.1.

3.12.2.2 Leaf Na⁺ and K⁺ contents
The Na⁺ and K⁺ contents in leaves were determined by flame photometer (USDA Laboratory Staff, 1954).

3.12.2.3 Leaf Cl⁻ contents
The hot water treatment was used for estimation of leaf Cl⁻ contents (USDA Laboratory Staff, 1954).

3.12.2.4 Total soluble protein (mg g⁻¹)
Total soluble proteins were determined by Bradford (1976) as described in section 3.8.3.2.

3.12.2.5 Enzymatic antioxidants (IU min⁻¹ mg protein⁻¹)
For determination of enzymatic antioxidants (IU min⁻¹ mg protein⁻¹), the same protocol was followed as described in section 3.8.3.3.

3.12.2.5.2 Superoxide dismutase (SOD) (IU min⁻¹ mg protein⁻¹)
SOD activity was determined by Giannopolitis and Ries (1977) as described in section 3.8.3.3.1.
3.12.2.5.3. Peroxidase (POD) (m.mol min\(^{-1}\) mg protein\(^{-1}\))
POD activity was determined by following the protocol of Chance and Maehly (1955) as explained in section 3.8.3.3.2.

3.12.2.5.4. Catalase (CAT) (µ mol min\(^{-1}\) mg protein\(^{-1}\))
Catalase activity was determined according to Chance and Maehly (1955) as given in section 3.8.3.3.3.

3.12.2.6. Non enzymatic antioxidants
3.12.2.6.1. Total phenolic contents (TPC) mg g\(^{-1}\)
Total phenolic contents in leaf samples were determined as mentioned in section 3.8.3.4.1. (Ainsworth and Gillespie, 2007).

3.12.2.6.2. Ascorbic acid (mmole g\(^{-1}\))
Ascorbic acid of leaf samples was measured according to Yin et al. (2008) as given in section 3.8.3.4.2.

3.12.3. Yield parameters:
At maturity plants were harvested and threshed manually to record following yield related parameters.

3.12.3.1. Number of grains per spike
The procedure mentioned in section 3.6.4.1 was followed to estimate number of grains per spike.

3.12.3.2. 100 Grain weight (g)
100 grain weight was determined as described in section 3.6.4.2.

3.13. Experiment VII: Mitigating effects of drought stress in wheat by MLE application

<table>
<thead>
<tr>
<th>Design</th>
<th>Factorial Completely Randomized Design (Factorial CRD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replications</td>
<td>3</td>
</tr>
<tr>
<td>Medium</td>
<td>Soil+ compost + sand (1+1+1) in earthen pots (soil analysis given in Table 3.5)</td>
</tr>
<tr>
<td>Stage of stress induction:</td>
<td>1 week old plants</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Factor A</th>
<th>Drought Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>i.</td>
<td>Well watered</td>
</tr>
<tr>
<td>ii.</td>
<td>Moderate drought</td>
</tr>
</tbody>
</table>
iii. Severe drought (50% Field capacity)

**Factor B** Treatments

i. Control

ii. MLE foliar application (optimized dilution level i.e. 30 times MLE)

iii. Benzyl amino purine foliar application (BAP, 50 mg L\(^{-1}\)) (Amin *et al.*, 2007)

iv. K\(^+\) (SOP 2%) (Ismail, 2005)

Foliar application was started one week after imposition of stress and repeated on weekly basis @ 25 mL plant\(^{-1}\) up to maturity.

During course of experiment, the following observations were recorded.

**3.13.1 Leaf area plant\(^{-1}\) (cm\(^2\))**

Leaf area was measured as mentioned in section 3.8.2.3.

**3.13.2 Analytical parameters**

Leaves were randomly selected from each treatment 75 days after sowing (DAS) and analysed for following parameters.

**3.13.2.1 Leaf chlorophyll ‘a’ and ‘b’ contents (mg g\(^{-1}\))**

Chlorophyll contents were analysed as mentioned in section 3.8.3.1. Chlorophyll contents were calculated with the formula described by Arnon (1949).

**3.13.2.2 Leaf Cl\(^-\) contents**

The hot water treatment was used for estimation of leaf Cl\(^-\) contents following the protocol of USDA Laboratory Staff (1954).

**3.13.2.3. Total soluble protein (mg g\(^{-1}\))**

Total soluble proteins were determined by Bradford (1976) as described in section 3.8.3.2.

**3.13.2.4. Enzymatic antioxidants (IU min\(^{-1}\) mg protein\(^{-1}\))**

For determination of enzymatic antioxidants (IU min\(^{-1}\) mg protein\(^{-1}\)), extraction and sample preparation was done as described in section 3.8.3.3.

**3.13.2.4.1. Superoxide dismutase (SOD) (IU min\(^{-1}\) mg protein\(^{-1}\))**

SOD activity was determined by following the protocol of Giannopolitis and Ries (1977) as described in section 3.8.3.3.1.

**3.13.2.4.2. Peroxidase (POD) (mmol min\(^{-1}\) mg protein\(^{-1}\))**

POD activity was determined by following protocol of Chance and Maehly (1955) as explained in section 3.8.3.3.2.
3.13.2.4.3. Catalase (CAT) (µ mol min⁻¹ mg protein⁻¹)

Catalase activity was determined according to Chance and Maehly (1955) as given in section 3.8.3.3.3.

3.13.2.5. Non enzymatic antioxidants

3.13.2.5.1. Total phenolic contents (TPC) (mg g⁻¹)

Total phenolic contents in leaf samples were determined as mentioned in section 3.8.3.4.1. (Ainsworth and Gillespie, 2007).

3.13.2.5.2. Ascorbic acid (m. mole g⁻¹)

Ascorbic acid of leaf samples was measured according to Yin et al. (2008) as given in section 3.8.3.4.2.

3.13.2.6. Leaf K⁺ contents

The K⁺ content in leaves were determined by flame photometer (USDA Laboratory Staff, 1954).

3.13.3. Grain yield per plant (g)

At maturity plants were harvested and threshed manually to record grain yield per plant.

3.14. Statistical analysis

All the data collected for above-mentioned parameters in all experiments were statistically analysed by determining significance of variance (P<0.05) using the MSTAT Computer Program (MSTAT Development Team, 1989). Differences among means were compared by using Duncan’s New Multiple Range test (Steel and Torrie, 1997).
Chapter 4

Result and Discussion

4.1 Experiment 1. Optimization of MLE dilution

In this experiment, moringa leaf extract (MLE) was diluted to 10, 20 and 30 times (MLE10, MLE20 and MLE30) and applied to germinating wheat seeds to optimize MLE diluted dose. Response was evaluated on the basis of seedling vigor. Exogenous application of varying dilutions of MLE significantly enhanced the seed germination of wheat (Table 4.1.1, 4.1.2). Among dilutions MLE30 was the most effective owing to maximum enhancement in seed germination. MLE30 also induced earliness in germination as depicted by less mean time to complete germination and time to 50% germination as compared to control (Fig. 4.1.1 b and c). Likewise, exogenous application of MLE significantly enhanced shoots and roots fresh and dry weights. Maximum enhancement in these growth attributes of wheat seedlings was observed by MLE30 treatment (0.229 and 0.035 g; 0.201 g and 0.058 g, respectively). In the same way, shoot and root length of wheat seedlings increased by addition of MLE in the growth medium. However, maximum enhancement in both shoot and root length was observed when MLE30 was applied (Fig. 4.1.2). Nonetheless, 30 times diluted MLE was the most effective in enhancing wheat seedling vigour in present studies. On the basis of these findings MLE 30 was selected for further experiments in pot and field conditions.

Discussion

Plant extracts of some trees and crop residues have been reported to influence crop growth and yield (Guenzi and McCalla, 1962; Chung and Miller, 1995; El-Atta and Bashir, 1999; Ahmed and Nimer, 2002; Farooq et al., 2008). Leaf extract of *M. oleifera* is one such example. It has been reported that foliar application of *M. oleifera* leaf extract accelerated growth of tomato, peanut, corn and wheat at early vegetative growth stage, improve resistance to pests and diseases and enhanced more and larger fruits and generally increase yield by 20 to 35% (Fuglie, 2000). In our studies, the exogenously applied MLE also effectively improved seed germination and seedling vigour as compared to untreated control. However, improving effect of MLE on growth was concentration dependent. Of various dilutions, exogenous application of 30 times dilution was the most effective in improving
growth of wheat seedlings as has earlier been observed in cotton and sugarcane (Foidle et al., 2001). While Fuglie (2000) reported yield enhancement by 25-30% in onions, bell pepper, soya, maize, sorghum, coffee, tea, chili, melon by MLE application. He suggested that this growth and yield enhancement was due to presence of zeatin, a cytokinin in moringa leaves. In another study with sorghum, maize and wheat, Phiri (2010) found that spray with 10 times diluted MLE to germinating seeds of these cereals increased germination of sorghum, length of maize radicals and hypocotyls of wheat. However, seed germination of wheat reduced significantly due to spray of 10 times dilution of MLE. In the present study, 30 time dilution was more effective in germination and seedling growth of wheat than other dilutions. So, it is suggested that MLE has potential to enhance the seed germination, and seedling growth in wheat. However, effectiveness of exogenous application of MLE depends on type of species, dilution of MLE and plant developmental stage. In view of published reports, it is suggested that Moringa leaf extract posses a rich and rare combination of nutrients, amino acids, antioxidants and cytokinin as observed in the present study (Table 3.1), and exogenous use of MLE might have enhanced plant’s endogenous hormonal levels thereby resulting in increased seed germination and seedling growth.
Table 4.1.1 Mean sum of squares of data for germination index (GI), mean germination time (MGT), and time to 50% germination (T50) of wheat grown in petri plates treated with *Moringa oleifera* leaf extract (MLE).

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Germination index (GI)</th>
<th>Mean germination time (MGT)</th>
<th>Time to 50% germination (T50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>3</td>
<td>14.572*</td>
<td>0.262*</td>
<td>2.814*</td>
</tr>
<tr>
<td>Error</td>
<td>8</td>
<td>6.396</td>
<td>0.349</td>
<td>0.838</td>
</tr>
</tbody>
</table>

Table 4.1.2 Mean sum of squares of data for final germination percentage, shoot fresh weight and shoot dry weight of wheat seedling grown in petri plates treated with MLE.

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Final germination percentage</th>
<th>Shoot fresh weight</th>
<th>Shoot dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>3</td>
<td>100.100**</td>
<td>0.0022**</td>
<td>0.0003**</td>
</tr>
<tr>
<td>Error</td>
<td>8</td>
<td>88.978</td>
<td>0.00004</td>
<td>0.00003</td>
</tr>
</tbody>
</table>

Table 4.1.3 Mean sum of squares of data for shoot length, root fresh weight and root dry weight of wheat seedling grown in petri plates treated with MLE.

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Shoot length</th>
<th>Root fresh weight</th>
<th>Root dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>3</td>
<td>3.1724**</td>
<td>0.0143**</td>
<td>0.00093**</td>
</tr>
<tr>
<td>Error</td>
<td>8</td>
<td>0.00004</td>
<td>0.0001</td>
<td>0.00001</td>
</tr>
</tbody>
</table>

**P<0.01    *P<0.05
Table 4.1.4 Mean sum of squares of data for root length of wheat seedling grown in petri plates treated with MLE.

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Root length</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>3</td>
<td>1.0849**</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Error</td>
<td>8</td>
<td>0.00020</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

**P < 0.01
Fig 4.1.1. Effect of different dilutions of *Moringa oleifera* leaf extract (MLE) on germination index (a), mean germination time (MGT) (b), time to 50% germination ($T_{50}$) (c) and final germination percentage of wheat cv. Sehar-2006 under laboratory conditions.
Fig. 4.1.2. Effect of different dilutions of *Moringa oleifera* leaf extract (MLE) on shoot fresh and dry weight (a), root fresh and dry weight (b) and shoot, root length (c) of wheat cv. Sehar-2006 under laboratory conditions.
4.2. Experiment II: Evaluation of MLE as priming agent

In this experiment, effects of optimized dose of MLE i.e., MLE30 as seed priming agent was compared with other priming agents (Hydropriming, CaCl₂, on-farm priming) and emergence, seedling vigour, antioxidants status and yield of wheat were assessed. The results show that seed priming with different priming agents significantly increased the emergence index and time to 50% emergence (T₅₀) of wheat seed (Table 4.2.1). Maximum seed emergence index was obtained in seeds primed with MLE30 followed by CaCl₂ and hydropriming. The on-farm priming remained at bottom and statistically at par with control for emergence index (Fig. 4.2.1a).

Analysis of variance (ANOVA) of the data for mean time taken by the seeds to emerge show that seed priming with different priming agents did not affect mean time to emerge (Fig. 4.2.1b). In contrast, time to 50% emergence (T₅₀) showed that seed priming significantly reduced time to attain Tₕ₀. However, maximum reduction in time to 50% emergence in wheat was observed in MLE30 (Fig.4.2.1c).

Seed priming showed a variable but significant stimulatory effect on seedling vigor attributes (Table 4.2.2 and Table 4.2.3). The maximum improvement in shoot fresh and dry weight was observed in seedlings raised from seeds primed with MLE30 followed by MLE10. All of the other priming treatments under study were statistically different from each other but better than control. Minimum shoot fresh and dry weight was obtained in control (Fig. 4.2.2 a, b). Similarly significantly highest root fresh and dry weight was produced when seeds were primed with MLE30 (Fig.4.2.2 d,e) followed by hydropriming and MLE10. MLE10 seed priming ranked third regarding root fresh weight.

The significantly highest increment in shoot length was found with CaCl₂ seed priming followed by MLE30 seed priming (Fig. 4.2.2 c). The shoot length was observed decreased in order of hydropriming > on- farm priming > MLE10 priming > control.

MLE30 and MLE10 primed seeds outperformed regarding root length, however, MLE30 was the superior one (Fig. 4.2.2 f). The root length observed in case of CaCl₂ shorter than MLE priming but longer than hydropriming and on farm priming. The maximally reduced root length was found in non primed seed.

The largest leaf area was produced in seedlings emerged from MLE30 primed seeds followed by MLE10 (Fig. 4.2.3 a). The hydropriming and on-farm priming showed significantly
similar values for leaf area. While comparing priming treatments CaCl$_2$ produced minimum value for leaf area, although, it was lesser than other priming treatments still larger than control. Overall MLE30 produced comparatively more vigorous seedlings.

Priming also showed significant influence on leaf chlorophyll contents (Table 4.2.4) which was maximally increased by MLE30 followed by CaCl$_2$ seed priming (Fig. 4.2.3b). The MLE10 exhibited chlorophyll $a$ content statistically lesser than MLE30 and CaCl$_2$ but higher than on-farm priming and hydropriming. Nevertheless the on-farm priming and hydropriming were significantly better for chlorophyll $a$ contents as compared to control. The lowest recorded value of chlorophyll $a$ was found in leaves of plants raised from non primed seeds.

Similar to chlorophyll $a$ the significantly highest quantities of chlorophyll $b$ were also obtained in case of MLE30 primed seeds (Fig. 4.2.3c). The CaCl$_2$ and hydropriming were statistically similar although inferior to MLE30 but superior than other priming treatments. The performance of non primed seeds was better than on-farm priming. The more pronounced effects of seed priming were observed with MLE30 and CaCl$_2$ for leaf chlorophyll $a$ and $b$ contents.

In case of yield attributes effects of priming treatments were found significant (Table 4.2.5). More and heavier grains were obtained from MLE30 primed plants which resulted in highest grain yield per plant. All the priming treatments gave more yield than non primed control (Fig. 4.2.4 a, b and c)

Total leaf soluble protein contents showed positive response towards seed priming treatments (Table 4.2.6). MLE30 gained significantly highest protein contents whereas MLE10 significantly lesser than MLE30 but higher than other treatments (Fig. 4.2.5a). Hydropriming exhibited better leaf protein contents than CaCl$_2$ and on-farm priming. The protein contents obtained in CaCl$_2$ and on-farm priming were even lesser than control.

More enhancement in super oxide dismutase (SOD), peroxidase (POD) and catalase (CAT) were resulted from MLE30 seed priming agent as compared to other priming and non primed seed treatments. The hydropriming followed the MLE30 in case of SOD (Fig. 4.2.4 b) and POD but in case of CAT MLE30 was followed by MLE10 seed treatment (Fig. 4.2.5 c and d). The least activity of SOD, CAT and POD was observed when seeds were on-farm primed.
and primed with CaCl$_2$, respectively. Nevertheless, the seed enhancement techniques significantly affect the enzymatic antioxidants. A significant increase in ascorbic acid was also found in case of MLE10 seed priming followed by MLE30 (Fig. 4.2.5 e). The plants raised from hydro primed seed produced ascorbic acid lesser than moringa priming but higher than CaCl$_2$ and on- farm priming. Although least ascorbic acid but maximum total phenolic content were produced in CaCl$_2$ priming treatments (Fig. 4.2.5 f).

**Discussion**

In this study, effects of different priming agents on growth and yield of wheat were evaluated. From the results of the present study, it is obvious that seed priming enhanced speed and total final germination count of wheat. Maximum increase in seed emergence and speed of seed germination was observed in plants raised from MLE30 treatment (Table 4.2.1, Fig. 4.2.1 a, b, c). It has already reported that 30 times diluted moringa leaf extract significantly increased seed and seedling vigor in wheat (Afzal *et al.*, 2008), maize (Basra *et al.*, 2011) and range grasses i.e. *Cenchrus ciliaris*, *Panicum antidotale* and *Echinochloa crus-galli* (Nouman *et al.*, 2011) reported that seed priming with MLE30 significantly increased germination of all three range grasses. All seed priming agents enhanced the seedling shoot and root fresh and dry weight of wheat plants, particularly when seeds were primed with MLE30. However, seed priming with CaCl$_2$ caused maximum increase in shoot length while longer roots were observed with MLE30 priming (Fig. 4.2.2 c and f). Seed priming enhances rate of metabolism which results in increase in speed of germination and emergence (Ashraf *et al.*, 2008). The effectiveness of moringa is because its leaves are rich source of PGR hormone, zeatin, ascorbic acid, Ca and K (Fuglie, 1999; Foidle *et al.*, 2001), which are involved in modifying the seed germination and seedling establishment related metabolism.

Osmohardening with CaCl$_2$ was previously successfully used to improve emergence and plant height in rice (Farooq *et al.*, 2006). So, its effectiveness was also confirmed in wheat by present studies. It might be due to the fact that higher or enhanced mobilizations of metabolites/inorganic solutes to germinating plumule result in enhanced growth (Taiz and Zeiger, 2002).
The crop yield is ultimate goal for cereal cultivation and numbers of strategies are underway to increase the productivity. Crop yield in cereals is mainly determined by optimum plant population, number of grains and size of grains. A number of reports are available which show that seed priming enhanced the crop productivity by either increasing the emergence, number of grains or size of grain or by combination of these traits (Khan et al., 2006; Ashraf et al., 2008; Athar et al., 2008). Farooq et al. (2006) reported that highest value of 1000 kernel weight in rice was produced by KCl followed by CaCl₂ seed priming. In the present study, seed priming with different priming agents increased the number of grains spike⁻¹ and 100 grain weight. Better partitioning of photoassimilates in developing grains at grain filling stage causes increase in size of grain (Taiz and Zeiger, 2002). The level of cytokinin is reported positively correlate to final grain weight in maize (Dietrich et al., 1995) and since moringa leaves are rich in zeatin, a cytokinin (Foidle et al., 2001) so, MLE priming effectiveness might be due to its other growth promoting factors. Gupta et al. (2003) also reported that benzyl adenine, a cytokinin, could be used to improve sink and source capacity of wheat in increasing grain yield. In some earlier studies it has been observed that seed priming with plant growth regulators, inorganic salts, compatible solutes or sugar beet extract improved seed germination by providing physiological and biochemical adaptations (Pill and Savage, 2008; Afzal et al., 2006a). Similarly, priming with cytokinins like kinetin or benzyl amino purine (BAP) increased salt tolerance in wheat at seedling stage (Iqbal and Ashraf, 2006).

The enhanced yield by seed priming arise from the events taking place during earlier stages of crop growth such as faster production of more vigorous seedlings (Farooq et al., 2006). Ruan et al. (2002) also observed an improvement in seedling vigor with CaCl₂ and CaCl₂ + NaCl priming in greenhouse conditions.

Seed priming with MLE30 followed by CaCl₂ also improved the photosynthetic pigments. CaCl₂ priming is well known priming tool for rice (Farooq et al., 2006), we tried in present study for wheat and found effective in enhancing the speed and spread of emergence and seedling vigor as well, though not as affective as MLE30 results. The vigorous seedling as exhibited by more chlorophyll in leaves. Afzal et al. (2011) also found CaCl₂ as effective priming agent in tomato.
In the present study, seed priming with different priming agents caused an enhancement in leaf total soluble protein. However, this effect was more in MLE30 priming. While working with wheat Al-Hakimi and Hamada (2001) observed that seed priming with ascorbic acid increased leaf soluble proteins. According to Price (2000) moringa leaf extract contain ascorbic acid in appreciable quantities. Thus, increased leaf protein due to MLE30 seed priming was one of the reasons that contributed in improved growth of wheat. Similarly, remarkable increase in protein content was obtained from CaCl₂ seed priming under salinity (Afzal et al., 2006b) which may be due to better defense of membrane and membrane bound enzymes.

It is evident that priming with antioxidant compounds such as ascorbic acid and tocopherol can increase free radical scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidase in seeds (Chang and Sung, 1998). Moringa leaves are richest source of antioxidants. It is reported that about 46 antioxidants are present in moringa leaves. The major ones are ascorbate, carotenoids, phenols and flavonoid (Iqbal and Bhanger, 2006). So the pretreatment of seeds with MLE improved the total phenolic contents of maize seedlings (Basra et al., 2011). In the present study, it was observed that most of priming treatments were effective in not only improving seedling vigor and may be attributed to the counteraction of free radicals and synthesis of membrane-bound enzymes as in other non-primed seeds.

Seed priming improved the seedling vigor and increased the activity of scavenging enzymes in leaves of wheat and as indicated by increase in SOD and POD by MLE30, CAT and TPC by CaCl₂ and ascorbic acid by MLE10 seed priming (Fig. 4.2.5 b, c, d, e and f). Catalase, which is involved in the degradation of H₂O₂ into water and oxygen, is the major H₂O₂ scavenging enzyme in all aerobic organisms. Previous reports show that priming resulted in a great enhancement in CAT and SOD activities in plants (Basra et al., 2004). The hydropriming decreased CAT activity in the present study which confirms the findings of Srinivasan and Saxena (2001) who reported that CAT activity was not increased after hydropriming in radish. Therefore, it is likely that enhanced antioxidant enzyme activity in wheat cultivars due to MLE30 and CaCl₂ priming was due to highest contents of antioxidants found in MLE (Iqbal and Bhanger, 2006).
Table 4.2.1 Mean sum of squares of data for emergence index (EI), mean emergence time (MET), time to 50% emergence ($E_{50}$) of wheat raised from seeds primed with different priming agents.

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Emergence index (EI)</th>
<th>Mean emergence time (MET)</th>
<th>Time to 50% emergence ($E_{50}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Priming Treatments</td>
<td>5</td>
<td>3.100*</td>
<td>0.629*</td>
<td>0.282**</td>
</tr>
<tr>
<td>Error</td>
<td>12</td>
<td>0.985</td>
<td>0.646</td>
<td>0.016</td>
</tr>
</tbody>
</table>

Table 4.2.2 Mean sum of squares of data for shoot fresh weight, shoot dry weight and shoot length of wheat raised from seeds primed with different priming agents.

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Shoot fresh weight</th>
<th>Shoot dry weight</th>
<th>Shoot length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Priming Treatments</td>
<td>5</td>
<td>0.070**</td>
<td>0.088**</td>
<td>7.324**</td>
</tr>
<tr>
<td>Error</td>
<td>12</td>
<td>0.001</td>
<td>0.001</td>
<td>0.032</td>
</tr>
</tbody>
</table>

Table 4.2.3 Mean sum of squares of data for root fresh weight, root dry weight and root length of wheat raised from seeds primed with different priming agents.

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Root fresh weight</th>
<th>Root dry weight</th>
<th>Root length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Priming Treatments</td>
<td>5</td>
<td>0.128**</td>
<td>0.045**</td>
<td>16.902**</td>
</tr>
<tr>
<td>Error</td>
<td>12</td>
<td>0.001</td>
<td>0.001</td>
<td>0.163</td>
</tr>
</tbody>
</table>

**P< 0.01  *P<0.05  ns Non significant
Table 4.2.4 Mean sum of squares of data for leaf area, leaf chlorophyll $a$ and chlorophyll $b$ contents of wheat raised from seeds primed with different agents.

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Leaf area</th>
<th>Chlorophyll $a$</th>
<th>Chlorophyll $b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Priming</td>
<td>5</td>
<td>932.835**</td>
<td>0.605**</td>
<td>0.304**</td>
</tr>
<tr>
<td>Treatments</td>
<td></td>
<td>7.238</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table 4.2.5 Mean sum of squares of data for number of grains per spike, 100 grain weight and grain yield per plant of wheat raised from seeds primed with different priming agents.

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Number of grains per spike</th>
<th>100 grain weight</th>
<th>Grain yield per plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Priming</td>
<td>5</td>
<td>353.428**</td>
<td>0.147**</td>
<td>0.870**</td>
</tr>
<tr>
<td>Treatments</td>
<td></td>
<td>0.039</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Error</td>
<td>12</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table 4.2.6 Mean sum of squares of data for total soluble protein, and enzymatic antioxidants i.e. superoxide dismutase (SOD) and peroxidase (POD) of wheat raised from seeds primed with different agents.

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Total soluble protein</th>
<th>Super oxide dismutase (SOD)</th>
<th>Peroxidase (POD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Priming</td>
<td>5</td>
<td>0.090**</td>
<td>160.045**</td>
<td>56.769**</td>
</tr>
<tr>
<td>Treatments</td>
<td></td>
<td>0.001</td>
<td>0.160</td>
<td>0.011</td>
</tr>
<tr>
<td>Error</td>
<td>12</td>
<td>0.001</td>
<td>0.011</td>
<td>0.011</td>
</tr>
</tbody>
</table>

** $p < 0.01$
Table 4.2.7 Mean sum of squares of data for enzymatic antioxidant catalase (CAT) and non-enzymatic antioxidants i.e. total phenolic content and ascorbic acid of wheat raised from seeds primed with different agents.

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Catalase (CAT)</th>
<th>Total phenolic content</th>
<th>Ascorbic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Priming Treatments</td>
<td>5</td>
<td>117.126**</td>
<td>1.100**</td>
<td>0.0009**</td>
</tr>
<tr>
<td>Error</td>
<td>12</td>
<td>0.007</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

**P< 0.01
Fig 4.2.1. Effect of seed priming on emergence index (EI) (a), mean emergence time (MET) (b) and time to 50% emergence (E50) (c) of wheat cv. Sehar-2006. (MLE10 = 10 times diluted MLE, MLE30 = 30 times diluted MLE, HP = Hydro priming, OFP = On-farm priming)
Fig 4.2.2. Effect of seed priming on shoot fresh weight (a), shoot dry weight (b), shoot length (c), root fresh weight (d), root dry weight (e) and root length (f) of wheat cv. Sehar-2006. (MLE10= 10 times diluted MLE, MLE30= 30 times diluted MLE, HP=Hydro priming, OFP=On farm priming)
Fig. 4.2.3. Effect of seed priming on leaf area (a), chlorophyll a (b) and chlorophyll b (c) of wheat cv. Sehar-2006. (MLE10= 10 times diluted MLE, MLE30= 30 times diluted MLE, HP= Hydro priming, OFP= On farm priming)
Fig. 4.2.4. Effect of seed priming on number of grains per spike (a), 100 grain weight (b) and grain yield per plant (c) of wheat cv. Sehar-2006 (MLE10= 10 times diluted MLE, MLE30= 30 times diluted MLE, HP= Hydro priming, OFP= On-farm priming)
Fig. 4.2.5: Effect of seed priming on leaf total soluble protein (a), enzymatic antioxidants (superoxide dismutase, peroxidase and catalase) (b, d, c) and non enzymatic antioxidants (ascorbic acid and total phenolic contents) (e, f) of wheat cv. Sehar-2006. (MLE10 = 10 times diluted MLE, MLE30 = 30 times diluted MLE, HP = Hydro priming, OFP = On farm priming)
4.3. Experiment III:

Evaluating the growth and development of tomato to exogenous application of MLE (pot study)

In a preliminary research study tomato plants were treated with different dilutions of (MLE0, MLE10, MLE20, and MLE30) and BAP (50 mg L⁻¹). The exogenous application was done either through soil or foliar. The data collected for different growth, yield and antioxidants status are discussed below:

Data presented in Table 4.3.1 and Fig. 4.3.1a elucidate that exogenous application of MLE and BAP significantly affected the number of vegetative branches per plant. The foliar application of BAP along with soil and foliar application of MLE30 showed maximum enhancement in number of vegetative branches. The soil application of control was superior to foliar application of control, MLE0 and MLE10. The minimum number of vegetative branches was observed where plants were sprayed with undiluted MLE.

Foliar application of BAP, MLE30 and soil application of MLE20 exhibited higher number of flowering branches (Table 4.3.1a and Fig. 4.3.1. b). Regarding control, MLE0 and MLE10 soil application showed more flowering branches as compared to their foliar application. The lowest number of flowering branches was found in case of foliar applied MLE0 and MLE10. The significant differences were found for number of flowers plant⁻¹ (Table 4.3.1) with largest number of flowers obtained in foliar spray of MLE30 alone (Fig.4.3.2a). Although the number of flowering branches obtained in MLE30 was lesser than BAP foliar spray, however, more number of flowers per branch was obtained in MLE30 than BAP.

The more flowering branches in foliar application of BAP and more number of flowers in foliar applied MLE30 resulted in highest number of fruits in both the treatments (Fig. 4.3.2 b). The lesser number of flowers in foliar spray of MLE10, MLE0 and control lead to the least number of fruits in these treatments.

Data on the response of fresh weight of fruit to the exogenous treatments indicate that the foliar application of MLE30 not only exhibited large number of flowers and more number of fruits but also heaviest fruits were observed in the same treatment (Fig.4.3.2c). Although the same number of flowers were produced by foliar application of BAP and MLE30 but lighter weight fruits in BAP as compared to MLE30 were obtained. The minimum fruit weight was recorded in case of foliar applied MLE10, MLE0 and control.
Analysis of variance (ANOVA) of the data in Table 4.3.2 and 4.3.3 depict that exogenous application significantly affected leaf chlorophyll \( a \) and chlorophyll \( b \) contents. The foliar application of BAP maximally raised chlorophyll \( a \) contents followed by foliar application of MLE30 (Fig. 4.3.3 a, b). In case of all exogenous applications irrespective of their application method more chlorophyll \( a \) contents were observed as compared to control. In contrast, the highest chlorophyll \( b \) contents were found in case of foliar spray of water.

Data presented in Table 4.3.3 and Fig. 4.3.4a showed that maximum leaf total soluble proteins were obtained by foliar application of BAP, MLE30 and MLE20. Non significant difference between soil and foliar application was found in case of MLE10 and control. The lowest quantity of total soluble protein was obtained under foliar application of MLE0.

The exogenous applications of different concentrations of MLE significantly affected the antioxidant activities of tomato leaves (Table 4.3.4). The MLE30 foliar application ranked first in case of enzymatic antioxidants i.e. SOD, POD and CAT (Fig. 4.3.4 b, c, d). Other treatments such as foliar applied BAP and MLE20 exhibited SOD value statistically at par with MLE30. The least quantity of SOD was obtained under foliar application of MLE0. The foliar applied BAP and MLE20 produced statistically similar POD value which was lesser than foliar applied MLE30. The POD lowest value was found when plants received foliar spray of water (control).

Nevertheless, the quantity of CAT observed in tomato leaves was lesser than POD but higher than SOD. The foliar applied MLE30 was superior for CAT and its soil application showed CAT lower than MLE foliar but better than other treatments under study. All the treatments performed better than control for CAT contents. Similar trend were observed in case of leaf total phenolics and fruit lycopene contents in which foliar application of MLE30 outperformed all the growth enhancers under study (Fig. 4.3.4 c and f ).

Overall, foliar mode of exogenous application and MLE30 dilution were more effective regarding growth, yield and biochemical parameters of tomato.

**Discussion**

The exogenous application of growth promoting compounds i.e. PGR, antioxidants, vitamins, minerals, osmoprotectants etc. are being used to enhance the crop growth and economic yield (Adams and Adams, 2002; Al-Hakimi and Hamada, 2001; Azeem and Ahmad, 2011). Researchers are always interested to find natural compounds containing these growth
promoting factors as cheap, natural and environmentally friendly compounds. Foliar application of yeast, a natural source of cytokinin, sugar, vitamins, amino acid and proteins in appreciable quantities increased growth, chlorophyll contents and green pod yield of *Phaseolus vulgaris* (El-Tohamy and El- Gready, 2007). Azeem and Ahmad (2011) observed that both individual and combined foliar application of K, Fe and B caused the significant improvement in number of fruits, weight of fruits, leaf chlorophyll and protein contents of tomato. We tried to use moringa leaf extract as a natural source of cytokinin, nutrients and antioxidants. In present study the maximum vegetative growth was obtained under foliar application of BAP, however, the yield related parameters such as number of flowering branches, number of flowers, number of fruits and fruit weight per plant were higher by foliar application of 30 times diluted moringa leaf extract (Fig. 4.3.1 and 4.3.2). It may be attributed to the higher nutrient requirements of reproductive phase fulfilled by application of macro and micro nutrient containing MLE. However, the response of plant to MLE depends upon both plant growth stage (as more response was observed during reproductive growth) and MLE concentration. The more diluted MLE spray provides nutrients to the plant through stomata and enhances the yield. These results confirmed the findings of Wu and Lin (2000) who found that higher concentration of seaweed extract resulted in sticky brown cotyledons due to drop in biological activity of extract. According to Crouch and Staden (1991) hormones at low concentration often promote physiological response while inhibit it at higher concentration. But in case of soil application concentrated MLE performed better than diluted MLE.

In our studies the plants treated with BAP and MLE in either method of exogenous application showed a significant enhancement in photosynthetic pigment chlorophyll *a* as compared to control but the maximum chlorophyll *b* was observed in case of soil application of water (Fig.4.3.3). These findings are in accordance to Kumari *et al.* (2011) in which largest content of chlorophyll *a*, *b* and carotenoids in tomato were obtained by foliar application and soil drench + foliar application treatments. Previously, increased levels of photosynthetic pigments in tomato leaves with application of *Ascophyllum nodosum* extract (Whapham *et al.*, 1993), and effective chlorophyll syntheses in maize and *phaseolus mungo* by application of *Sargassum* seaweed extract (Lingakumar *et al.*, 2004) was observed.
In earlier reports the high level of soluble protein in maize can be maintained by application of 1% and 0.5% *Sargassum* seaweed extract (Lingakumar *et al.*, 2004) and in sorghum with 1.5% liquid extract obtained from *Hydroclathrus clathratus* (Ashok *et al.*, 2004). In present research the highest protein content was achieved in plants where foliar application of MLE30 was done (Fig. 4.3.4). This increase protein contents may be due to enhanced availability and absorption of minerals which facilitated the source efficiency of leaves. The use of diluted extract or lower concentration caused more increase in protein contents may be due to enhanced absorption of necessary elements in such a concentration (Anantharaj and Venkatesalu, 2001).

In the present research MLE and BAP significantly improved the enzymatic and nonenzymatic antioxidants of tomato leaves depending upon their concentration and method of application. Under the exogenous application of concentrated and diluted MLE and BAP, the maximum activities of SOD, POD, CAT and total phenolics were obtained with foliar application of MLE30.

Dorais *et al.* (2008) reported that the injurious effects e.g. age-related molecular degeneration, cancer and cardiovascular disorders in humans caused by various substances can be counteracting by improving lycopene contents. Due to its importance there is increasing trend for developing lycopene rich food product and ingredient (Choudhary *et al.*, 2009). In our study foliar application of MLE30 produced highest lycopene contents while in case of soil application MLE0 or higher concentration of MLE showed larger lycopene contents as compared to control (Fig. 4.3.4f). Similarly highest contents of lycopene in tomato fruit were produced by soil application of 10% seaweed extract (Kumari *et al.*, 2011).

From such report it is evident that cytokinin like substances or cytokinin itself present in aqueous extract of moringa leaf or *Sargassum johnstonii* extract facilitate mobilization of nutrients to the fruit and improve lycopene contents. Although it was a preliminary research study, however, the present research findings strongly suggested that foliar application of MLE30 was most effective and has a potential to be used as plant growth enhancer in tomato crop.
Table 4.3.1 Mean sum of squares of data for number of vegetative branches, number of flowering branches and number of flowers of tomato under exogenous application of different plant growth enhancers.

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Number of vegetative branches plant(^{-1})</th>
<th>Number of flowering branches plant(^{-1})</th>
<th>Number of flowers plant(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Application method</td>
<td>1</td>
<td>16.333(^*)</td>
<td>38.521(^*)</td>
<td>218.180(^**)</td>
</tr>
<tr>
<td>Treatment</td>
<td>5</td>
<td>41.683(^**)</td>
<td>99.571(^**)</td>
<td>1135.229(^**)</td>
</tr>
<tr>
<td>Application method x Treatment</td>
<td>5</td>
<td>37.133(^**)</td>
<td>46.271(^**)</td>
<td>582.126(^**)</td>
</tr>
<tr>
<td>Error</td>
<td>36</td>
<td>3.042</td>
<td>3.021</td>
<td>9.011</td>
</tr>
</tbody>
</table>

Table 4.3.2 Mean sum of squares of data for number of fruits, fruit yield plant\(^{-1}\) and leaf chlorophyll \(a\) of tomato under exogenous application of different plant growth enhancers.

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Number of fruits plant(^{-1})</th>
<th>Fruit yield plant(^{-1})</th>
<th>Leaf chlorophyll (a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Application method</td>
<td>1</td>
<td>164.628(^*)</td>
<td>0.261(^*)</td>
<td>0.440(^**)</td>
</tr>
<tr>
<td>Treatment</td>
<td>5</td>
<td>703.421(^**)</td>
<td>2.471(^**)</td>
<td>0.797(^**)</td>
</tr>
<tr>
<td>Application method x Treatment</td>
<td>5</td>
<td>346.885(^**)</td>
<td>1.308(^**)</td>
<td>0.197(^**)</td>
</tr>
<tr>
<td>Error</td>
<td>36</td>
<td>10.589</td>
<td>0.019</td>
<td>0.001</td>
</tr>
</tbody>
</table>

\(^*\) p < 0.05 \hspace{1cm} \(^**\) p < 0.01 \hspace{1cm} ns = non significant
Table 4.3.3 Mean sum of squares of data for leaf chlorophyll $b$, plant height and leaf total soluble protein of tomato under exogenous application of different plant growth enhancers.

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Leaf chlorophyll $b$</th>
<th>Plant height</th>
<th>Leaf total soluble protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Application method</td>
<td>1</td>
<td>0.266**</td>
<td>1.346**</td>
<td>0.266*</td>
</tr>
<tr>
<td>Treatment</td>
<td>5</td>
<td>0.505**</td>
<td>2.443**</td>
<td>0.505**</td>
</tr>
<tr>
<td>Application method x Treatment</td>
<td>5</td>
<td>0.551**</td>
<td>0.123**</td>
<td>0.551**</td>
</tr>
<tr>
<td>Error</td>
<td>36</td>
<td>0.002</td>
<td>0.003</td>
<td>0.002</td>
</tr>
</tbody>
</table>

** p < 0.01    * p < 0.05

Table 4.3.4 Mean sum of squares of data for super oxide dismutase (SOD), peroxidase (POD) and catalase (CAT) of tomato leaf under exogenous application of different plant growth enhancers.

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Leaf superoxide dismutase (SOD)</th>
<th>Leaf peroxidase (POD)</th>
<th>Leaf catalase (CAT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Application method</td>
<td>1</td>
<td>0.063*</td>
<td>74.018**</td>
<td>0.314*</td>
</tr>
<tr>
<td>Treatment</td>
<td>5</td>
<td>0.128**</td>
<td>122.737**</td>
<td>2.168**</td>
</tr>
<tr>
<td>Application method x Treatment</td>
<td>5</td>
<td>0.028*</td>
<td>9.502**</td>
<td>0.198*</td>
</tr>
<tr>
<td>Error</td>
<td>36</td>
<td>0.014</td>
<td>0.016</td>
<td>0.314</td>
</tr>
</tbody>
</table>

** p < 0.01    * p < 0.05
Table 4.3.5 Mean sum of squares of data for leaf total phenolic contents (TPC) and fruit lycopene of tomato under exogenous application of different plant growth enhancers.

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Total Phenolic contents (TPC)</th>
<th>Fruit lycopene</th>
<th>--</th>
</tr>
</thead>
<tbody>
<tr>
<td>Application method</td>
<td>1</td>
<td>135.445**</td>
<td>9.882**</td>
<td>--</td>
</tr>
<tr>
<td>Treatment</td>
<td>5</td>
<td>41.857**</td>
<td>24.828**</td>
<td>--</td>
</tr>
<tr>
<td>Application method x Treatment</td>
<td>5</td>
<td>15.446**</td>
<td>7.914**</td>
<td>--</td>
</tr>
<tr>
<td>Error</td>
<td>36</td>
<td>3.143</td>
<td>0.023</td>
<td>--</td>
</tr>
</tbody>
</table>

** p < 0.01
Fig 4.3.1. Effect of exogenous application of growth enhancer on number of flowering branches (a) and number of vegetative branches (b) of tomato cv. Sahil (MLE0, MLE10, MLE20, MLE30 =0, 10, 20, 30 times diluted MLE respectively, BAP= benzyl amino purine)
Fig 4.3.2. Effect of exogenous application of growth enhancer on number of flowers plant$^{-1}$ (a), number of fruits (b) and fruit weight (c) of tomato cv. Sahil (MLE0, MLE10, MLE20, MLE30 = 0, 10, 20, 30 times diluted MLE respectively, BAP= benzyl amino purine).
Fig 4.3.3. Effect of exogenous application of growth enhancer on total soluble protein (a), SOD (b), total phenolics (c), POD (d), catalase (e) of leaf and fruit lycopene (f) of tomato cv. Sahil (MLE0, MLE10, MLE20, MLE30 = 0, 10, 20, 30 times diluted MLE respectively, BAP = benzyl amino purine)
Fig 4.3.4. Effect of exogenous application of growth enhancer on chlorophyll $a$ (a), chlorophyll $b$ (b) and plant height (c) of tomato cv. Sahil (MLE0, MLE10, MLE20, MLE30 = 0, 10, 20, 30 times diluted MLE respectively, BAP= benzyl amino purine)
4.4. Experiment IV:
Evaluating the growth and development of pea to exogenous application of MLE (pot study)

The growth and yield response of pea under soil and foliar application of different growth enhancers was studied. The results obtained are mentioned and discussed below:

The soil and foliar application of different growth enhancers significantly affect the vegetative, reproductive and biochemical parameters of pea (Table 4.4.1, 4.4.2 and 4.4.3). The foliar spray of MLE30 produced maximum number of vegetative branches which were significantly superior to all other treatments under study (Fig. 4.4.1 a). The foliar spray of BAP, MLE20 and soil application of MLE30 exhibit statistically similar vegetative branches lesser than MLE30 but higher than control. More number of vegetative branches was observed in foliar application as compared to soil application. Among the MLE dilutions and BAP, the MLE30 performed best. The least number of vegetative branches similar to control were obtained in case of soil applied MLE10 along with both foliar and soil applied MLE0. The highest number of vegetative branches turned in to reproductive branches and produced highest number of flowers when the plants received foliar spray of MLE30 (Fig. 4.4.1 b, c). It was followed by the foliar spray of MLE20, BAP and soil applied MLE20. There was no significant difference among soil applied MLE20, MLE10, MLE0 and control in case of reproductive branches and number of flowers per plant.

The exogenous application in the form of soil and foliar spray significantly affect the number of vegetative, reproductive branches and number of flowers. It leads to the highest number of pods and pod yield (weight) under foliar application of MLE30 (Fig. 4.4.2). The soil application did not perform better than foliar spray in any growth and yield determining attributes.

Although the different growth enhancers significantly affect growth and yield, however, their effect in case of chlorophyll $a$ and $b$ was non significant. All the growth enhancers effect leaf chlorophyll contents in similar way, nevertheless largest chlorophyll $a$ and $b$ were obtained when leaves were sprayed with MLE30.

While observing effect of soil and foliar applied growth enhancers on antioxidant status of pea, highest quantities of total phenolic contents were found under foliar spray of BAP (Fig. 4.4.3c). It was statistically at par with soil applied 20 or 30 times diluted MLE. Unlike, other
growth and yield parameters more enhancements in total phenolic content were obtained by the soil application as compared to foliar application. However, all soil and foliar treatments were significantly better than control.

**Discussion**

Exogenous applications of plant extract are known to enhance crop growth and yield. Pea crop yield like majority of grain legumes greatly depends on the number of flowering branches, number of flowers, and number of pods per plant or per unit area. In present research the exogenous application of nutrient rich MLE affected these traits significantly. The application of BAP exhibited slight effect on the growth and yield attributes of pea as compared to MLE. The plants that were sprayed with MLE30 showed 37 and 60% increase in flowering branches and pod yield respectively, as compared to control. Accordingly, Duval and Shetty (2001) reported that 1.5 % anise root extract (AR-10) showed enhancement in pea growth. The probable reason may be the water soluble characteristics of cytokinin found in AR-10 root tissues (Andarwulan and Shetty, 1999) similarly the zeatin a cytokinin in MLE might be reason of 25-30% growth and yield enhancement in onions, bell pepper, soya, maize, sorghum, coffee, tea, chili, melon (Fuglie, 2000).

The important attributes of a food product considered by a consumer are its physical appearance and colour. The colour of foods depends upon the presence of various natural or artificial pigments produced during growth or after harvest. The green color of vegetables is due to chlorophyll whereas carotenoids and/or anthocyanins provide yellow, red and orange colors to fruits and vegetables. All the organisms capable of carrying out photosynthesis possess carotenoids and chlorophylls, however, the chlorophylls often masked the bright colors of many carotenoids in photosynthetic tissues (Bartley and Scolnik, 1995). In green peas chlorophylls a and b were identified as the major chlorophylls (Edelenbos et al., 2001). In our study both method of exogenous application and concentration of growth enhancers exhibit non significant difference in case of chlorophyll a and b (Fig. 4.4.3 a and b). It may be attributed that MLE is a rich source of Fe and Mg (Nambiar, 2006). Both these elements important for chlorophyll biosynthesis (Marschner, 1995). Mg as a component of chlorophyll and Fe although not constituent of chlorophyll but involved in chlorophyll biosynthesis i.e. in conversion of Mg proporphyrin in to chlorophyllide (Marschner, 1995). So enhanced chlorophyll a and b constituent under application of Fe and Mg rich MLE may not be
affected by the concentration and method of application. Furthermore, the chlorophyll contents in pea much influenced by genotype (Kidmose and Grevsen, 1992). Polyphenols are important redox-active antioxidants found in high concentration in vegetables and fruits (Odukoya et al., 2007). Phenolic compounds considered as powerful chain-breaking antioxidants (Shahidi and Wanasundara, 1992) and their scavenging ability based upon their hydroxyl groups (Hatano et al., 1989). In the present study, the leaf total phenolic content of pea in foliar application of BAP was found to be highest i.e. 5.82 mg GAE g\(^{-1}\) (Fig. 4.4.3) followed by soil applied MLE 30 (5.592 mg GAE g\(^{-1}\)) as compared to the control (3.152 mg GAE g\(^{-1}\)). In previous studies the AR-10-yeast extract treatments improved the phenolic content in pea seedlings as compared with the control (Duval and Shetty, 2001).

From the above discussion it become clear that MLE as a blend of mineral nutrients, antioxidants and PGR like cytokinin is an effective natural plant growth enhancer for promotion of vegetative and reproductive growth and antioxidant phenols in pea.
Table 4.4.1 Mean sum of squares of data for number of vegetative branches, number of reproductive branches and number of flowers of pea under exogenous application of different plant growth enhancers.

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Number of vegetative branches plant(^{-1})</th>
<th>Number of reproductive branches plant(^{-1})</th>
<th>Number of flowers plant(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Application method</td>
<td>1</td>
<td>30.250**</td>
<td>40.111**</td>
<td>0.111ns</td>
</tr>
<tr>
<td>Treatment</td>
<td>5</td>
<td>32.028**</td>
<td>54.067**</td>
<td>65.711**</td>
</tr>
<tr>
<td>Application method x Treatment</td>
<td>5</td>
<td>3.183*</td>
<td>5.178*</td>
<td>16.978**</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>0.583</td>
<td>1.444</td>
<td>1.639</td>
</tr>
</tbody>
</table>

**P < 0.01       *P < 0.05
ns = non significant

Table 4.4.2 Mean sum of squares of data for number of pods, pods weight plant\(^{-1}\) and leaf chlorophyll \(a\) of pea under exogenous application of different plant growth enhancers.

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Number of pods plant(^{-1})</th>
<th>Pods weight plant(^{-1})</th>
<th>leaf chlorophyll (a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Application method</td>
<td>1</td>
<td>42.250**</td>
<td>9.020*</td>
<td>0.0001(^{ns})</td>
</tr>
<tr>
<td>Treatment</td>
<td>5</td>
<td>82.583**</td>
<td>114.518**</td>
<td>0.001**</td>
</tr>
<tr>
<td>Application method x Treatment</td>
<td>5</td>
<td>20.050**</td>
<td>7.027**</td>
<td>0.0001(^{ns})</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>1.472</td>
<td>0.412</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

**P < 0.01       *P < 0.05
ns = non significant
Table 4.4.3 Mean sum of squares of data for leaf chlorophyll $b$ and total phenolic contents (TPC) of pea under exogenous application of different plant growth enhancers.

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Leaf chlorophyll $b$</th>
<th>Total phenolic contents (TPC)</th>
<th>--</th>
</tr>
</thead>
<tbody>
<tr>
<td>Application method</td>
<td>1</td>
<td>0.001**</td>
<td>3.361**</td>
<td>--</td>
</tr>
<tr>
<td>Treatment</td>
<td>5</td>
<td>0.0001*</td>
<td>3.686**</td>
<td>--</td>
</tr>
<tr>
<td>Application method x</td>
<td>5</td>
<td>0.0001ns</td>
<td>0.662**</td>
<td>--</td>
</tr>
<tr>
<td>Treatment</td>
<td>24</td>
<td>0.0001</td>
<td>0.049</td>
<td>--</td>
</tr>
</tbody>
</table>

**$P < 0.01$   *$P < 0.05$
ns = non significant
Fig 4.4.1 Effect of exogenous application of different growth enhancers on number of vegetative branches (a), reproductive branches (b) and number of flowers per plant (c) of pea cv. Climax. (MLE0, MLE10, MLE20, MLE30 = 0, 10, 20, 30 times diluted MLE respectively, BAP= benzyl amino purine)

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(a) LSD 0.05\(p=1.28\)

(b) LSD 0.05\(p=2.02\)

(c) LSD 0.05\(p=1.082\)
Fig 4.4.2  Effect of exogenous application of different growth enhancers on number of pods (a) and pod yield plant$^{-1}$ (b) of pea cv. Climax. (MLE0, MLE10, MLE20, MLE30 = 0, 10, 20, 30 times diluted MLE respectively, BAP= benzyl amino purine)
Fig 4.4.3 Effect of exogenous application of different growth enhancers on chlorophyll a (a), b (b) and total phenolics (c) of pea cv. Climax. (MLE0, MLE10, MLE20, MLE30 = 0, 10, 20, 30 times diluted MLE respectively, BAP = benzyl amino purine)
4.5. Experiment V:
Response of late sown wheat to foliar application of MLE under field conditions

Foliar application of MLE at different growth stages revealed significant improvement in growth, development and yield as compared to control (water spray) (Table, 4.5.1, 4.5.2, 4.5.3, 4.5.4, 4.5.5, 4.5.6, 4.5.7). The crop attained the maximum leaf area index (LAI) 75 days after sowing (DAS) with the highest value of LAI under foliar applications of MLE at all critical stages i.e. tillering, jointing, booting and heading (T + J + B + H). While MLE application at other growth stages produced LAI although lesser than T + J + B + H while check remained at bottom (Fig. 4.5.1). Nonetheless, a decreasing trend was observed in LAI after 75 DAS but this reduction was minimal in plants with MLE applied at all growth stages followed by foliar application at heading, while maximum reduction was observed in control plants (Fig. 4.5.1). Effect of MLE application on seasonal leaf area duration (SLAD) was also significant (P<0.05), and plants having MLE foliar application stayed green longer than control (4.5.1). However, higher SLAD (65.4 d) were recorded in plants sprayed at all stages closely followed by MLE spray at heading alone (62.63 d) than control (56.18 d) (Fig. 4.5.1). Foliar spray of MLE caused a gradual rise in crop growth rate of late sown wheat crop and showed maximum growth rate (9.74 g m$^{-2}$day$^{-1}$) in foliar spray at 4 stages (Fig. 4.5.2). Afterwards, the growth rate of crop decreased but the minimum reduction (5.97 g m$^{-2}$ day$^{-1}$) was observed in case of 4 MLE foliar sprays followed by CGR produced in MLE application only at heading (5.47 g m$^{-2}$ day$^{-1}$) while the maximum reduction (4.41 g m$^{-2}$ day$^{-1}$) was in untreated plants (Fig. 4.5.2). Maximal gain in net assimilation rate was observed up to 75 DAS as compared to control under MLE foliar spray at any growth stage with subsequent reduction and the crop subjected to 4 foliar sprays showed least reduction (2.46 g m$^{-2}$ day$^{-1}$) in net assimilation rate followed by NAR produced in MLE at heading (2.35 g m$^{-2}$ day$^{-1}$), the highest reduction (2.20 g m$^{-2}$ day$^{-1}$) was exhibited by foliar spray of water (Fig. 4.5.2). Similarly, response of MLE on yield and its related traits was significant. Maximum number of fertile tillers and grains per spike, 1000-grain weight, biological, and economic yield and harvest index were recorded when MLE was sprayed at tillering + jointing + booting + heading crop stages as compared to control (Fig. 4.5.3 & 4.5.4). Nonetheless, similar number of fertile tillers and grains per spike were recorded where MLE was applied at T+J and T+J+B stages (Fig. 4.5.3). But the response in case of 1000-grain weight, biological and
economic yield as well harvest index varied among the foliar applications at different stages and was highest in tillering + jointing + booting + heading spray followed by heading treatment. However, there was no significant (P>0.05) difference for these traits when MLE was applied at tillering, tillering + jointing and tillering + jointing and booting crop stages (Fig. 4.5.3, 4.5.4), whilst minimum values for these traits were observed in plants with only water application. Nevertheless, the performance of MLE at any growth stage was better than control and the pronounced effects of MLE were observed when it was sprayed at four crop stages (T + J + B + H) with the maximum contribution being observed under MLE application at heading.

Discussion
Late sowing of wheat shortens the growth period a pre-requisite for harvesting higher yield (Farooq et al., 2008). The postponement of wheat sowing after mid November induces yield reduction by 50 kg ha⁻¹ per day (Khan, 2004). Abrupt rise in temperature during early spring further intricate the problem (Wardlaw and Wrigley, 1994). In cool season cereal species, heat stress turns down the chlorophyll contents leading to many physiological damages; leaf senescence the major one (Xu and Huang 2008). Spano et al. (2003) emphasized that when assimilates supply to the grain decreased due to acceleration in senescence as in case of late sown wheat then delaying leaf senescence may be an advantageous attribute. MLE foliar spray at tillering, jointing, booting and heading stages showed highest value of seasonal leaf area duration (Fig. 4.5.1) due to delayed leaf senescence which resulted in 10.70% increment in grain yield. It might be due to cytokinin in the MLE, which has stay green induction quality as indicated by exogenously applied BAP enhanced the grain yield of Kalyansona and HD 2285 cultivars of wheat by 8.8 and 13.70%, 5.66 and 13.33%, under normal and late sown conditions, respectively (Gupta et al., 2003). When the transportation of cytokinins is reduced to leaves under heat stress, it increases the degree of senescence (early maturity). Cytokinin containing MLE application may negates the early maturing effects of heat stress exhibiting longer leaf area duration. In addition, moringa leaf is also rich in ascorbate, carotenoids, phenols, potassium and calcium like other plant growth enhancers (Foidle et al., 2001). Antioxidants such as ascorbic acid and glutathione are found at high concentrations in moringa chloroplasts and other cellular compartments are crucial for plant defense against oxidative stress (Noctor and Foyer, 1998). Under combined heat and drought stress a rise in
wheat grain yield and more stable cell membrane and chlorophyll were observed by exogenous application of cytokinin (Gupta et al., 2000). Thermotolerance and yield stability in maize was reported by Cheikh and Jones (1994) as a result of high cytokinin content in maize kernel for the heat stress period. Under late planting of wheat, maintenance of photosynthetic activity due to increased temperatures during maturation (Paulsen, 1994) and efficient utilization of these photosynthates indicated by high harvest index, (Gifford and Thorne, 1984; Blum et al., 1994) are two important determinants of grain yield. The maximum recorded harvest index (Fig. 4.5.4) by MLE sprayed at all stages supported that photosynthetic activity was maintained up to maturity which was result of longer seasonal leaf area duration and more stay green period. This delayed onset of leaf senescence is reported to provoke about 11% more carbon fixation in *Lolium temulentum* (Thomas and Howarth, 2000). An extension in active photosynthetic period may enhance total photosynthates availability in annual crops life cycle and higher mass per grain can be achieved if assimilated carbon supply be maintained to grain during grain filling period (Spano et al., 2003).

The MLE application at single stage of heading although was statistically different but closely followed the MLE treatment at four growth stages regarding 1000 grain weight (40.85 g), biological yield (13.65 t ha\(^{-1}\)), grain yield (3.19 t ha\(^{-1}\)) and harvest index (23.39), which may be due to the ability to maintain green leaf area duration “stay green” throughout grain filling period, remobilization of soluble carbohydrates during grain filling (Stoy, 1965) and by significant increment in grain weight of wheat by attracting more assimilates towards the developing grain with the application of benzyl adenine at anthesis (Warrier et al., 1987). In later phases of grain filling, leaf senescence caused shortage of assimilate then extended duration of photosynthesis provides more photoassimilate translocated to the grain, improved the grain weight as an outcome of amplified carbohydrate content.
Table 4.5.1 Mean sum of squares of data for LAI (50 DAS), LAI (57 DAS) and LAI (75 DAS) of wheat by exogenous application of MLE under late sown conditions.

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>LAI (50 DAS)</th>
<th>LAI (57 DAS)</th>
<th>LAI (75 DAS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>2</td>
<td>0.001</td>
<td>0.001</td>
<td>0.006</td>
</tr>
<tr>
<td>Treatment</td>
<td>5</td>
<td>0.001*</td>
<td>0.009*</td>
<td>0.032*</td>
</tr>
<tr>
<td>Error</td>
<td>10</td>
<td>0.000</td>
<td>0.000</td>
<td>0.003</td>
</tr>
</tbody>
</table>

**P<0.01    *P<0.05

LAI = Leaf area index
DAS = Days after sowing
CGR = Crop growth rate g m⁻² day⁻¹

Table 4.5.2 Mean sum of squares of data for LAI (85 DAS), LAI (95 DAS) and seasonal leaf area duration of wheat by exogenous application of MLE under late sown conditions.

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>LAI (85 DAS)</th>
<th>LAI (95 DAS)</th>
<th>Seasonal leaf area duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>2</td>
<td>0.001</td>
<td>0.001</td>
<td>0.037</td>
</tr>
<tr>
<td>Treatment</td>
<td>5</td>
<td>0.102**</td>
<td>0.048**</td>
<td>28.069**</td>
</tr>
<tr>
<td>Error</td>
<td>10</td>
<td>0.000</td>
<td>0.000</td>
<td>0.155</td>
</tr>
</tbody>
</table>

**P<0.01    *P<0.05

Table 4.5.3 Mean sum of squares of data for CGR (57 DAS), CGR (75 DAS) and CGR (85 DAS) of wheat by exogenous application of MLE under late sown conditions.

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>CGR (57 DAS)</th>
<th>CGR (75 DAS)</th>
<th>CGR (85 DAS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>2</td>
<td>0.005</td>
<td>0.001</td>
<td>0.005</td>
</tr>
<tr>
<td>Treatment</td>
<td>5</td>
<td>0.066*</td>
<td>0.621**</td>
<td>1.121**</td>
</tr>
<tr>
<td>Error</td>
<td>10</td>
<td>0.022</td>
<td>0.003</td>
<td>0.019</td>
</tr>
</tbody>
</table>

**P<0.01    *P<0.05

LAI = Leaf area index
DAS = Days after sowing
CGR = Crop growth rate g m⁻² day⁻¹
Table 4.5.4 Mean sum of squares of data for CGR (95 DAS), NAR (57 DAS) and NAR (75 DAS) of wheat by exogenous application of MLE under late sown conditions.

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>CGR (95 DAS)</th>
<th>NAR (57 DAS)</th>
<th>NAR (75 DAS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>2</td>
<td>0.007</td>
<td>0.002</td>
<td>0.003</td>
</tr>
<tr>
<td>Treatment</td>
<td>5</td>
<td>0.874**</td>
<td>0.006</td>
<td>0.019*</td>
</tr>
<tr>
<td>Error</td>
<td>10</td>
<td>0.011</td>
<td>0.010</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table 4.5.5 Mean sum of squares of data for NAR (85 DAS), NAR (95 DAS) and number of fertile tillers of wheat by exogenous application of MLE under late sown conditions.

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>NAR (85 DAS)</th>
<th>NAR (95 DAS)</th>
<th>Number of fertile tillers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>2</td>
<td>0.001</td>
<td>0.001</td>
<td>0.703</td>
</tr>
<tr>
<td>Treatment</td>
<td>5</td>
<td>0.026*</td>
<td>0.021*</td>
<td>447.051**</td>
</tr>
<tr>
<td>Error</td>
<td>10</td>
<td>0.003</td>
<td>0.003</td>
<td>2.774</td>
</tr>
</tbody>
</table>

Table 4.5.6 Mean sum of squares of data for number of grains per spike, 1000 grain weight and biological yield of wheat by exogenous application of MLE under late sown conditions.

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Number of grains per spike</th>
<th>1000 grain weight</th>
<th>Biological yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>2</td>
<td>0.222</td>
<td>0.002</td>
<td>0.001</td>
</tr>
<tr>
<td>Treatment</td>
<td>5</td>
<td>5.256*</td>
<td>5.345**</td>
<td>0.232**</td>
</tr>
<tr>
<td>Error</td>
<td>10</td>
<td>0.356</td>
<td>0.014</td>
<td>0.001</td>
</tr>
</tbody>
</table>

**P<0.01  *P<0.05  
CGR = Crop growth rate g m\(^{-2}\) day\(^{-1}\)  
NAR = Net assimilation rate g m\(^{-2}\) day\(^{-1}\)  
DAS = Days after sowing
Table 4.5.7 Mean sum of square summaries of the data for grain yield and harvest index of wheat crop by exogenous application of MLE under late sown conditions.

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Grain yield</th>
<th>Harvest index</th>
<th>--</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>2</td>
<td>0.001</td>
<td>0.007</td>
<td>--</td>
</tr>
<tr>
<td>Treatment</td>
<td>5</td>
<td>0.033**</td>
<td>0.342**</td>
<td>--</td>
</tr>
<tr>
<td>Error</td>
<td>10</td>
<td>0.000</td>
<td>0.004</td>
<td>--</td>
</tr>
</tbody>
</table>

**P<0.01  *P<0.05
Fig. 4.5.1. Effect of exogenous application of MLE on leaf area index (a) and seasonal leaf area duration (b) of wheat cv. Sehar-2006 under late sown conditions.

LSD 0.05p (50 DAS) = 0.019, LSD (57 DAS) = 0.019, LSD 0.05p (75 DAS) = 0.099, LSD 0.05p (85 DAS) = 0.019, LSD 0.05p (95 DAS) = 0.019

LSD 0.05p = 0.7162
Fig. 4.5.2. Effect of exogenous application of MLE on crop growth rate (a) and net assimilation rate (b) of wheat cv. Sehar-2006 under late sown conditions.
Fig. 4.5.3. Effect of exogenous application of MLE on number of fertile tillers (a), number of grains per spike (b) and 1000 grain weight (c) of wheat cv. Sehar-2006 under late sown conditions.
Fig. 4.5.4. Effect of exogenous application of MLE on biological yield (a), grain yield (b) and harvest index of wheat cv. Sehar-2006 under late sown conditions.
4.6. Experiment VI:

Response of wheat to exogenous application of MLE under saline stress conditions

The growth, yield and antioxidant status of wheat was studied as affected by exogenous application of MLE and other growth enhancers under saline conditions. The data collected during course of research are mentioned and discussed below:

The increased salinity significantly affected the shoot and root growth of wheat seedlings (Table 4.6.1 and 4.6.2). The reduction in shoot length while enhancement in root length was found with increased salinity (Fig. 4.6.1 a, d). BAP and MLE foliar spray showed maximum shoot length under low saline conditions while the longest roots were observed with similar treatments under highly saline conditions. All the treatments produced shoot fresh and dry weight better than control under each salinity levels (Fig. 4.6.1 b, c). The exogenous application of BAP and MLE improved shoot fresh and dry weight as compared to control under moderate or high salinity levels. Impostion of salt stress also reduced biomass accumulation in roots (Fig. 4.6.1 e, f). The exogenous application of different growth enhancers minimized the effect of salinity under moderate and high salinity levels. As a result more root fresh and dry weights as compared to control were observed under 8 and 12 dS m$^{-1}$ by BAP and MLE foliar spray, respectively. The analysis of variance for the leaf area indicates a significant difference among plants treated with exogenous application of different growth enhancers under salinity (Table 4.6.3). The largest leaf area was produced by MLE application under less saline conditions (Fig. 4.6.2 a). While BAP performed better than other growth enhancers under moderate or high salinity as exhibited by more leaf area. The chlorophyll $a$ and $b$ contents were decreased by salinity. Under nonsaline conditions highest contents were obtained from MLE treated plants (Fig. 4.6.2 b, c) whereas in saline condition either moderate or high BAP showed maximum enhancement in the chlorophyll contents.

The MLE and BAP were statistically at par with respect to total soluble proteins (Fig. 4.6.3 a). The protein contents were increased with increasing salinity stress and more protein being observed at highest salinity level. At 12 dS m$^{-1}$ largest total soluble proteins observed in BAP foliar spray and minimum total soluble proteins produced in control under low saline conditions. Both the qualitative and quantitative difference in antioxidant contents were
observed in salinized medium in response to application of growth enhancers (Fig. 4.6.3 a, b, c, d, e, f). Increasing salinity increased the contents of enzymatic antioxidants up to 8 dS m$^{-1}$. MLE treatment showed higher content of enzymatic antioxidants i.e. catalase, peroxidase and superoxide dismutase (SOD) as compared to control under low and moderately saline conditions. The higher salinity levels caused a reduction in enzymatic antioxidant contents and minimum effects were observed in MLE foliar spray. The minimum recorded value of SOD, POD and catalase were under less saline conditions in control treatment. In non enzymatic antioxidant salt stress showed a gradual rise in ascorbic acid contents (Fig. 4.6.3c). The significantly maximum content of ascorbic acid was obtained by MLE treated plants under highly saline conditions. Lowest ascorbic acid was found in control under normal conditions. The higher salinity showed phenolics lesser than moderate salinity but better than less saline conditions. The peak value of total phenolic content was observed under MLE foliar application at moderate salinity. The minimum phenolics value was observed at 4 dS m$^{-1}$ in case of control. Accumulation of Na$^+$ and Cl$^-$ in the leaves was significantly increased under saline conditions (Fig 4.6.4 a, c). Exogenous application of MLE showed minimum Na$^+$ and Cl$^-$ with maximum K$^+$ in leaves under highly saline conditions. The maximum Cl$^-$ contents were also found in control but under highly saline conditions (Fig. 4.6.4 c.). As far as yield attributes are concerned the increasing salinity show a decreasing trend in number of grains and 100 grain weight (Fig. 4.6.5 a, b). The BAP application produced maximum number of grains whereas highest grain weight was obtained under MLE treated plants in less saline conditions. The minimum number of grains and 100 grain weight was observed in control at 12 dS m$^{-1}$.

Discussion

It is widely believed that salt stress greatly reduces wheat growth at all growth stages, particularly during germination and seedling stage (Munns et al., 2006). Suppression of growth in different plant organs occurs differently under saline conditions (Shoresh et al., 2011). Salinity caused similar reduction in biomass of both root and shoot (fresh and dry weight) but proportionally shoots length was decreased more as compared to roots length in present study. However, exogenous application of essential elements enriched MLE through foliar spray was found promising to accelerate the growth of different plant organs both under saline and non saline conditions. Previously, reduction in growth of all the vegetative,
reproductive and biochemical parameters of tomato was found proportional with increasing salinity of irrigation but foliar application of essential minerals like K, Fe and B minimized the deleterious effects of salinity up to various extents. In the same study spray of individual mineral was less effective as compared with their mixture but still better than non spray control (Azeem and Ahmad, 2011).

The yield of a crop is mostly affected by its photosynthetic ability (Akram et al., 2002). The smallest leaf area observed in present investigation under highest salinity level may be due to reduction in water uptake and the nutritional imbalance causing toxicities or deficiencies of ions under salinity leading to leaf injuries (Munns et al., 2006). The salinity not only reduced the leaf area but also decreased chlorophyll contents. Nevertheless, the foliar spray of growth enhancers partially alleviates this reduction and minimum reduction in chlorophyll contents was observed with foliar spray of MLE during moderate salinity. Shoresh et al. (2011) observed that salinity caused reduction in chlorophyll content in combination with the reduced leaf area and growth. He further found that negative effects of salinity can be ameliorated partially with improvement of plant growth by supplemental Ca\(^{2+}\) application. It has been reported that moringa leaves have four times more calcium than milk, three times the potassium of banana and seven times the ascorbic acid of oranges (Foidle et al., 2001), so the application of MLE improve chlorophyll contents under salinity. Hanaa et al. (2008) also earlier reported that total chlorophyll, chlorophyll \(a\) and chlorophyll \(b\) contents were significantly improved in wheat plants sprayed with bioregulator (bezyladenine and ascorbic acid), but this increase was less pronounced, when compared with that induced in wheat stressed plant treated with algal antioxidant extracts. While foliar spray of ascorbic acid also minimized the reduction of chlorophyll \(a\) by salinity in wheat (Athar et al., 2008). Foliar application of potassium containing materials can minimize the antagonistic effects of soil salinity (Ahmad and Jabeen, 2005). Moringa leaf extracts foliar application prevents premature leaf senescence and resulting in more leaf area with higher photosynthetic pigments (Rehman and Basra, 2010).

The incomplete reduction of \(O_2\) to form water under abiotic stresses such as salinity leads to generation of reactive oxygen species (ROS) such as singlet oxygen, superoxide (\(O_2^-\)), hydrogen peroxide (\(H_2O_2\)) and hydroxyl radical (OH\(^•\)) (Mittler, 2002; Dat et al., 2000). As they are in ionic forms or being radicals so highly reactive and a threat to the cell membrane
and other important cellular organelle DNA, proteins and lipids etc. In plant defense mechanism both enzymatic (Superoxide dismutase (SOD), Catalase (CAT), Peroxidase (POD) etc.) and non enzymatic (ascorbates, phenols etc.) antioxidants as scavenger of ROS have gained primary consideration (Asada and Takahashi, 1987; Noctor and Foyer, 1998; Sgherri et al., 2004). We found enhancement in antioxidants contents under exogenous application of growth enhancers was more at 8 dS m$^{-1}$ as compared to 12 dS m$^{-1}$ MLE exhibit highest antioxidant status. The continuous improvements in ascorbic acid contents with increasing salinity were obtained by MLE application. Ascorbates are powerful primary antioxidant and direct scavenger of ROS (Buettner and Jurkiewicz, 1996). Ascorbic acid induced ionic changes might have triggered the antioxidant system. Thus, enhanced salt tolerance in wheat plants was due to ascorbic acid producing a better antioxidant system for the effective removal of ROS in plants, and help in maintenance of ion homeostasis (Mittler, 2002). Overall, exogenous application of ascorbate increased endogenous level of ascorbic acid which exerted a protective effect on growth and also improved photosynthetic capacity of wheat against salt induced oxidative stress (Athar et al., 2008). The increased contents of antioxidants in response to enhanced level of ROS generation under salinity have been studied in wheat, cotton, maize and rice (Sairam et al., 2001; Vaidyanathan et al., 2003). According to Zhang and Ervin (2008) stress tolerance in creeping bent grass was improved with increased SOD level by the application of cytokinin containing sea weed extract. Since moringa is a rich source of zeatin (Foidle et al., 2001), so the effectiveness of MLE in mitigating salinity by better chlorophyll, antioxidants and plant growth might be due to cytokinin mediated stay green effect. Similarly, H$_2$O$_2$ exogenous application also plays an important role in improving plant’s tolerance against oxidative stress by activating plant antioxidants system (Gechev et al., 2002).

Salt tolerance in sugarcane leaves improved with enhanced level of soluble phenolics (Wahid and Ghazanfar, 2006). It may be justified that under moderate salinity ROS were cellular indicators playing roles of secondary messengers to activate plant defense system against salt injuries (Knight and Knight, 2001) resulting in increased antioxidants level by these treatment. But under highly saline conditions injuries caused by ROS exceeded their intracellular stress monitoring role and resulted in enzyme inhibition, oxidation and peroxidation of proteins and lipids respectively leading to programmed cell death (Mittler,
So the lesser quantities of antioxidants at 12 dS m\(^{-1}\) as compared to 8 dS m\(^{-1}\) were found except ascorbates which continue to increase up to 12 dS m\(^{-1}\).

Salinity also produces ionic stress leading to ionic imbalances and impairment of root membrane selectivity. In our research the application of MLE, BAP and H\(_2\)O\(_2\) decreased the concentration of Na\(^+\) and Cl\(^-\) along with increased concentration of K\(^+\) in leaves against control. The maximum exclusion of Na\(^+\) and Cl\(^-\) with highest K\(^+\) accumulation was observed in MLE foliar spray up to moderate salt level. Similarly, increased K\(^+\) accumulation in leaves under salinity was obtained by ascorbic acid application in wheat (Athar \textit{et al.}, 2008). Makkar \textit{et al.} (2007) reported that MLE contains significant quantities of calcium, potassium, and cytokinin in the form of zeatin, antioxidants proteins, ascorbates and phenols. Such compositions of MLE not only enhanced seedling vigor and provide a good start to plant even under saline conditions but also improved the grain yield.
Table 4.6.1 Mean sum of squares of the data for shoot length, shoot fresh weight and shoot dry weight of wheat by exogenous application of growth enhancers under saline conditions.

<table>
<thead>
<tr>
<th>SOV</th>
<th>Df</th>
<th>Shoot length</th>
<th>Shoot fresh weight</th>
<th>Shoot dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salinity</td>
<td>2</td>
<td>100.107**</td>
<td>9.003**</td>
<td>1.581**</td>
</tr>
<tr>
<td>Treatment</td>
<td>3</td>
<td>38.547**</td>
<td>0.433**</td>
<td>0.198**</td>
</tr>
<tr>
<td>Salinity x Treatment</td>
<td>6</td>
<td>2.698**</td>
<td>0.047**</td>
<td>0.016**</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>1.036</td>
<td>0.011</td>
<td>0.000</td>
</tr>
</tbody>
</table>

** p < 0.01 ns = non significant

Table 4.6.2 Mean sum of squares of data for root length, root fresh weight and root dry weight of wheat by exogenous application of growth enhancers under saline conditions.

<table>
<thead>
<tr>
<th>SOV</th>
<th>Df</th>
<th>Root length</th>
<th>Root fresh weight</th>
<th>Root dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salinity</td>
<td>2</td>
<td>98.045**</td>
<td>3.096**</td>
<td>0.184**</td>
</tr>
<tr>
<td>Treatment</td>
<td>3</td>
<td>30.592**</td>
<td>0.655**</td>
<td>0.028**</td>
</tr>
<tr>
<td>Salinity x Treatment</td>
<td>6</td>
<td>1.110</td>
<td>0.026**</td>
<td>0.002**</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>1.738</td>
<td>0.004</td>
<td>0.000</td>
</tr>
</tbody>
</table>

** p < 0.01 ns = non significant
Table 4.6.3 Mean sum of squares of the data for leaf area, leaf chlorophyll $a$ and chlorophyll $b$ of wheat by exogenous application of growth enhancers under saline conditions.

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Leaf area</th>
<th>Chlorophyll $a$</th>
<th>Chlorophyll $b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salinity</td>
<td>2</td>
<td>1333.117**</td>
<td>0.259**</td>
<td>0.010**</td>
</tr>
<tr>
<td>Treatment</td>
<td>3</td>
<td>745.538**</td>
<td>0.290**</td>
<td>0.003**</td>
</tr>
<tr>
<td>Salinity x Treatment</td>
<td>6</td>
<td>35.512**</td>
<td>0.003**</td>
<td>0.000**</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>1.437</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

** p < 0.01 ns = non significant

Table 4.6.4 Mean sum of squares of the data for total soluble protein, enzymatic antioxidants SOD and POD of wheat by exogenous application of growth enhancers under saline conditions.

<table>
<thead>
<tr>
<th>SOV</th>
<th>Df</th>
<th>Total soluble protein</th>
<th>Superoxide dismutase (SOD)</th>
<th>Peroxidase (POD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salinity</td>
<td>2</td>
<td>0.012**</td>
<td>4076.635**</td>
<td>51.660**</td>
</tr>
<tr>
<td>Treatment</td>
<td>3</td>
<td>0.040**</td>
<td>16694.870**</td>
<td>272.886**</td>
</tr>
<tr>
<td>Salinity x Treatment</td>
<td>6</td>
<td>0.000*</td>
<td>771.991**</td>
<td>8.307**</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>0.000</td>
<td>15.810</td>
<td>0.340</td>
</tr>
</tbody>
</table>

** p < 0.01 ns = non significant
Table 4.6.5 Mean sum of squares of the data for enzymatic antioxidants (Catalase), non-enzymatic antioxidants i.e. total phenolic contents and ascorbic acid of wheat by exogenous application of growth enhancers under saline conditions.

<table>
<thead>
<tr>
<th>SOV</th>
<th>Df</th>
<th>Catalase (CAT)</th>
<th>Total phenolic content (TPC)</th>
<th>Ascorbic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salinity</td>
<td>2</td>
<td>24.339**</td>
<td>0.173**</td>
<td>0.023**</td>
</tr>
<tr>
<td>Treatment</td>
<td>3</td>
<td>38.528**</td>
<td>3.229**</td>
<td>0.126**</td>
</tr>
<tr>
<td>Salinity x Treatment</td>
<td>6</td>
<td>2.629**</td>
<td>0.032**</td>
<td>0.011**</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>0.040</td>
<td>0.008</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Table 4.6.6 Mean sum of square of the data for Na⁺, K⁺ and Cl⁻ contents of wheat by exogenous application of growth enhancers under saline conditions.

<table>
<thead>
<tr>
<th>SOV</th>
<th>Df</th>
<th>Na⁺</th>
<th>K⁺</th>
<th>Cl⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salinity</td>
<td>2</td>
<td>0.036**</td>
<td>0.151**</td>
<td>5.510**</td>
</tr>
<tr>
<td>Treatment</td>
<td>3</td>
<td>0.161**</td>
<td>0.735**</td>
<td>8.680**</td>
</tr>
<tr>
<td>Salinity x Treatment</td>
<td>6</td>
<td>0.247**</td>
<td>0.106**</td>
<td>2.534**</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>0.001</td>
<td>0.002</td>
<td>0.034</td>
</tr>
</tbody>
</table>

** p < 0.01 ns = non significant
Table 4.6.7 Mean sum of squares of the data for number of grains per spike and 100 grain weight of wheat by exogenous application of growth enhancers under saline conditions.

<table>
<thead>
<tr>
<th>SOV</th>
<th>Df</th>
<th>Number of grains per spike</th>
<th>100 grain weight</th>
<th>--</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salinity</td>
<td>2</td>
<td>1043.084**</td>
<td>1.451**</td>
<td>--</td>
</tr>
<tr>
<td>Treatment</td>
<td>3</td>
<td>353.688**</td>
<td>1.154**</td>
<td>--</td>
</tr>
<tr>
<td>Salinity x Treatment</td>
<td>6</td>
<td>17.847**</td>
<td>0.032**</td>
<td>--</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>1.084</td>
<td>0.008</td>
<td>--</td>
</tr>
</tbody>
</table>

** p < 0.01  ns = non significant
Fig 4.6.1. Effect of exogenous application of different plant growth enhancer on shoot length (a), shoot fresh weight (b), shoot dry weight (c), root length (d), root fresh weight (e) and root dry weight (f) of wheat cv. Sehar-2006 under saline conditions.
Fig 4.6.2. Effect of exogenous application of different plant growth enhancers on leaf area (a), chlorophyll \( a \) (b) and chlorophyll \( b \) (c) of wheat cv. Sehar-2006 under saline conditions
Fig 4.6.3. Effect of exogenous application of different plant growth enhancers on total soluble protein (a), SOD (b), ascorbic acid (c), catalase (d), POD (e) and total phenolics (f) of wheat cv. Sehar-2006 under saline conditions.
Fig 4.6.4. Effect of exogenous application of different plant growth enhancer on leaf Na$^+$ (a), K$^+$ (b) and Cl$^-$ (c) of wheat cv. Sehar-2006 under saline conditions

**Legend:**
- Control
- MLE foliar spray
- BAP foliar spray
- H2O2 foliar spray

**LSD 0.05p = 0.05329**

(a) Na$^+$ (mg g$^{-1}$ dry weight)

(b) K$^+$ (mg g$^{-1}$ dry weight)

(c) Cl$^-$ (mg g$^{-1}$ dry weight)
Fig 4.6.5. Effect of exogenous application of different plant growth enhancers on number of grains (a) and 100 grain weight (b) of wheat cv. Sehar-2006 under saline conditions.
4.7. Experiment VII: Response of wheat to exogenous application of MLE under drought stress conditions

Increasing drought stress levels significantly affected plant growth and yield (Table 4.7.1 and 4.7.4). But improvement in antioxidants status was obtained by exogenous applications (Table 4.7.2, 4.7.3). The detailed results are mentioned and discussed below:

Comparison of means indicate that leaf area was gradually reduced with increasing drought stress (Fig. 4.7.1a). The minimum leaf area was obtained under extreme drought conditions. The interaction of drought and foliar application on leaf area was found to be significant (Table 4.7.1). The leaf area produced by the foliar application of MLE and BAP was better than control under well watered, moderate and severe drought stress. The minimum leaf area was recorded in control under severe drought stress.

The increasing water stress decreased leaf chlorophyll \( a \) contents (Fig. 4.7.1b). The maximum quantity of chlorophyll \( a \) was observed under well watered conditions by exogenous application of MLE and BAP. While in moderate and severe drought, foliar application of K and MLE exhibit higher and statistically similar leaf chlorophyll \( a \). The similar trend were followed by all the treatments in case of chlorophyll \( b \) under each level of water stress (Fig. 4.7.1a). The minimum quantity of chlorophyll \( a \) and chlorophyll \( b \) were produced by control under extreme water stress.

A direct relationship was evident between drought stress level and leaf total soluble protein (Fig. 4.7.2a). The largest leaf protein content was produced at moderate followed by severe with minimum value under well watered conditions. The BAP foliar spray showed significantly highest value of leaf protein in well watered and under both drought stress levels. Nevertheless, the exogenous application of growth enhancers performed better than control in case of leaf total soluble protein.

The rise in drought stress amplified the antioxidants status of wheat plant (Fig. 4.7.2b, c, d). The enzymatic and nonenzymatic antioxidants contents produced by exogenous application of all growth enhancers were better than control under each water level. The contents of enzymatic antioxidants were increased up to moderate drought stress. The application of BAP produced maximum superoxide dismutase (SOD) under severe drought. In case of POD, water stress increased POD contents as compared to non stress conditions. Largest
increase was found under medium drought stress by MLE application. Similar trend as that of POD was followed by catalase. Decreasing water level increased ascorbic acid contents in all the treatments (Fig. 4.7.2 e). The highest value of ascorbic acid obtained under moderate drought by exogenous application of MLE30. However, ascorbic acid remains unaffected by severity of drought and produced in similar quantity under moderate and severe drought by foliar application of MLE30.

The TPC contents were found in comparatively lesser quantities as compared to ascorbic acid (Fig. 4.7.2 e). The maximum TPC produced during severe water stress under MLE30. The application of K⁺ and BAP showed similar TPC contents under well watered and severe drought. The minimum TPC were obtained in case of control under well watered conditions MLE application accumulated largest leaf K⁺ ions under moderate drought stress (Fig. 4.7.3 a). Under severe water stress foliar application of MLE produced K⁺ ion in similar quantity as that of its respective value under well watered conditions. The least K⁺ ion was observed in control under severe drought stress.

Highest grain yield plant⁻¹ observed in the absence of water stress by MLE foliar spray (Fig. 4.7.3b). However, deficiency of water decreased grain yield but all the treatments ameliorated the negative effects of water stress significantly better than control. MLE application produced more grain yield under moderate and severe water stress as compared to BAP, K⁺ and control.

Discussion

There is a faster expansion in arid zones on our planet as an impact of climate change. The dual challenges of expanding arid land and faster world’s population growth exerts direct impact on water reservoirs and water availability. This aridity and water stress have direct impact on crop growth and yield reduction. We also reported that water stress caused reduction in growth and grain yield of wheat. Reduction in plant growth under restricted water availability might have been due to reduction in photosynthesizing tissue and photosynthetic capacity (Athar and Ashraf, 2005; Chaves et al., 2011). Chlorophyll and protein contents affect the photosynthetic capacity of plants and considered as important adaptation traits under drought stress conditions (Chernyad’ev and Monakhova, 2003). The level of chlorophyll must be essentially maintained in water deficit situation for supporting
photosynthetic capacity of plants (Sairam et al., 1997). Nevertheless, in the present study, reduced yield due to water stress was not only correlated with leaf area but also with chlorophyll \( a \) and chlorophyll \( b \) (Fig. 4.7.1). Exogenous application caused increase in yield under normal or water stress conditions, except that of \( K^+ \) as a foliar spray. However, foliar application of MLE caused maximum increase in grain yield of wheat under control or water stress conditions. These results are similar with those of Zhang et al. (2004) who found that chlorophyll contents were significantly improved by exogenous application of growth regulators under water deficit conditions. The foliar application of algal extract, MLE and BAP may also stimulate earlier cytokinin formation thus preventing premature leaf senescence resulting in more leaf area with higher photosynthetic pigments (Hanaa et al., 2008; Rehman and Basra, 2010; Ali et al., 2011). The phytoregulators exhibiting cytokinin activity showed protective effects in water deficit and prevent reduction in chlorophyll and protein contents. In view of Schachtman and Goodger (2008) plant growth regulators such as cytokinins, activated under water stress conditions initiate defensive responses in plants to protect plant’s important processes from water stress injuries. In the presence of cytokinin like activity compounds e.g. Kartolin less reduction were observed in protein contents (Chernyad’ev and Monakhova, 2003). The positive effects of BAP on protein pool was also pronounced under drought stress. Besides adaptation role, hormones also regulate yield potential, by controlling floret survival and grain filling capacity in cereals (Young et al., 1997). Less reduction in growth and yield observed in present study by foliar spray of MLE and BAP in drought stressed wheat plants could be attributed to hormonal influence especially rich zeatin contents of moringa.

Moringa leaves have been reported to be a rich source of \( \beta \)-carotene, protein, vitamin C, calcium and potassium and act as a good source of natural antioxidants such as ascorbic acid, flavonoid, phenolics and carotenoids (Dillard and German, 2000; Siddhuraju and Becker, 2003). So the exogenous applications of MLE improve the antioxidant status and yield of wheat under drought stress.
Table 4.7.1 Mean sum of squares of data for leaf area, leaf chlorophyll $a$ and chlorophyll $b$ contents of wheat by exogenous application of growth enhancers under drought conditions.

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Chlorophyll $a$</th>
<th>Chlorophyll $b$</th>
<th>Leaf area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drought</td>
<td>2</td>
<td>0.323**</td>
<td>0.293**</td>
<td>21294.976**</td>
</tr>
<tr>
<td>Treatments</td>
<td>3</td>
<td>0.726**</td>
<td>0.028**</td>
<td>23144.557**</td>
</tr>
<tr>
<td>Drought x Treatments</td>
<td>6</td>
<td>0.064**</td>
<td>0.004**</td>
<td>848.006**</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>0.001</td>
<td>0.001</td>
<td>51.686</td>
</tr>
</tbody>
</table>

Table 4.7.2 Mean sum of squares of data for total soluble proteins, enzymatic antioxidants i.e. super oxide dismutase (SOD) and peroxidase (POD) of wheat by exogenous application of growth enhancers under drought conditions.

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Total soluble proteins</th>
<th>Superoxide dismutase (SOD)</th>
<th>Peroxidase (POD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drought</td>
<td>2</td>
<td>0.052**</td>
<td>158.854**</td>
<td>5.973**</td>
</tr>
<tr>
<td>Treatments</td>
<td>3</td>
<td>0.511**</td>
<td>523.489**</td>
<td>7.031**</td>
</tr>
<tr>
<td>Drought x Treatments</td>
<td>6</td>
<td>0.025**</td>
<td>17.088**</td>
<td>0.681**</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>0.004</td>
<td>1.338</td>
<td>0.013</td>
</tr>
</tbody>
</table>

** $P < 0.01$
### Table 4.7.3 Mean sum of squares of data for enzymatic antioxidant catalase (CAT), non-enzymatic antioxidant ascorbic acid and total phenolics (TPC) of wheat by exogenous application of growth enhancers under drought conditions.

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Catalase (CAT)</th>
<th>Ascorbic acid</th>
<th>Total phenolics (TPC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drought</td>
<td>2</td>
<td>1357.425**</td>
<td>364.912**</td>
<td>2.689**</td>
</tr>
<tr>
<td>Treatments</td>
<td>3</td>
<td>642.191**</td>
<td>486.082**</td>
<td>6.214**</td>
</tr>
<tr>
<td>Drought x Treatments</td>
<td>6</td>
<td>64.105**</td>
<td>15.383**</td>
<td>0.208**</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>1.550</td>
<td>0.819</td>
<td>0.013</td>
</tr>
</tbody>
</table>

** P < 0.01

### Table 4.7.4 Mean sum of squares of data for leaf K⁺ contents and grain yield of wheat by exogenous application of growth enhancers under drought conditions.

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Leaf K⁺ contents</th>
<th>Grain yield per plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drought</td>
<td>2</td>
<td>0.111**</td>
<td>1.924**</td>
</tr>
<tr>
<td>Treatments</td>
<td>3</td>
<td>0.287**</td>
<td>0.265**</td>
</tr>
<tr>
<td>Drought x Treatments</td>
<td>6</td>
<td>0.008**</td>
<td>0.007*</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>0.001</td>
<td>0.004</td>
</tr>
</tbody>
</table>

** P < 0.01
Fig. 4.7.1. Effect of exogenous application of different plant growth enhancers on leaf area (a), chlorophyll $a$ (b) and chlorophyll $b$ (c) of wheat cv. Sehar-2006 under drought conditions.
Fig. 4.7.2. Effect of exogenous application of different plant growth enhancer on total soluble protein (a), super oxide dismutase (b), ascorbic acid (c), catalase (d), peroxidase (e) and total phenolic contents (f) of wheat cv. Sehar-2006 crop under drought conditions.
Fig. 4.7.3. Effect of exogenous application of different plant growth enhancer on leaf K⁺ contents (a) and grain yield plant⁻¹ (b) of wheat cv. Sehar-2006 under drought conditions.
General Discussion

This dissertation examines the potential of moringa leaf extract (MLE) as a natural plant growth enhancer in various crops under normal and abiotic stresses. Moringa belongs to family Moringaceae and is a natural as well as cultivated variety of the genus *Moringa* (Mahmood *et al*., 2010). It is the richest plant source of vitamins A, B, C, D, E and K (Anwar and Bhanger, 2003; Babu, 2000; Caceres *et al*., 1992; Dayrit *et al*., 1990). The vital minerals present in moringa leaves include calcium, potassium, copper, iron, magnesium, zinc and manganese. From the analysis of moringa leaves it become clear that they have appreciable quantities of mineral nitrogen, phosphorus, potassium, calcium, magnesium, zinc, copper, iron, manganese (Table, 3.1). Moreover presence of total soluble protein, enzymatic antioxidants (SOD, POD and CAT) and non enzymatic antioxidants (total phenolics and ascorbates) was observed during analysis of moringa leaf extract (Table, 3.1). Foidle *et al*. (2001) also reported that MLE have plant growth enhancing capabilities as it is rich in zeatin, ascorbates, carotenoids, phenols, potassium and calcium. Moringa truly appears a gift of nature and a "Miracle" plant having countless benefits for humanity so it can be used to fight against hunger, poverty and malnutrition.

Leaf extracts of *M. oleifera* (MLE) have been reported to accelerate growth of tomato, peanut, corn and wheat at early vegetative growth stage, improve resistance to pests and diseases and enhanced more and larger fruits and generally increase yield by 20 to 35% (Fuglie, 2000). The germination and growth promotion of wheat seeds was found to be concentration depended and MLE diluted up to 30 times proved effective as compared to other dilutions. The reduced germination of wheat seeds by application of concentrated (10 times diluted) MLE was also reported by Phiri (2010). The growth enhancing characteristics of MLE was also noted to be affected by its method of exogenous application (priming, soil or foliar). In some earlier studies it has been observed that seed priming with plant growth regulators, inorganic salts, compatible solutes or sugar beet extract caused improvement in seed germination by providing physiological and biochemical adaptations (Pill and Savage, 2008; Afzal *et al*., 2006a). The seed priming with MLE30 enhances seed germination and seedling vigour along with improvement in antioxidant status of wheat. Likewise, while working with three range grasses (*Cenchrus ciliaris*, *Panicum antidotale* and *Echinochloa crusgalli*) Nouman *et al*. (2011) reported that seed priming with MLE30 significantly increased germination of all the
range grasses. It may be attributed that MLE is a rich source of zeatin, ascorbic acid, Ca\(^{2+}\), and K\(^{+}\) (Fuglie 1999; Foidle et al. 2001), which are involved in improving several plant growth and development processes. Al-Hakimi and Hamada (2001) observed that seed priming with ascorbic acid increased leaf soluble proteins. According to Price (2000) moringa leaf extract contain ascorbic acid in appreciable quantities. Thus, increased leaf protein due to MLE30 seed priming was one of the reasons that contributed in improved growth of wheat. The foliar applied MLE30 as compared to soil application caused more enhancements in antioxidants, growth and yield of tomato and pea crop.

Besides crop production under normal conditions, abiotic stresses cause significant crop losses with the 50% reduction in average yield of major crops (Bray et al., 2000). Among abiotic stresses drought, salinity and extreme temperature along with chemical toxicity and oxidative stress becoming major threats to agriculture and natural environment sustainability (Wang et al., 2003). As plants produce significant amount of antioxidants to prevent the oxidative stress, they represent a potential source of compounds with antioxidant activity such as ascorbic acid, total phenols, and vitamins in addition to mineral elements K\(^{+}\), Ca\(^{2+}\) and PGRs. Alternatively, toxicological effects of synthetic antioxidants and consumer preference for natural products have resulted in increased interest in the application of natural antioxidants (Castenmiller et al., 2002; Kaur and Kapoor, 2001; Koleva et al., 2002; Pizzale et al., 2002). Siddhuraju and Becker (2003) observed antioxidant properties in the solvent extract of moringa leaves and reported that leaves are potential source of natural antioxidants. According to Arabshahi et al. (2007) the extracts from drumstick and carrot had a higher antioxidant activity (83% and 80%) than α-tocopherol (72%). The presence of antioxidants in MLE was also observed during current studies (Table 3.1).

Under late planting of wheat, maintenance of photosynthetic activity due to increased temperatures during maturation (Paulsen, 1994) and efficient utilization of these photosynthates indicated by high harvest index, (Gifford and Thorne, 1984; Blum et al., 1994) are two important determinants of grain yield. The maximum recorded harvest index by MLE sprayed from start up to end of wheat growth (tillering + jointing + booting + heading) supported that photosynthetic activity was maintained up to maturity which was result of longer seasonal leaf area duration and more stay green period. This delayed onset of leaf senescence is reported to provoke about 11% more carbon fixation in *Lolium temulentum*
(Thomas and Howarth, 2000). An extension in active photosynthetic period may enhance total photosynthates availability in annual crops life cycle and higher mass per grain can be achieved if assimilated carbon supply be maintained to grain during grain filling period (Spano et al., 2003). MLE extended the seasonal leaf area duration (SLAD) by 9.22 d and 6.45 d over control when applied at all growth stages and single spray at heading, respectively.

The increased contents of antioxidants in response to enhanced level of ROS generation under salinity have been studied in wheat, cotton, maize and rice (Sairam et al., 2001; Vaidyanathan et al., 2003). The foliar application of MLE exhibit highest antioxidant status as compared to other growth enhancers up to moderate salinity (8 dS m\(^{-1}\)) except ascorbic acid which continued to be increased up to highest salinity level (12 dS m\(^{-1}\)). Moreover, the increase in ascorbate under MLE application in salinity stress was largest than all other enzymatic and nonenzymatic antioxidants. As reported earlier MLE is a rich source of ascorbate and exogenous application of ascorbate increased endogenous level of ascorbic acid which exerted a protective effect on growth and also improved photosynthetic capacity of wheat against salt induced oxidative stress (Athar et al., 2008). The phytoregulators exhibiting cytokinin activity showed protective effects in water deficit and prevent reduction in chlorophyll and protein contents. In view of Schachtman and Goodger (2008) plant growth regulators such as cytokinins, activated under water stress conditions initiate defensive responses in plants to protect plant’s important processes from water stress injuries. Moringa leaves have been reported to be a rich source of β-carotene, protein, vitamin C, calcium and potassium and act as a good source of natural antioxidants such as ascorbic acid, flavonoid, phenolics, carotenoids and PGR like zeatin (Dillard and German, 2000; Siddhuraju and Becker, 2003). So the exogenous applications of MLE improve the antioxidant status and yield of wheat under drought stress.

Finally it is concluded that MLE was effective in improving growth and yield of wheat, tomato and pea. Thirty times diluted MLE was found to be optimum dose in laboratory studies and confirmed in pot and field studies. The exogenous application of MLE as priming agent or foliar spray improve the antioxidant status chlorophyll contents, leaf area, grain weight and finally yield of wheat crop under normal and abiotic stress (late sowing, drought and salinity) conditions.
Summary

Crop productivity is affected by many abiotic stresses i.e. temperature extremes, drought and salinity and each of these limit plant growth and development. Plants have internal system of regulation to modify these responses. Plants also respond to the exogenous application of antioxidants, PGRs and certain nutrients for abiotic and biotic stress tolerance that results in higher economic return. This dissertation investigated the role of exogenous application of MLE in vivo and in vitro as plant growth enhancer and amelioration of devastating affects of high temperature stress in late sown wheat and under drought and salinity stresses. The physiological and biochemical basis for improved performance of peas and tomato by MLE application was also assessed. Wheat cultivar Sehar-2006, tomato cv.Sahil and pea cv.Climax were used in the study. Seven independent experiments were performed to examine the effect of exogenous application of MLE at the germination, seedling, vegetative and adult growth stages. Experiments at the germination and seedling stages were conducted in a laboratory, while those of at the adult growth stage were conducted in a wire-net house and field conditions of the Department of Crop Physiology, University of Agriculture, Faisalabad, Pakistan, during 2008-2009. In first experiment wheat seeds were allowed to germinate supplemented with different dilutions of MLE. The second was comprised of MLE evaluation as priming agent. The third and fourth experiment were involve the assessment of MLE exogenous application through soil and foliar in tomato and pea. In the fifth experiment potential of MLE was evaluated in growth and yield of wheat under late sowing conditions. The sixth and seventh experiment was conducted in saline and drought conditions respectively to assess the comparative effect of foliar applied growth enhancers along with MLE. From the results of seed germination experiment it is evident that MLE30 (30 times diluted MLE) was the optimum dose. MLE30 priming excelled all other priming agents used in study and showed 23% more grain weight which leads to highest (35%) grain yield per plant with enhanced antioxidant status of plant. The foliar application was superior to soil application; however both modes of exogenous application performed significantly better than control in all growth, yield and biochemical parameters of tomato and pea crop. The foliar application method of MLE30 dilution was most effective and has a potential to be used as plant growth enhancer in tomato and pea crop. The foliar MLE spray delayed the crop maturity, extend SLAD (9.22 d and 6.45 d over control) and grain filling period there by
leading to greater seed and biological yields in late sown wheat. The performance of MLE at any growth stage under late sown wheat was better than control and the pronounced effects of MLE were observed when it was sprayed at four critical crop stages (tillering + jointing + booting + heading) with the maximum contribution being observed under MLE application at heading. MLE foliar spray enhanced yield under normal conditions but more yield was observed from MLE foliar spray in moderately saline conditions. MLE foliar application produced more grain yield under 75% and 50% field capacity as compared to other treatments in wheat.

In conclusion, MLE diluted up to 30 times was the most effective. MLE delayed senescence, extended seasonal leaf area duration, leaf area, regulation of antioxidant system, improved physiological parameters i.e. chlorophyll under abiotic stresses. Improvement in growth and yield of wheat due to exogenous application of MLE under salinity and drought was the result of improved antioxidant level and increased accumulation of K⁺ and reduced levels of Na⁺ and Cl⁻ in leaves.

These studies provide preliminary data and were first attempts to evaluate the potential of MLE as growth enhancer under normal and stress conditions; however, more research is needed to investigate the cause of enhancement. Moreover, further investigations are also needed for quantification of cytokinin in MLE. The simple method of extraction and exogenous application were used, so that it can be easily adopted by community, however, more refinements like extraction methods and blending of MLE with other natural and synthetic compounds, of these techniques be required in future research.
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