In this chapter an attempt has been made to review the work done on the effects of abiotic stresses on *Withania somnifera*. The general objective of the present study is to understand the response of *W. somnifera* to two abiotic stresses (temperature and light stress) and evaluation of relative tolerance of plant in term of morphological and physiological traits. Herbicidal role of *Withania* against some prominent weeds of the Himalaya were also studied and related literature is reviewed. Although not much research has been undertaken in relation to studies done herewith, literature from related plants is also reviewed to understand the physiological aspects.

2.1 *Withania somnifera*

*Withania somnifera* (L.) Dunal popularly known as ashwagandha, ginseng and winter cherry, belongs to family Solanaceae is an important medicinal plant of India (Vakeswaran, 2003). It is a herb used in Ayurveda for about 3000 years. *W. somnifera* possesses antioxidant, antitumor, anti-inflammatory, antistress, immunomodulatory, hematopoetic, anti-ageing, anxiolytic, anti-depressive rejuvenating properties and is also found to influence various neurotransmitter receptors in the central nervous system (Gajalakshmi *et al.*, 2011). It plays a significant role in the prevention of different central nervous system (CNS) disorders, especially under stress and neurodegenerative diseases which includes Parkinson’s and Alzheimer’s disorders, tardive dyskinesia, cerebral ischemia, and also in the management of the drug addiction. It is classified in the traditional Ayurveda as rasayana, which means a group of plant-derived drugs that is reputed for promoting the physical and mental health, augmenting the resistance of the body against various diseases and diverse adverse environmental factors and found to be helpful in revitalizing the body in debilitated conditions and in increasing the longevity. Its isolated pure compounds have gained importance in various therapeutic areas and gained attention of the industrialists and farmers because of the increasing demand for its roots in pharmaceutical industries (Senthil, 2011).

The plant is widely distributed throughout the drier and subtropical parts of India (Hooker, 1885) and is well represented in Maharastra, Gujarat, Rajasthan, Madhya Pradesh, Uttar Pradesh, Punjab plains and extending to the mountainous regions of Himachal Pradesh and Jammu & Kashmir where it ascends up to an elevation of 1800m above sea level (Nigam
and Kandalkar, 1995). The species dwells in a variety of phyto-geographic regions differing from each other in climate and edaphic characters (Singh and Kumar, 1998). It has also been reported from Pakistan, Afghanistan, Palestine, Egypt, Jordan, Morocco, Spain, Canary Island, Eastern Africa, Congo, Madagascar and South Africa and occupies areas, which differ in their soil, rainfall, temperature and altitudinal profiles (Dymock et al., 1981).

*W. somnifera* is a perennial, small shrub of about 1m in height and to half across (Fig.2.1). Fleshy roots of the plant are found to be cylindrical gradually tapering down brightly with a brownish white surface and are pure white inside when it is broken. The roots possess wide number of the therapeutic agents. Leaves are simple, ovate, glabrous and found to be of about 10 cm long with dense beneath and sparse above. Flowers are inconspicuous, greenish or as lubrid-yellow, in the axillary, umbellate cymes. Berries are small, globose and orange-red when it is matured, they get enclosed in the persistent calyx. Seeds are yellow and reniform. The bright red fruit is usually harvested in the late fall and the seeds are dried for plantation in the following spring.

![Fig. 2.1: Withania somnifera growing in nursery area of Shoolini University.](image)

The plant possess certain aphrodisiac, rejuvenative and life prolonging properties (Khare, 2000). Fruits, leaves and seeds have been used traditionally in the Ayurvedic system as aphrodisiacs, diuretics and also for treatment of memory loss (Singh, 2008). Bioactive
constituent isolated from ashwagandha are withanolide A, withanolide D and withaferin A reported to be neuroactive and have antitumour activity (Singh et al., 2010). It also prevents the tardive dyskinesia neurological syndrome. The drug is mild sedative and helps in reducing the excitement and pain. Ashwagandha is found to be a major ingredient of various adaptogenic and anti-stress tonics. Roots of Ashwagandha are widely used to source a restorative drug (Asthana and Raina, 1989). Cellular growth inhibitory effect of W. somnifera extract was found to be most effective against different prostrate cancer cell lines of varying metastatic potential and showed a moderate effect on normal skin fibroblasts (Rao et al., 2004). Plants produces antioxidants in order to control the oxidative stress. Ashwagandha was found to have anti-ageing properties, diuretic, hypoglycemic, and hypocholesterolemic effects by means of the clinical studies (Narinderpal et al., 2013). Ashwagandha was found to produce profound effects on the production of white blood cells, which proves that it is an effective immunoregulator and also a chemoprotective agent (Singh et al., 2011). The studies have revealed a significant positive effect of plant on the sexual function and behavior in the diabetic peoples. Its root is capable of reversing the reductive effect of diabetes against the progesterone (Belal et al., 2014).

Ethnobotanically Ashwagandha was used to increase the energy, vigour, endurance, muscle fat, blood, lymph, semen and cell production. The plant was known to counteract chronic fatigue, weakness, strength, health, bone weakness, premature aging emaciation, convalescence and muscle tension (Verma and Kumar, 2011). Leaves of plants are used for tumor and tubercularis glands in Ayurvedic and Unani system (Chopra et al., 1958). The roots are used in constipation, senile debility and rheumatism. It infuses fresh energy and vigour and is good for the treatment of syphilis, rheumatic fever etc. The root of the plant are used as nutrients and health restorative in pregnant women. The decoction of the root boiled with milk and vegetable oil is recommended for curing sterility in women. Leaves have been used both internally for fever and haemorrhoids and externally for wounds, haemorrhoids, tumours, tuberculosis glands, anthrax pustules, syphilitic sores, erysipelas, and ophthalmitis. Fruits are used as remedy of ringworm (Sharma and Sood, 2013).

In Withania somnifera total alkaloidal content in the roots varies between 0.13 and 0.31%, but higher yields upto 4.3% has been reported by Sharada et al. (2014). The wide
variations in the alkaloidal and steroidal yield is attributed to environmental and genetic factors (Dhalla et al., 1961; Schwarting et al., 1963). Methanolic and chloroform extract of aerial part and root have antimicrobial activity against gram positive bacteria (Jaffer et al., 1988) and extract contain numerous alkaloids and secondary metabolites which has GABA-mimetic activity (Mehta et al., 1991). The pharmacological activity in roots is attributed to the presence of withanolides and alkaloids (Ray and Gupta, 1994).

2.2 Abiotic Stress

Plants are sessile organisms and their life cycle is dependent upon environment. Plant growth and productivity are adversely affected by nature’s wrath in the form of biotic and abiotic factors (Jaleel et al., 2009). Abiotic factors are the prime cause to affect growth and productivity and if not favorable can decrease growth and productivity by 50 percent (Valliy and Nguyen, 2006; Afsharmanesh, 2009). Abiotic factors influence the character, composition, growth and development of individual plant and plant communities. When any of environmental factor exceed the optimum tolerance of a plant, the result is stress to that plant (Jaleel et al., 2007). Biological stress is an adverse condition that inhibits the normal functioning and well-being of biological system of plants (Wang et al., 2011). Plants are frequently exposed to many stresses such as drought, low temperature, heat, flooding, salt, heavy metals and oxidative stress while growing in nature (Jaleel et al., 2009). The abiotic stresses will increase in the near future because of global climate change, according to reports from the Intergovernmental Panel of Climate Change (2014). In the European heatwave of 2003, crop production was reduced by around 30% (Ciais et al., 2005).

Abiotic stress can induce a wide number of responses in the plants ranging from re-adjustment of transport and metabolic process leading to growth inhibition. The primary effect of abiotic stress is ion imbalance and hyper-osmotic stress. A direct result of these primary effects is the enhanced accumulation of reactive oxygen species (ROS) which is harmful to the plant cells at higher concentrations. Oxidative stress occurs when there is serious imbalance in any cell compartment among the production of ROS and antioxidant defence, leading to significant physiological challenges (Gill and Tuteja, 2010). The excess ROS cause damage to proteins, lipids, carbohydrates, DNA, and ultimately results in cell
death. They activate cytoplasmic Ca\(^{2+}\) and protein signaling pathways leading to stress responsive gene expression and physiological changes (Debnath et al., 2011).

Plant response to environmental stress include temperature stress, light stress, water stress, osmotic, heavy metal and salinity stress which are of critical importance. According to Blum (1996) plant response is complex because it reflects over space and time over the integration of stress effects and responses at all underlying levels of organization. Tolerance or susceptibility to these abiotic stresses is a complex phenomenon, in part, because stress may occur at multiple stages of plant development and often more than one stress simultaneously affects the plant which leads to stress tolerance. Various abiotic stresses result in both general and specific effects on plant growth and development. For example, chilling (temperatures below optimal but above freezing) and freezing temperatures can also cause osmotic stress in addition to their direct effect on metabolism (Thomashow, 1994). High temperature limits plant growth due to photosynthetic decline, osmotic stress-imposed constraints on plant processes and interference with nutrient availability as the soil dries. Therefore, osmotic stress and the associated oxidative stress appear to be common consequences of exposure to high and low temperature. As part of plant stress responses, regulation of gene expression also involves both universal and unique changes in transcript levels of certain plant genes (Shinozaki and Yamaguchi-Shinozaki, 2000). In addition, more than one abiotic stress can occur at one time and one abiotic stress can decrease a plant’s ability to resist a second stress. For example, low water supply can make a plant more susceptible to damage from high irradiance due to the reduction in plant’s ability to reoxidize NADPH and thus maintain an ability to dissipate energy delivered to the photosynthetic light-harvesting reaction centers (Boyer, 1982; Araus et al., 2002). The possibilities for increasing tolerance of plants to abiotic stresses are reviewed in textbooks (e.g. Taiz and Zeiger, 2002) and in a range of review articles (Thomashow, 1994; Raghothama, 1999; Masclaux et al., 2001; Blokhina et al., 2003; Samac and Tesfaye, 2003; Shinozaki et al., 2003; Tester and Davenport, 2003), correlating particular traits with increased tolerance, and molecular and cellular processes involved in adaptive plant responses to abiotic stresses.
2.2.1 Temperature Stress

Temperature is one of the most important environmental factor that affects growth and development of plants (Noohi et al., 2009). Heat stress is often defined as temperature beyond a threshold level for a period of time sufficient to cause irreversible damage to growth and development (Wahid et al., 2007). High temperatures can cause an array of morpho-anatomical, physiological and biochemical changes in plants, which affect plant growth and development and may lead to a drastic reduction in economic yield (Badu-Apraku et al., 1983; Commuri and Jones, 2001). Global climatic variability has put into foray several important stresses impeding the production and productivity scenario. Heat stress due to rising temperature is one of the prominent concern. Agriculture systems are extremely vulnerable to climate change and especially high temperature in tropics (Boyer, 1982; Niyogi et al., 1999; Rodell et al., 2009). Stebbins (1985) viewed that climatic vulnerability has led to higher temperatures. It has been predicted that growing season temperatures in the tropics and subtropics will exceed even the most extreme seasonal temperatures so far (Battisti and Naylor, 2009). Heat waves have already struck Western Europe and Asiatic regions leading to major crop losses (Ciais et al., 2005). Several climate modeling studies have suggested future increases in both day and night temperatures, which would adversely impact plant production (Lobell et al., 2011; Cairns et al., 2012). The US Environmental Protection Agency (EPA, 1998) predicted decrease in maize yields from 4-42% under conditions of future climatic change due to temperatures rising above the range of tolerance for the maize crop. The greatest challenge to understand the problems associated with heat stress, especially in terms of interactions between plant structure, function and the environment needs study at various phases of plant development at the organismal, cellular and molecular levels (Barnabas et al., 2008).

Plant stress responses are very complex and various plant organs in a definite hierarchy and interaction with each other are involved in determining crop yield under stress (Barnabas et al., 2008). High temperatures have been reported to cause severe impediment to panicle emergence, anthesis, pollen viability and germination capacity and overall photosynthetic efficiency of plants, which are often manifested in their final grain yield potential (Prasad et al., 2006). Downey et al. (2003) opined that C4 plants will be
advantaged by warmer temperatures through slightly higher assimilation rates and more efficient enzyme use and hence higher nitrogen use. Higher temperatures can also influence the quantity and synthesis of polypeptide components of photosynthetic enzymes – Phosphoenol pyruvate carboxylase (PEPC) and Ribulose bis-phosphate carboxylase and oxygenase (RUBISCO) influencing the enzymatic efficiency (Ashraf and Harris, 2013). High temperatures have been reported to cause depreciation of certain protein. High temperature caused a marked decrease in several growth parameters. Temperatures above 35°C result in reduction of leaf area by upto 33%. Low chlorophyll stability, leaf firing, tassel blast, male and female sterility have also been observed frequently in plants exposed to heat stress. Plant cells have an integrated network of temperature sensing devices (cellular features) that trigger short term responses and long term adaptation for living with ever changing temperatures. Changes in morphology of plant system are a result of cross-talk between several cellular and molecular processes that are induced or repressed at elevated environmental stress. High temperatures can result in repression of several important cellular pathways by repressing the synthesis and activities of protein and antioxidant defense system (Farooq et al., 2009) (Fig.2.2.). Several studies have examined the effects of increased temperatures on secondary metabolite production of plants, but most of these studies have contradictory results (Jochum et al., 2007). Some report that secondary metabolites increase in response to elevated temperatures (Litvak et al., 2002), while others report that they decrease (Snow et al., 2003).

Cold Stress effect the plants and their products as it reduce quality and loss of plant product utilization following exposure to low but nonfreezing temperatures. Despite considerable efforts in this field of study, there is no general agreement on the cause or nature of cold stress, or even the primary event triggering low temperature damage (Parkin et al., 1989). It has been reported that low temperature induced membrane lipid phase transitions leading to a loss of membrane integrity and physiological dysfunction. Membranes and changes in their physical characteristics are further implicated as having a role in cold stress by the discovery that chilling stress evokes an elaborate membrane retailoring response that leads to increased fluidity at reduced temperatures. It is also speculated that membrane integrity may have a role in the development of irreversible injury.
during low temperature stress. Its effect would be similar to the senescent processes of free radical damage to tissue and progressive membrane rigidification (Parkin et al., 1989).

Temperature stress can affect secondary metabolites and other compounds that plants produce, which are usually the basis for their medicinal activity (Zobayed et al., 2005; Salick et al., 2009). Generally when plants are stressed, secondary metabolite production may increase because growth is often inhibited more than photosynthesis, and the carbon fixed not allocated to growth is instead allocated to production of secondary metabolites (Mooney et al., 1991). However, it is hypothesized that chilling stress alter growth cycles of plants and active constitutes of the plants may change due to physiological changes (Chaturvedi et al., 2007). Therefore, studies on abiotic stress in medicinally important plant of the Himalaya needs greater attention to understand the underlying phenomenon. Cold (<20°C) or freezing (<0°C) temperatures can induce ice formation in plant tissues which leads to cellular dehydration (Chinnusamy et al., 2007). To cope with this adverse condition, plants adopt several strategies such as producing more energy by activation of primary metabolism, raising the level of antioxidants and chaperones, and maintaining osmotic balance by altering membrane structure (Uemura et al., 1995; Prasad, 1996; Sharma et al., 2005). Comparative proteomics performed on cold tolerant and sensitive plants helped to understand the overall response as well as recovery mechanism against cold stress (Fig. 2.3). For instance, activation of metabolic processes was observed in rice roots upon 24–72 hrs of chilling stress as indicated by the enhanced levels of several metabolism-associated proteins (Lee et al., 2009). These include a group of carbohydrate metabolism enzymes, such as phosphogluconate dehydrogenase, fructokinase, NADP-specific isocitrate dehydrogenase, and cytoplasmic malate dehydrogenase. In addition, higher abundance of pyruvate orthophosphate dikinase precursors (PPDK), glycine dehydrogenase, aconitate hydratase, and enolase were also identified in chilling stress-related studies (Lee et al., 2009). Similarly, higher abundance of adenylate kinase protein under chilling stress is an indication of enhanced ATP synthesis and energy metabolism. Additionally, peptidylprolyl isomerase Cyp2 and cysteine proteinase was preferentially accumulated in rice roots upon chilling stress (Pradet and Raymond, 1983; Hashimoto and Komatsu, 2007). Quantitative analysis of PM proteome of rice roots grown under cold stress condition revealed that proteins related to
membrane permeability and signal transduction through the membrane enhanced level of annexin and hypersensitive-induced response (HIR) protein families (Hashimoto et al., 2009).

On the other hand, cold stress causes oxidative damage to the cells by generating ROS or their precursors. For protection against this damaging effect, several anti-oxidants are produced in the root. For example, oxalyl-CoA decarboxylase, the second enzyme of oxalate catabolism pathway, was enriched in rice roots under chilling stress (Lee et al., 2009). Gradual increase of glyoxalase I protein level throughout the cold stress period indicated detoxification of methylglyoxal produced during the stress condition (Espartero et al., 1995; Lee et al., 2009), thus providing another example of antioxidant generation. In addition, ROS scavengers induced commonly in all abiotic stresses, such as superoxide dismutase, catalase, and ascorbate peroxidase, were found to be in abundance in a study with chicory roots under chilling stress (Lee et al., 2009). Moreover, in chilling stress, heat shock proteins (HSPs) were found to be higher in roots of rice (Cui et al., 2005; Yan et al., 2006), chicory (Degand et al., 2009), maize (Kollipara et al., 2002), and poplar (Renaut et al., 2004), with HSP70 family being the most abundant. These proteins act as molecular chaperones and thus prevent aggregation of the denatured proteins as well as facilitate protein refolding (Lee et al., 2009). In addition, a putative calreticulin precursor with chaperone activity was also detected in a study with rice seedlings under low temperature stress (Hashimoto and Komatsu, 2007). Defense-related proteins such as protein disulfide isomerase and disease resistance response protein were also detected in relatively high abundance in pea roots under chilling stress (Dumont et al., 2011) indicating that defense-related pathways are activated in the root when combating cold stress.
Fig. 2.2: Possible mechanism of heat stress to produce Reactive oxygen species and antioxidant defense system to reduce photosynthesis (Farooq et al., 2011).

Fig. 2.3: Mechanism of low temperature in plant metabolism (Yan et al., 2006).
2.2.2 Light Stress

Light as an energy source for plant life is known to affect plants dually. It affects photosynthetic rate and assimilate accumulation, thereby playing a substrate role, but also controls plant growth and development, in that way playing a regulatory role (Sysoeva et al. 2010). One of the most important environmental factor that affect floral transition is the change in daylength (photoperiod). A role of photoperiod was originally proposed by Tournois (1912) and Klebs (1913). In the 1920s, Garner and Allard (1920, 1923) were the first who discovered that flowering and other developmental responses could be controlled by exposure to short day (SD) or long day (LD) depending on the plant species. They introduced the terms “photoperiod,” which defines the recurring duration of daily light and dark periods, and “photoperiodism,” which defines the responses to photoperiod. Many studies have been devoted to understanding the molecular mechanisms of the photoperiodic flowering pathway (Imaizumi and Kay, 2006; Kobayashi and Weigel, 2007). This pathway, which consists of a circadian clock and a circadian-regulated day length measurement mechanism which promotes flowering specifically under LD and continuous light. Continuous light was shown to increase the developmental rate in 30 spring wheat (LDP) cultivars of different geographical origin (Zhukov and Romanovskaja, 1980), barley, radish (LDP) (Lisovskij and Dolgu-shev, 1986; Moshkov, 1987), LD and neutral day (ND) pea varieties (Berry and Ait-ken, 1979; Dolgushev, 1986). For LD legume chickpea, supplemental light was recommended under Indian conditions (Sethi et al., 1981). There are several indications of the role of continuous light spectrum in plant development. Kasajima et al. (2007) investigated the developmental rate of wheat under continuous light of four different kinds of fluorescent lamps (white, blue, purplish red and ultraviolet-A), results suggested that green and red lights played important roles in the regulation of the developmental rate having a promotive effect (Yanagi et al., 2006). Stutte et al. (1996) have shown that in potato plants grown under 24-hrs photoperiod, photosynthesis was 33% lower than in plants grown under 12-hrs photoperiod. According to Gestel et al. (2005) in Allium species, 24-hrs plants had much lower N levels compared with 12-hrs plants. Reduced rubisco activity, potentially initiated by higher foliar carbohydrate concentration in A. fistulosum, could have been primarily responsible for the observed photosynthetic downregulation (Gestel et al., 2005). This is consistent with Paul and Foyer (2001) who
stated that when carbohydrate levels reached a threshold, downregulation of photosynthesis was initiated to rebalance the source-sink ratio. The effect of photoperiod on leaf pigment content was varied.

According to Schopfer and Brennicke (1999) each plant species has a specific tolerance range for the light intensity in which their growth is possible. Irradiation below that range will not provide enough energy to drive photosynthesis to maintain their metabolism. Excessive light on the other hand damages the organism due to high levels of ultra-violet radiation, which causes damages to the DNA, and because of toxic photosynthesis byproducts, which are accumulating when the photosynthetic machinery is overloaded under high light conditions, this condition is termed photooxidation or light stress. Many responses to varying light intensities take place on molecular and biochemical level. Demmig and Adam, (1992) opined that composition of the proteins and other compounds were constantly arranged to maximize the plants photosynthetic performance and overall fitness. When plants are exposed to irradiance far above the saturation point of photosynthesis, the solar energy is not exclusively used for photosynthesis in plants as it cannot be fully utilized for it. Chlorophyll molecules absorb light and with this they enter the singlet state (1Chl). This energy can be transferred very rapidly between juxtaposed chlorophyll molecules by resonance transfer (Hall and Rao, 1999) and so harvested to the reaction center of the photosynthetic complexes. When a rising number of neighboring chlorophylls enter the excited state, it becomes increasingly difficult to transfer excitation energy and the average lifetime of the singlet state increases (Niyogi, 1999). This can lead to the transition to the relatively long-lived triplet state of chlorophyll (Chl) which can interact directly with O2 forming singlet oxygen (O2) which itself has the potential to generate more oxygen radicals (ROS: reactive oxygen species), which altogether have a very high potential of oxidizing and damaging all kinds of cell-components as proteins, lipids and DNA (Mittler, 2002)(Fig.2.4). The most prominent ROS are superoxide radicals, perhydroxyl radicals, hydrogen peroxide, hydroxyl radicals. Hydrogen peroxide particularly can easily pass biological membranes and cause damage in the whole cell (Liszkay, 2005; Breusegen and Dat, 2006). Being a major source of ROS formation, the photosynthetic machinery is also the structure most likely to be damaged. Oxidative damage done to any part of the photosystems with deficiency of light,
decreases the efficiency and maximum rate of photosynthesis, a process called photooxidation or photoinhibition (Kok, 1956; Matsubara and Chow, 2004).

![Mechanism of light stress on plant parts and processes](image)

**Fig. 2.4** Mechanism of light stress on plant parts and processes (Mittler, 2002).

### 2.3 Growth

#### 2.3.1 Stem length

Abiotic stresses cause a reduction in stem length. Morphological variation depends on seasonal or developmental changes that affect many individuals in a population regardless of genotype. High temperatures severely affected shoot length and resulted in poor kernel set in *Zea mays* L. (Herrero and Johnson, 1980). High temperature significantly decreased the stem length in wild-type and transgenic plants of *Zea mays* L. (Yang *et al.*, 2005). Stem length decreased in rice plant due to high temperature stress (Shah *et al.*, 2011). Barbara *et al.* (2011) studied the effect of differential temperature and photoperiod on growth of *Ocimum basilicum*. The effect of elevated temperature on seedling growth was investigated on three Nigerian cereal crops: maize, rice and sorghum. Results showed an increase in the shoot length of maize at 37°C and 40°C after 96 hrs of exposure to these temperature regimes but a drastic decrease at 42°C, 45°C and 50°C, respectively. Sorghum plantlets also showed shoot increase at 37°C, 40°C and 42°C temperature regimes but decreased at 45°C and 50°C, rice plantlets exposed to different temperature regimes showed an increase in shoot length at 37°C, 40°C, 42°C and 45°C, respectively with a decrease only at 50°C (Iloh *et al.*, 2014).

Low temperature also causes dehydration, mainly due to reduction in water uptake by roots and an impediment to close stomata. At freezing temperature, membrane damage was caused by severe cellular dehydration, associated with ice formation. The accumulation of ice in the intracellular spaces causes the physical disruption of cells and tissues. Freezing temperature also caused protein denaturation and precipitation of solutes as a result of freezing induced
dehydration (Uemura *et al.*, 1995; Salinas, 2002). Low temperature result in the oxidative stress due to generation of reactive oxygen species (ROS). These ROS disequilibrates the electron transfer reactions and disturb accompanying biochemical reactions. Thus generation of ROS leads to cellular injury, which ultimately leads to death of plant due to damage of photosystem II reaction center and membrane lipids (Prasad *et al.*, 1994; Suzuki and Mittler, 2006).

Light stress had significantly affected the stem length of plants. Silva *et al.* (2006) verified marked shoot etiolation, large internodal spaces and a small number of leaves in *Passiflora edulis* seedlings grown at 70% shading in comparison with plantlets maintained under full-sunlight condition. As the temperature raised the ratio of rootlength to stem length and seed vigor index also reduced significantly (Arves *et al.*, 2013). Effect of minute amounts of raditions on the elongation of mesocotyle was studied in *Avena* and other grass seedlings (Dubuy and Nuernberg, 1929). Excessive elongation of mesocotyl was correlated with a 50% higher auxin production in coleoptile tips of plants kept in continuous darkness, in comparison with plants which received occasional light (Van overweek, 1937). Similar results in pea showed taller stem in complete darkness than those grown in the dark room where orange light was used for some hours per day (Went, 1939). Continuous light had shown reduced stem growth in *Jatropha curcus* (Wadhwa *et al.* 2010). Variation in shoot height in Cowpea (*Vigna unguiculata* (L.) Walpin light stress conditions was verified statistically by the coefficient of variation (Adelusi and Aileme, 2006). The light stress resulted in retarding growth, yellowing of the leaves and finally plant death after a very short period of time (Elfadil and Abdallah, 2013). Hasanuzzaman ( 2013) reported that growth of roots and shoots in hydroponically grown *Phaseolus aureus* seedlings was not inhibited at 35/25°C (day/night temperature), but at 40/30°C and 45/35°C, 18% and 34% reduction of shoot growth was observed. Light stress resulted in retarding growth in *Sorghum* (Elfadil and Abdallah, 2013).

2.3.2 Root length

Roots are often reported to play a key role in the temperature tolerance of plants as they represent the first organ to control the uptake and translocation of nutrients throughout
the plant (Manivannan et al., 2007). Low soil temperature adversely affected the initiation and elongation of new roots. The branching and morphology of the new roots were also influenced by soil temperature (Nambiar et al., 1979). High temperature resulted in loss of water and drought. Decreased root length was reported in Albizzia seedlings (Nanjo, 1999), in Eucalyptus microtheca seedlings (Marron et al., 2002), in Populus species (Nautiyal et al., 2002) and in Erythrina seedlings (Silva et al., 2010) due to drought stress. A significant negative linear relation between root biomass and increased temperature was detected in wheat (Ferris et al., 1998). The loss of root length at a mean temperature of 6°C was threefold greater than at 17°C. Reduction in root growth, and particularly root viability, under high temperature and low temperature contributed to the declines and cultivar variations in bentgrass quality (Huang et al., 1998).

Root elongation rate was linearly related to changes in soil temperature. At soil temperatures less than 17°C, temperature was found to be dominant factor affecting rate of growth, but at temperature greater than 17°C soil water potential became the important factor. Decrease in root elongation rate was observed but the number of growing roots and root growth intensity increased at 8°C (Teskey and Hinckley, 1981). Macduff (1986) worked on effects of temperature on root length and to nutrient uptake including measurements on oilseed rape and barley grown in flowing nutrient solution. Hund et al. (2004) revealed that soil temperature influenced the functioning of roots in many ways as if soil moisture and nutrient availability are adequate, rates of root length extension and root mortality increased with increasing soil temperature, at least up to an optimal temperature for root growth, which seems to vary among taxa. Contradictorily, high temperature cycle increased the seedling growth of both wheat and ryegrass. The root growth and seedling biomass of ryegrass increased dramatically at high temperature even under high interference of wheat (Sultana et al., 2013). High percentage of root length among the genotypes was at 10°C–20°C temperature however it was less at 20°C–30°C temperature in Vigna mungo (Dash and Shree, 2013). In Caragana korshinskii role of high temperature in relation to root morphological traits, including mean root diameter, specific root length and root tissue density was studied (Lai et al., 2014). Temperature had severely affected root length of Plantago psyllium, Althaea officinalis and Nigella sativa (Jamian et al., 2014).
Richards (1991) found that low temperature imposed fundamental limits to forest productivity and significantly reduced root length. Lennart Eliasson (1997) worked on effects of nutrients and light on growth and root formation in *Pisum sativum* and showed that no roots were formed on cuttings kept in the dark. The number of roots increased with increasing irradiance given to the leafy part of the cutting. At a low level of irradiance sucrose supply through the rooting medium increased the number of roots. Carbohydrate level easily became a limiting factor for root formation in growing pea cuttings (Reich et al. 2003).

In low light, all species allocated proportionally more biomass to stems and less to roots, but the same to foliage, compared with the high-light environment (Reich et al., 1998). Variation in physiological and morphological traits among tree species has been related to their differences in regeneration habitat conditions, including light availability (e.g. Loach 1970; Bazzaz 1979; Pompa and Bongers 1988; Walters et al., 1993; Jones et al., 1994; Kitajima 1994; Walters and Reich 1996). A related issue concerns whether rankings of relative growth rate reverse between shade intolerant and tolerant species when compared at high vs low light levels (e.g. Kitajima 1994; Walters & Reich 1996). A study was conducted to describe the effects of different photoperiods (16-8, 14-10 and 12-12 h light/dark) on some growth characters of *Hippeastrum johnsonii* cultured on MS basal medium. Maximum root number (2.25) and longest root length (2.70 cm) were observed in explants treated with 16-8 h light/dark (Zakizadeh, 2013). Despite growing underground, largely in darkness, roots emerge to be very sensitive to light. In *Arabidopsis*, illumination of roots speeds-up root growth via reactive oxygen species-mediated and F-actin dependent process and activated the phytohormones with light-related signaling cascade. Particularly, light-exposed roots are less effective in their salt-avoidance behavior known as root halotropism (Yokawa et al., 2014). In rice seedlings, strawberry plantlets, sprouting broccoli, grapes, roses and Cymbidium plantlets it was observed that light reduced root length and root biomass in seedlings (Chang Chen et al., 2014).

### 2.4 BIOMASS AND PRODUCTIVITY

A decrease in total dry matter is generally due to the decrease in plant growth,
photosynthesis and canopy structure under abiotic stress conditions. Progressive loss of water due to high temperature resulted in a significant reduction in early allocation of dry matter and decreased biomass in *Populus davidiana* (Zhang et al., 2004). A significant negative linear relation between root biomass and increased temperature was detected in wheat (Ferris et al., 1998). Wheeler et al. (1997) found that the root:total biomass ratio declined more rapidly in a high-temperature sensitive, compared to a high-temperature tolerant genotype of groundnut (*Arachis hypogaea* L.) exposed to high temperature. Also, in both pot-grown plants of wheat (Mitchellet et al., 1993) and field-grown crops of wheat (Batts et al., 1998), the decline in root biomass at anthesis was greater following exposure to warmer seasonal mean temperatures from sowing. Wheat single grain weight decreased as temperature rose above 20°C (Jenner, 1991; Mohammadi et al., 2004). It had been observed that the wheat grain weight and yield was diminished at high temperature (Jenner, 1994). Nieto-Sotelo et al. (2002) found that a heat shock protein (HSP) is involved in the development of thermo tolerance and HSP also reduced the primary root growth which in turn decreased fresh weight in maize. However, the consistent main effect of heat stress was a significant reduction in root growth, fresh weight and dry weight at high temperature i.e. 36/32°C day /night. High temperature above 35°C decreased nodule weight and number, nitrogenase activity and shoot dry matter production in pigeon pea and soybean (Lindermann et al., 1974; Dart et al., 1976; Bansal et al., 2014). Geetika et al. (2014) reported that maximum decrease was found in seedlings treated with 44°C temperature which was 14% in fresh weight and 18% in dry weight.

Cold stress significantly reduced cell division and cell elongation, which eventually resulted in stunted growth (Miedema et al., 1982). There was a significant reduction in fresh weight of shoots (15–23%) in wheat (Davis et al., 1988). Pinhero (1995) showed that high temperature reduced the growth, fresh weight and dry weight in potato. Gunawardhana and Silva (2011) found that temperature stress treatments were highly significant on the fresh weight of okra pods. In case of dry matter of the fruit, the maximum weight was observed at 34°C temperature.

According to Burns and Winn (2006), light restriction was more effective for photoassimilate transport under shading conditions, resulting in a major accumulation of
shoot mass at the expense of root development. In *Anoectchilus formosanus*, a medicinal plant, dry weight was lowest for the plants which were at low light period whereas fresh weight was also lowest for the plants which were at high light. Dry weight was 1.4 times greater in control than in the low light. An increase in light intensity increased the ratio of dry weight to fresh weight of plant (DW/FW) (Zengqiang et al., 2010). It was observed that dry mass partitioning of plants of the hybrid *Cattleya. Forbesii X Laelia tenebrosa* was affected by the light conditions in which they were cultivated. The plants that grew under higher light intensity showed decreased fresh and dry weight (Mackay et al., 1990), antioxidants (Senaratna et al., 1988) and chlorophyll content (Fletcher and Hofstra, 1988). According to Eliasson (1978) increase in dry weight was strongly dependent on light supply. Plants were grown 31 days in the chambers equipped with LEDs matrices of the same intensity of light (200 μmol m⁻²s⁻¹), but of different spectral composition: white (WL), white-blue (WBL) and red-green-blue (RGBL). It was found that the WBL as compared to RGBL inhibited the growth of the whole gametophytes of *Plagiomnium cuspidatum* and *Polytrichastrum formosum*. Effect of spectral composition of light on the length and weight of the gametophyte depends on the species of mosses (Mozdzen et al., 2014).

In a study, the effects of raising seedlings with different light spectra such as with blue, red, and blue plus red light-emitting diode (LED) lights on seedling quality and yield of red leaf lettuce plants was determined (Johkan et al., 2014). The light treatments used were applied for a period of 1 week and consisted of 100 μmolm⁻²s⁻¹ of blue light, simultaneous irradiation with 50 μmolm⁻²s⁻¹ of blue light and 50 μmolm⁻²s⁻¹ of red light, and 100 μmolm⁻²s⁻¹ of red light. The dry weight of the shoots and roots of the lettuce seedlings treated with blue-containing LED lights increased by 29% and 83% compared with seedlings grown under a white fluorescent lamp (FL). The compact morphology of lettuce seedlings treated with blue LED light reduced the dry weight (Johkan et al., 2014). The effect of solar radiation on plant growth of *Geranium* was observed and found that plant height and number of branches increased with increasing levels of curtailment of solar radiation from 50% to 65%. Radiation was most effective at 75% level of solar radiation for plant height. However, plant spread was found highest at 65%, but increased number of branches was found at 75% of solar radiation (Kumar et al., 2014).
Relative water content (RWC) of leaves is an important character, which is directly related to soil water content (Sarker et al., 1999). The maintenance of favorable plant water status is an essential strategy for plant tolerance to stresses that result in cellular water-deficit and loss of turgor pressure. RWC is the major tool for assessing changes in plant water relations for studying plant responses to stress and subsequent relation to stress tolerance. RWC has been used as a trait for screening different environmental stresses, where plants that maintain higher RWC tend to be more tolerant to stress compared to plants that cannot efficiently control leaf water status. It has been observed that the species which are better adapted to dry environment have higher relative water content (RWC) at given water potential (Jarvis and Jarvis, 1963). According to Nava et al. (2002) RWC was significantly reduced by high temperature to greater than control conditions in maize landraces. In tolerant genotype of maize the water potential was reduced in 84%, however in the susceptible genotype the reduction was in 26%.

The relative content of water in the leaves depend on light conditions (Ivanov, 2010). Experiments were conducted to investigate the growth, morphological, anatomical and biochemical responses to light and drought stresses in Spilanthes acmella (toothache plant). It was evident from the study that both light and drought treatments retarded plant growth. The extent of salt stress effect expressed as a decrease in the rate of the photosynthetic release of oxygen and the relative content of water and chlorophyll in the leaves greatly depended on light conditions of growing (Ivanov, 2010). Relative water content markedly increased in light treated plants and reduced in drought (Reshmi and Rajalaxmi, 2012).

Reduction in leaf area by temperature variation and light stress is an important cause of reduced crop yield through reduction in photosynthesis. High temperature cause loss of water in the cells and the total leaf area per plant decreased significantly in Eragrostis curvula, Oryza sativa, Abelmoschus esculentum, and Asteriscus maritimus due to water deficit (Rucker et al., 1995; Sadras et al., 1993; Passioura, 1977; Rose et al., 1993; Shubhra and Ooswami, 2003). Heat stress reduced the leaf area (Warrington et al., 1977), the duration of vegetative growth (Noohi et al., 2009 and Saini et al., 1988) and leaf number (Acevedo et al., 1990).
According to Tadesse et al. (2000) reducing temperature increased leaf area. A higher temperature in some cultivars had negative effects on the relative increase in leaf area after acclimatization. Leaves of maize, which develop under low temperature conditions, were characterized by lower leaf area than leaves which developed under more favorable conditions (Nie et al., 1992; Leipner et al., 1999). Miedema et al. (1987) studied the effect of low temperature on seedling growth from germination to the leaf stage and suggested that resistance to reduction in leaf area, chlorosis and rapid leaf expansion at low temperature were considered major selection criteria for the improvement of low-temperature adaptation (Ying et al. 2000).

Leaf area growth was also reported to be a function of prevailing temperature. The optimum temperature for leaf extension in maize was 30°C. Genetic variation was reported in leaf extension rate at day/night temperatures of 15/10°C, 20/15°C, and 25/20°C in maize (Zaidi et al., 2010). As cold stress increased from 0 to 8 hrs, leaf area decreased linearly. Leaf area decreased curvilinearly as cold increased to 54 hrs. Findings are related to relative growth rate of corn (Wolfe, 1991), cucumber (Bulder et al., 1987) and muskmelon (Korkmaz and Dufault, 2001). Wolfe (1990) opined that important advantages exhibited by Pisum sativum and Spinacia oleracea chilling-sensitive species showed 40–50 % reductions in leaf area. Lower leaf area at the cooler temperature was also observed in Zea mays. Visibly thicker leaves and increased leaf density were observed in all species when grown at 18/12°C in Zea mays (Stancato, 2002). The effects of light on Vallisneria americana were studied in outdoor mesocosms for an entire growing season. Leaf area index was affected by light, apparently because of morphological plasticity (increased leaf length and width), increased photosynthetic efficiency, and increased chlorophyll concentration under low light (Mascarini et al., 2006).

2.5. Physiochemical Aspects

2.5.1 Membrane stability

Plasma membrane because of its external position acts as a sensor to receive any kind of signal for stressful change that takes place in the external environment. Cell membranes are generally the first line of defense against the adverse environmental or abiotic stresses. A
major impact of plant environmental stress is cellular membrane modification, perturbed function or total dysfunction. The cellular membrane dysfunction due to stress is well expressed in the increased permeability and leakage of ions out, which can be readily measured by the efflux of electrolytes through determining the conductivity of the ambient solution (Dewir, 2015).

Under environmental stresses plant membranes are subject to changes often associated with increase in permeability and loss of integrity (Blokhina et al., 2003). Biological membranes are the first target of many abiotic stresses including temperature and light stress. It is generally accepted that the maintenance of integrity and stability of membranes under heat stress is a major component of drought tolerance in plants (Bajji et al., 2002). Heat injury to the plasmamembrane may be measured by ion leakage (Chaisompongpan et al., 1990; Hall, 1993). Injury to membranes from a sudden heat stress event may result from either denaturation of the membrane proteins or from melting of membrane lipids which leads to membrane rupture and loss of cellular contents (Ahrens and Ingram, 1988). According to Lee et al. (1983) heat stress may be an oxidative stress. Peroxidation of membrane lipids has been observed at high temperature conditions (Mishra and Singhal, 1992; Upadhyaya et al., 1990), which is a symptom of cellular injury. In Arabidopsis, exposed to high temperatures, total lipid content decreased to about one-half and the ratio of unsaturated to saturated fatty acids decreased to one-third of the levels (Somerville and Browse, 1991). Increase in saturated fatty acids of membranes increased their melting temperature and thus confers heat tolerance. In cotton, however, heat tolerance did not correlate with degree of lipid saturation (Rikin et al., 1993) and similar differences in genotypic differences in heat tolerance had been unrelated to membrane lipid saturation in other species (Kee and Nobel, 1985). In addition, by causing injuries to the cell membrane, organization of microtubules and ultimately to the cytoskeleton, heat stress changed membrane permeability and alters cell differentiation, elongation, and expansion (Smertenko et al., 1997; Potters et al., 2008; Rasheed, 2009). Plant tolerance to high temperature in a changing environment in crops was studied by producing heat stress resistant crops (Bita and Gerats, 2013). High temperature stress tolerance in Brassica juncea germplasm was evaluated by membrane stability index (Bhagirath Ram, 2014).
In order to tolerate high temperature, plants must maintain membrane fluidity within a biologically functional range i.e. membrane thermostability. Early work by Lyons and Raison (1970) highlighted the fact that tropical species have a higher proportion of saturated lipids than temperate species, but found that the full role of lipid composition in regulating membrane fluidity was complex. Changes in lipid composition during acclimation to high temperature, including increases in the proportion of saturated lipids, had been described in cyanobacteria (Los and Murata, 2004) and a number of plants from both warm and cool regions (Raison et al. 1982; Larkindale and Huang, 2004). Some of the changes in the physical properties of membranes are regulated by the activity of heat shock proteins (Sharkey, 2005). The integrity and functions of biological membranes are sensitive to high temperature, as heat stress alters the tertiary and quaternary structures of membrane proteins enhancing the permeability of membranes evident from increased loss of electrolytes (Al-Jebory, 2013). Much of the heat sensing occurred through protein unfolding. Since protein conformation changes with temperature, both temperature downshift and temperature upshift can lead to protein unfolding (Hemantaranjan et al., 2014).

Lukatkin (2003) observed a gradual increase in leakage of ions from the cells upon prolongation of chilling exposure, with the maximum attained by the end of 24 hrs chilling treatment. Farooq et al. (2008) found that reduced membrane permeability contributed towards chilling tolerance in maize hybrids. The overall effect of cold stress on various morpho-physiological traits eventually resulted in highly significant genotypic variability for grain yield under stress. The plasmalemma and membranes of cell organelles play a vital role in the functioning of cells. Any adverse effect of temperature stress on the membranes leads to disruption of cellular activity or death. Until recently, it was thought that freezing stress results in a total loss of membrane semipermeability and in membrane rupture. It had been demonstrated that freeze–thaw stress results in alteration of membrane transport properties. This alteration is an early manifestation of the injury (Palta, 2008).

Light stress significantly affected the membrane stability (Tardy and Havaux, 1997). The nuclear-encoded, thylakoid-bound early light-inducible protein (ELIP) reported to be related to the initial stages of chloroplast differentiation is synthesized in substantial amounts in leaves of mature plants exposed to light stress conditions (Adamska et
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(Adamska, 1992). Increase in ELIP content correlates with the photoinactivation of PSII, degradation of Dl protein and changes in the level of pigments. The thylakoid bound protein was stable in high light exposed leaves and was degraded only during recovery from light stress at low light intensity (Adamska, 1993).

2.5.2 Carbohydrate

Carbohydrates play a keen role in plant metabolism and regulate plant growth and development. Sucrose and its derivatives are the major transport forms of assimilated carbon in plants which is transported from source to sink organs via the phloem. Soluble carbohydrates represent about 50% of the total osmotically active organic solutes among all organic compounds (Ashraf and Harris, 2004). Lafta and Lorenzen (1995) conducted a study to know the effects of heat stress on enzymes of carbohydrate metabolism in developing shoots in potato. Heat stress increased accumulation of foliar sucrose and decreased starch accumulation in mature leaves but did not affect glucose. Heat stress alters carbohydrate partitioning in potato (Solanum tuberosum L.) plants from tubers to shoots and reduced overall plant yield (Borah and Milthorpe, 1962; Ewing, 1981; Wolf et al., 1990). Zimmerman and Whigham (1992) found that the water-soluble carbohydrates present in the underground corm of the orchid Tipularia discolor were hydrolysed in heat stress and were important as a support for the beginning of the new season of growth. Stancato et al. (2001) observed that water soluble carbohydrates (predominantly glucomannan) were present in pseudobulbs and leaves of plants of Cattleya forbesii X Laelia tenebrosa. The reduction in carbohydrate concentration of shoots was more pronounced than that of roots. Shoot glucose and sucrose concentrations were more sensitive to heat stress than other carbohydrates. It has been suggested that high carbohydrate availability, particularly glucose and sucrose, during heat stress was an important physiological trait associated with heat-stress tolerance in creeping bentgrass (Liu and Bingru, 2000).

Riikonen et al. (2013) demonstrated the effects of slightly elevated temperature on over-wintering buds of Betula pendula Roth. It was observed that after two growing seasons of exposure in the field, genotype-specific alterations in carbohydrate metabolism were found in the buds grown under elevated temperature. Effects of high temperature on the carbohydrate metabolism were studied in pepper seedlings (Capsicum annum L). Sucrose
concentration in the leaves of pepper seedlings was elevated significantly under heat stress but the increase in the sucrose content of these leaves exposed to 40°C and 45°C was dramatic. In the leaves of sugarcane seedlings exposed to 40°C, soluble sugar concentration increased significantly compared to control (28°C) (Wahid, 2006). Pepper seedlings responded to heat similarly and heat stress increased sucrose concentration in pepper leaves. Dramatic increase in the sucrose concentration under heat stress was probably resulted from reduction in the activity of acid invertase since invertase breaks down sucrose into glucose and fructose (Ipek, 2007). No significant difference was observed in carbohydrates concentration under heat stress and normal conditions in wheat grain (Zamani et al., 2014). According to Saghfi and Eivazi (2014) leaf soluble carbohydrates, glucose, ramnose, mannose and fructan increased in leaves exposed to cold stress in resistant cultivar during the treatment period. This increase was more prominent for the resistant cultivar as compared to the susceptible cultivar. The stressed light grown seedlings showed an elevated content of sugars in comparison with dark grown seedlings (Gill et al., 2001). Accumulation of sugars in different parts of plants was enhanced in response to the variety of environmental stresses (Macleod et al., 1958; Escalada et al., 1976; Garham et al., 1981; Prado et al., 2000; Wang et al., 2000). Presence of high amount of sucrose had been observed under low photon flux density by Kozal (1991). According to Hughes et al. (2005) seasonal comparison of total nutrients content showed that starch was significantly higher in summer sun leaves than winter sun leaves. Both sun and shade winter leaves exhibited significantly higher soluble sugar content than summer leaves, with a nearly two-fold increase. Light intensity is one of the most important environmental factors that determine the basic characteristics of rice development (Qi- Hua et al., 2014). Stress caused by low light often created severe meteorological disasters in some rice-growing regions worldwide. The light intensity have significant effect on the enzymes involved in starch synthesis in grains as well as the translocations of carbohydrate and nitrogen in rice (Qi-hua et al., 2014).

### 2.5.3 Protein

Various abiotic stresses usually cause protein dysfunction in the plants. Maintaining proteins in their functional conformations and preventing the aggregation of non-native proteins are particularly important for cell survival under stress conditions. Abiotic stress (e.g. heat) strongly affect senescence and the degradation of chloroplast proteins (Feller et
In general, such a stress caused an early or accelerated senescence (Wingler and Roitsch, 2008). Upon encountering oxidative stress, proteins are oxidized by highly reactive and oxidative species, and these damaged, oxidized proteins need to be degraded rapidly and effectively. Proteins synthesized in response to drought stress are called dehydrins (dehydration induced) and belong to the group II late embryogenesis abundant (LEA) proteins (Close and Chandler, 1990). The dehydrin family of proteins accumulates in a wide range of plant species under dehydration stress. Drought stress induced changes in protein synthesis in maize (Bewley et al., 1983). The accumulation of dehydrin like proteins was detected in the roots and leaves of drought-stressed plants, which could protect plants from further dehydration damage (Mohammadkhani and Heidari, 2008).

According to Castro et al. (2009a) heat stress did not noticeably influence the protein quality of a selected subset of six wheat genotypes, measured using high-performance liquid chromatography. The nuclear-encoded plastidic heat shock proteins and other photosynthetic proteins and precursor of small subunit of Rubisco, accumulated in cytosol of plants heat stressed wheat genotype at 43°C. However, it was readily reversible on return to normal growth temperature (Heckathorn et al., 1998).

High temperature was reported to increase the proportion of gliadins to glutenins and decrease the proportion of large polymer in flour from several wheat varieties grown in controlled environment experiments (Dupont and Altenbach, 2003). According to Jamieson et al. (2001) conditions that shorten the grain filling, such as high temperature or drought, affect the balance of protein fractions. Moderately high temperatures of 25°C to 32°C had a positive effect on dough properties, and have been reported to lead to variation of the composition of the gliadin fraction (Daniel and Tribbi, 2000; Martre et al., 2006). Study of Sharma et al. (2013) showed that osmotic and temperature stress generated significant reduction in total protein contents in Anabaena strains. The percent reduction in all the parameters was higher in A. ellipsospora indicated higher sensitivity than A. oryzae. Effects of high temperature stress include oxidative stress, enhanced transpiration ensuing permanent tissue collapse and cell death. A decrease in proteins content under induced temperature stress was attributed to escalation in biosynthesis of heat shock proteins, along with antioxidant, metabolites like polyphenols (Rutuja and Mala, 2014). Different
degrees of temperature treatment alone decreased protein concentration of seedlings in 10 days old seedlings of *Brassica juncea* L. (Geetika et al., 2014).

Barua et al. (2005) reported that protein content in field-grown plants, undergoing natural temperature stress, was greater in open sun than shaded environments. Supporting these results, both light and temperature significantly affected accumulation of protein *in vitro* condition also. This is the first study to show that the interaction of light microclimate and temperature significantly influenced protein accumulation in field-grown plants. B deficiency and B excess conditions together with high temperature treatment caused a range of effects in the cowpea plants. These include decrease in photosynthetic pigments and soluble proteins (Inbaraj and Muthuchelian, 2011). Effects of specific light regimes on protein content of *Apios americana* aimed to make recommendations on the light environments that may help maximize food production with increase amount of protein of plant in an agricultural setting (Michael et al., 2011).

According to Adamska (2006) light stress resulted in the release of reactive intermediates of reduced dioxygen such as superoxide radicals, hydroxyl radicals, hydrogen peroxide or singlet oxygen. In order to maintain their normal function under light stress conditions, chloroplasts have developed multiple repair and protection systems. The induction of specific light stress proteins, the ELIPs (for early light-induced proteins) can be considered to be part of these protective responses and its accumulation is correlated with the photoinactivation of PSII, degradation of the D_1-protein of PSII reaction centre and changes in the level of pigments (Heddad et al., 2006).

According to Michaela et al. (2000, 2014) plants of the first group exposed to white-light tubes (400–700 nm) 60w and UV(365nm) showed that protein content decreased significantly in both root and shoot in *Phaseolus vulgaris*. The protein content of grains increased significantly as it was 14.62% higher under 49% shade and 37.59% higher under 69% shade. The grains protein yield under low light were significantly lower than control (Huitao et al., 2014).
2.6 Plant Antioxidant Defence Systems

2.6.1 Nonenzymatic Antioxidants

Ascorbic acid is a naturally occurring organic compound with antioxidant properties. Ascorbic acid is one of the essential water-soluble antioxidants present in millimolar concentrations in the chloroplast, and is used as substrate to control the level of photosynthetically generated H$_2$O$_2$ (Nakano and Asada, 1981) as part of the ascorbate-glutathione cycle. Also, ascorbate is involved in the regeneration of the membrane-bound antioxidants α-tocopherol and zeaxanthin, affording protection against lipid peroxides and singlet oxygen, respectively. The pool of ascorbate in the cell is kept at a fairly constant level, and the loss of ascorbate might reflect the degree of stress imposed (Stegmann et al., 1991). As ascorbate functions in leaves mainly a reductant, its redox state is pivotal for its function as an antioxidant (Foyer, 1993). A study was carried out by Qin et al. (2009) to investigate the responses of ascorbic acid metabolism system in potato leaves to high and low temperature stresses. Under the exposure to 40°C, the leaf ascorbic acid content increased rapidly and reached the highest (43.7% higher than the control) at 6 hrs, followed by a rapid decrease. Under the exposure to 5°C, the ascorbic acid content also increased first, reached the highest (27.7% higher than the control) at 9 hrs, and then decreased. Total cellular ascorbic acid activity appeared to be more sensitive to heat stress in plants. Heat inducible transcriptional activation of cytosolic ascorbate genes corresponds with an increase in ascorbic acid activity (Almeselmani, 2006). Temperature had been shown to be one of the factors that affect the ascorbic acid synthesis in plants. Both types of thermal stress (high and low temperature) resulted in small but significant increase in ascorbic acid levels on a dry weight basis (Mogren et al., 2010). Ascorbic acid content increased in long day condition and delays flowering in LD-grown Arabidopsis and Brassica rapa (Daniela and Tullio, 2007). At low light intensity, an increase in photosynthetic carbon fixation had occurred, which varied depending on growth and light intensity, may lead to different susceptibilities to photoinhibition (Powles, 1984). A higher increase in the chloroplast ascorbate content in low light treated leaves was found in a tolerant pea genotype (Hernandez et al., 1995). However, decreased content of ascorbate in leaf tissue was found, especially in plants grown in high light.
Chlorophyll, known as the "green blood" of plants, is the natural plant pigment that lends its color to grass, leaves, and other vegetation. Light and temperature is one of the environmental factors that limit plant growth by affecting chlorophyll concentration (Levitt, 1980; Boyer, 1982; Frova, 1997). Photosynthesis is one of the physiological processes that are most sensitive to high temperature stress (Berry and Björkman, 1980). Inhibition of photosynthesis by high temperature stress is a common occurrence for plants in tropical and subtropical regions and the temperate zones where plants are exposed periodically to high temperatures (Larcher, 1995). It is well documented that high temperature stress caused damage to photosynthetic electron transport (Berry and Björkman, 1980). Chlorophyll concentration may be an indicator of plant physiological status or level of stress (Blackburn, 1998). The research has shown significant variation among wheat cultivars with respect to reduction in photosynthesis at very high temperature (Abrol and Ingram, 1996).

Heat stress induced changes in respiration and photosynthesis and thus leads to a shortened life cycle and diminished plant productivity (Barnabas et al., 2008). The early effects of thermal stress comprise of structural alterations in chloroplast protein complexes and reduced activity of enzymes (Ahmad et al., 2010). The photochemical modifications in the carbon flux of the chloroplast stroma and those of the thylakoid membrane system are considered the primary sites of heat injury (Wise et al., 2004), as photosynthesis and the enzymes of the Calvin–Benson cycle, including Rubisco and Rubisco activase are very sensitive to increased temperature and are severely inhibited even at low levels of heat stress (Maestri et al., 2002; Morales et al., 2003). The maintenance of cellular membrane function under high temperature stress is thus essential for a sustained photosynthetic and respiratory performance (Chen et al., 2010). The detrimental effects of heat on chlorophyll and the photosynthetic apparatus are also associated with the production of injurious reactive oxygen species (Camejo et al., 2006; Guo et al., 2007). By increasing chlorophyllase activity and decreasing the amount of photosynthetic pigments, heat stress ultimately reduced the plant photosynthetic and respiratory activity (Todorov et al., 2003; Sharkey and Zhang, 2010).

In wheat seedlings the increase in chlorophyll a amount as well as in both chlorophyll a/b and chlorophyll (a+b) carotenoids ratios after cold stress indicated that chlorophyll a participated in the resistance to low temperature. After cold stress the amount of chlorophyll a in 7-day-old seedlings diminished while heat stress caused an increase in the level of
chlorophyll b and decreased the level of carotenoids (Pyatygin, 2008; Stanetska et al., 2011). The most evident changes in the chlorophyll (a + b)/carotenoids ratio were observed after cold stress. In general, the changes in pigment complex under temperature stress suggest the involvement of pigments in the initial step of short-time adaptation (Babenko et al., 2014). An analysis to find out the relationship between altitude and the effect of temperature on chlorophyll content showed that at warm temperature, regressions between chlorophyll content and altitude of origin were negative. In contrast, positive relationships were found at cold temperature. Under heat stress, susceptible sorghum genotypes showed higher reduction in total chlorophyll content than tolerant sorghum genotypes. Heat tolerant and wild sorghum genotypes had higher levels of chlorophyll as compared to heat susceptible genotypes (Gosavi et al., 2014).

Cold stress reduced chlorophyll content in two light intensities, however, this effect was more noticeable at high light intensity for reduced chlorophyll value (21%) (Jenabiyan et al., 2014). Where light intensity increased from 2000 to 8000 lux, plants exhibited slightly increase in chlorophyll b. Chlorosis of leaves is the first visual symptom of stress leading to senescence (Fletcher and Hofstra, 1988) and is associated with a concomitant decline in concentration of photosynthetic pigments (Fletcher and Hofstra, 1990). The leaves of control plants after low temperature stress were chlorotic and the photosynthetic pigments – chlorophylls and carotenoids markedly decreased (Pinhero and Fletcher, 1994).

The chlorophyll concentration of plants plays an important role in the absorption of light during photosynthesis. The greatest chlorophyll concentration was estimated at a lowest PPF of 10 μmolm$^{-2}$s$^{-1}$ (Mitchell et al., 1991). Walters et al. (2003) found that leaves of plants growing at low PPF have relatively higher contents of chloroplastic pigments, electron carriers, and increased number of chloroplast. The result also demonstrates that plants could balance light absorption and translation by regulating chlorophyll synthesis (Bailey et al., 2001). The change of chlorophyll concentration was possibly an acclimation of plants to different light intensities. For many plants, changes in light intensity may elicit physiological responses at the level of leaf and chloroplast (Bailey et al., 2001). A study carried by Bailey et al.(2001) revealed that leaf responded to the light intensity by reducing the chlorophyll concentration. Chloroplast responded to light intensity by changing chlorophyll a/b. An
increase in light intensity was associated with an increase in chlorophyll a/b and a decrease in the size of the PSII light-harvesting antenna (Bailey et al., 2001; Masuda et al., 2002). Thus, interconversion of chlorophyll a and chlorophyll b is significant for the establishment of required chlorophyll a/b ratio during the adaption of leaves to high and low light intensity (Ito et al., 1995). The study by Sharma et al. (2013) revealed that osmotic and temperature stress generated significant reduction in photosynthetic pigments in two Anabena strains. The percent reduction in all the parameter was higher in Aspergillus ellipsospora indicated higher sensitivity than Aspergillus oryzae. Cold stress in either 2000 or 8000 lux adversely affected morphological parameters of soybean plants, however, the rate of damage increased at low light intensity (Jenabiyan et al., 2014).

Carotenoids are organic pigments that are found in the chloroplasts and chromoplasts of plants and some other photosynthetic organisms, including some bacteria and some fungi. The total carotenoid content was adversely affected by temperature stress as plants subjected to low temperature stress at 25ºC, and to high temperature at 75ºC exhibited lower content of total carotenoids. According to Fletcher and Hofstra (1990) the leaves of corn plants after low temperature stress were found chlorotic and carotenoids markedly decreased. Carotenoid levels decreased in both the control and the treated seedlings of corn and wheat plants which were subjected to high temperature and waterlogging (Webb and Fletcher, 1996). The levels of α-carotene and β-carotene were significantly reduced in the OsGH3-2-overexpressing lines under drought and cold tolerance in rice (Du et al., 2012). Tomato (Solanum lycopersicum cv. Kommeet) at low and intermediate temperature, plants grafted onto LA 1777 were capable of increasing carotenoid content in roots to higher levels than those grafted onto S. lycopersicum rootstocks (Ntatsi et al., 2014). Postharvest yellowing caused yellowing in rice endosperm during conditions of high temperature and moisture (Belefant et al., 1994). Transcription levels of phytoene synthase, the first committed step in carotenoid biosynthesis, were higher in carotenoid containing bran, indicating that carotogenesis is an ongoing process in mature bran. During Post harvesting yellowing, total carotenoid levels in bran increased while levels of the predominant xanthophyll carotenoid and lutein decreased (Belefant et al., 2014). Carotenoid levels decreased in low temperature treated seedlings of wheat plants (Webb and Fletcher, 1996). Interactive effects of high light
on carotenoid profiles in *Nannochloropsis oceanica* was studied. Nitrogen starvation under high light enhanced the production of reserve lipids at the expense of chloroplast lipids and carotenoids of pigment apparatus in *N. oceanica*. Regardless of cultivation conditions, the stress-induced changes in pigments were highly coordinated (Solovchenko, 2014).

Alkaloids are plant secondary metabolites that have a nitrogen-containing chemical ring structure, alkali-like chemical reactivity and pharmacologic activity. Higher temperatures result in a higher alkaloid content of the seeds. Seed alkaloid content is strongly influenced by the temperature during initiation of flowering up to pod ripening (Jansen *et al.*, 2009). According to Toivonen *et al.* (1992) cell suspension cultures of *Catharanthus roseus* were used to study the effect of temperature on plant cell lipids and indole alkaloid accumulation. The effects of temperature and humidity on the alkaloid content and nicotine conversion in curing barley and tobacco was also found. Cold treatments caused significant variations in the biosynthesis of tropane alkaloids in *Datura innoxia* (Lavieville, 2014). At cold temperature alkaloid content quinolizine in *Lupinus argenteus* increased to significant level (Carey and Wink, 1994).

According to Hoff *et al.*(1996) high light intensity lowered alkaloid content but promoted growth. Investigation was done by the relationship between primary production and the production of secondary metabolites with respect to relative and total alkaloid content. Under conditions of high temperature and low light, all plants allocated almost equal proportions of leaf nitrogen to alkaloids, regardless of fertilizer (Hoft *et al.*,1996). According to Ralphas *et al.*(1998) nortiterpenoid alkaloids in larkspur did not respond to short-term light stress. Alkaloid concentration was lower in larkspur plants growing beneath forest canopy and in potted plants in a long-term shade study (70% reduction in sun light) than plants growing in open sunlight. Long-term shade reduced synthesis of nortiterpenoid alkaloids, particularly in the earlier developmental stages of the plant. In *Catharanthus roseus*, an important herb used in traditional as well as modern medicine, exposed to water deficit stress and high temperature and possible changes in total alkaloid content and vincristine and vinblastine levels were studied. Total alkaloid content significantly increased to 187% compared to the control (Amirjani, 2013). The light-absorbing compounds as well as three alkaloids, vinblastine, vindoline, and catharanthine, were observed to have a
remarkable elevation. These compounds were considered to serve as protectants of light radiations (Gua et al., 2014).

Tocopherols are the best-studied class of lipid-soluble antioxidants and are produced only by photosynthetic organisms. Tocopherols can efficiently quench singlet oxygen, scavenge various radicals, particularly lipid peroxy radicals, and thereby terminate lipid peroxidation chain reactions (Liebler and Burr, 1992; Bramley et al., 2000; Schneider, 2005). In plants, tocopherols are synthesized and localized in plastid membranes that are also highly enriched in polyunsaturated fatty acids (PUFAs) (Bucke, 1968; Soll et al., 1980; Lichtenthaler et al., 1981; Soll et al., 1985, Soll et al., 1987; Vidi et al., 2006), and increased tocopherol content had been correlated in response of photosynthetic tissues to a various abiotic stresses, including high-intensity light (HL), drought, high and low temperatures (Munne-Bosch et al., 1999; Keles and Oncel, 2002; Bergmuller et al., 2003; Collakova and Della Penna, 2003). The specific response of tocopherols differed, α-tocopherol being increased by high temperature by as much as 752%, the reverse being observed for δ-tocopherol and γ-tocopherol in soybeans (Almonor et al., 1998). The temperature effect on the relative antioxidant activity of the tocopherols may be related to differences in stability or to differences in antioxidant potencies at various temperatures (Hove and Hove, 1944). Tocopherols have long been assumed to play crucial roles in high light protection, presumably by acting as singlet oxygen quenchers and lipid peroxy radical scavengers (Fryer, 1992; Munne-Bosch and Alegre, 2002; Trebst et al., 2002). Tocopherols are not essential for the adaptation and tolerance of photosynthetic tissues subjected to high light stress alone. Such a conclusion runs counter to long-held assumptions that a primary function of tocopherols is to protect photosynthetic tissues against high light stress (Fryer, 1992; Bosch andAlgeree, 2002).

The flavonoids content in plants is temperature dependent as it decreased at 50°C, whereas it was high at 75°C. There was also increase in loss of flavonoids with increase in temperature in Soybean (Chennupati et al., 2012). A study of phytochemistry, metabolite changes, and medicinal uses of the common food mung bean and its sprouts (Vigna radiata) revealed that lavone, isoflavone, flavonoids, and isoflavonoids are the important metabolites severely affected by light stress. Flavonoids are involved in stress protection (i.e., oxidative
and temperature stress), early plant development, signaling, and protection from insect and mammalian herbivores (Tang et al., 2014).

Higher the temperature greater is the total phenol content (Akowuah et al., 2009). The metabolism of soluble phenolics is regulated by the activity of various enzymes. The first step necessary for the synthesis of the phenyl-propanoid skeleton in higher plants is the deamination of the L-phenylalanine, giving rise to transcinnamic acid and ammonium. This reaction is catalysed by the enzyme PAL, which is commonly considered the principal enzyme in the biosynthesis of phenolic compounds. PAL activity is affected by a great number of factors, both biotic and abiotic, including light, temperature, growth regulators, protein synthesis, drought and mineral nutrition. It has been demonstrated that heat and cold stress induced the production of soluble phenolics and thereby increased PAL activity. The highest PAL activity in tomato plants and in watermelon plants was recorded at 35°C. Therefore, the PAL activity increased probability, in response to heat and cold stress (Rivero et al., 2001). These results are consistent with other authors who consider PAL to be one of the prime elements of cell acclimation against thermal stress in plants (Christie et al., 1994). The relationship between PAL activity and soluble phenolics concentration in tomato and watermelon plants indicated an accumulation of phenolics compounds in the plants in response to heat and cold stress respectively, caused by activation of enzyme PAL. In addition, the metabolism of phenolic compounds also includes the action of oxidative enzymes such as POD and PPO, which catalyse the oxidation of phenols to quinones (Rivero et al., 2001). Austin et al. (1960b) found a small effect of light on the rate of postharvest colour development in Sparkle strawberries. Kalt et al. (1993) observed that light increased the pigments of Blomidon strawberries. In the light, the accumulation of phenol over a temperature range of 12-28°C was observed while in the dark accumulation exhibited a monotonic relationship with temperature. Temperature also influenced the accumulation of phenol by algae (Newsted, 2014).

Sterols are a group of naturally occurring substances derived from hydroxylated polycyclic isopentenoids. They occur as a mixture of different compounds although their structures are closely related and varied depending on the extent of modifications of the ring system and side chain variations. Sterols are known to have a wide range of biological activities and physical properties such as: inhibition of cholesterol
absorption, lowering of plasma cholesterol, acting as useful emulsifiers for cosmetic manufacturers, supplying the majority of steroidal intermediates and precursors for the production of hormone pharmaceuticals (Abidi, 2001). Sterols in garlic and ginger contain very high percentage of sitosterol; 86% and 87% for garlic and ginger, respectively. Campesterol, stigmasterol, and 5-avenasterol are present in approximately 7%, 2%, 5% and 6%, 3%, 4% for garlic and ginger oil respectively. Sterols are known to inhibit oxidative deterioration of oils; serving as potential antipolymerization agent for frying oils (Abidi, 2011). The unsaturated analogoues of phytosterols and their esters have been suggested as effective cholesterol – lowering agent by decreasing low density lipoprotein (LDL) cholesterol, mostly through interfering with the intestinal absorption of cholesterol thereby offering cardiologic health benefit (Law, 2000; Mercy et al., 2014)

Phytosterols are present as free sterols or in conjugated forms (steryl esters, acyl steryl glycosides, and steryl glucosides). Free sterols are integral components of the membrane lipid bilayer where they interact with other membrane components and play a functional role in the regulation of membrane fluidity and permeability (Schaller, 2003; Carland et al., 2010). Modification of the plant sterol content in the plasma membrane was shown to alter the function of membrane bound proteins, including enzymes, channels, and receptors or other components of signal transduction pathways (Schaller, 2012). It has been suggested that at least part of the severe phenotype of sterol-deficient mutants may be due to impaired synthesis of cellulose (β-1,4-glucan), which normally assures the strength and flexibility of plant tissues (Peng et al., 2002). Lee et al. (2004) reported that the overexpression of Panax ginseng squalene synthase gene (PgSS1) due to high temperature in it resulted in remarkable increase of phytosterols as well as ginsenosides. These results demonstrated that PgSS1 was a key regulatory enzyme not only for biosynthesis of phytosterol but also for triterpene (Jiewen et al., 2014). Velasco (2014) has reviewed phytochemistry, metabolite changes, and medicinal uses of the common food mung bean and its sprouts (Vigna radiata). Roche et al. (2010) reported a large variability in total shoots among a collection of sixteen sunflower inbred lines as it varied almost twofold between extreme genotypes. A delay of sowing, giving higher temperatures during seed formation, induced a
general increase in total sterol concentration by up to 35 percent, as well as sterol composition but this varied according to genotype (Roche et al., 2010).

High light intensity increased the phenol and saponin content in sunflower (Ramakrishna and Ravishankar, 2011). The pattern of climatic temperature during the plant growth season was acknowledged as the crucial factor affecting the saponin level in yam *Dioscorea pseudojaponica*. Yam is a perennial herbaceous vine cultivated for the production of its edible starchy tubers containing steroidal furostanol and spirostanol saponins, which are regarded as the important functional compounds. The accumulation of saponins in plant reproductive organs fits with the hypothesis that these compounds play a role in chemical protection and the plant response to environmental factors (Lin et al., 2009).

The availability, intensity and quality of light are major components of the environment that can influence the saponin content of plants (Szakiel et al., 2011). The level of soya saponins in germinating soybean *Glycine max* seeds was found to be considerably higher in seeds subjected to day/night periods than in seeds germinating in total darkness, although light irradiation did not produce an equal effect on the biosynthesis of all the tested compounds (Shimoyamada and Okubo, 1991). The effect of light on ginsenoside content was investigated in forest-grown American ginseng *Panax quinquefolius* (Fournier et al., 2003). American ginseng plants exposed to longer sunflecks were found to have higher root ginsenoside contents than those exposed to shorter periods of direct sunlight. This phenomenon was explained by mild photooxidation stimulating plant defense mechanisms, such as the induction of jasmonate-response genes leading to the formation of methyl jasmonate which affects ginsenoside synthesis. Exposure to more than 36% of the solar radiation resulted not only in reduced ginsenoside accumulation, but also caused photoinhibition, photobleaching, and if the light conditions persisted, premature leaf death. Variation in light availability inside a tree crown can also influence the amount of saponins, as was demonstrated for the fruiting African tree *Diospyros abyssinica* (Hiern) F. White. Fruit quantity and quality vary vertically within the tree canopy according to the stratification of light and shadow during fruit development (Szakiel et al., 2011).

### 2.6.2 Enzymatic antioxidants

Activity of superoxide dismutase (SOD) significantly increased after exposure to high and low temperature and light treatments. SOD enzyme actively increased under low
and high temperature stresses. Schoner and Krause (1990) reported that only cytosolic SOD mRNA level significantly increased in levels of *Nicitiana plumbaginifolia* that exposed to high temperature. Similarly, mRNA level of chloroplastic SOD rose at combined effects of chilling and intense light conditions (Kels and Oncel, 2000). A significant increase was found in the activity of SOD on treatment with high temperature in five wheat genotypes viz., PBW 343, PBW 175, HDR-77, HD 2815 and HD 2865 (Almeselmani *et al.*, 2006).

Superoxide dismutase converts one form of ROS (O$_2$) to another equally toxic one (H$_2$O$_2$). However, higher activity of SOD alone is not sufficient for providing heat stress tolerance, as the enzymes for H$_2$O$_2$ scavenging (ascorbate peroxidase (APX), Glutathione reductase (GR), Catalase (CAT) and Peroxidase (POD)) are lacking in PBW 343 and other susceptible genotypes. Thus, the higher SOD under very high temperature condition in PBW 343 is of little consequence in the absence of inadequate H$_2$O$_2$ scavenging mechanism. Variable degree of stimulation was also reported in the activities of SOD and POD in leaves of 21 day old seedlings of groundnut (*Arachis hypogaea* L.) irradiated with selected doses of light (Sreedhar *et al.*, 2013). Exposure of plant cells to light led to the formation of ROS and therefore, SOD removed superoxide formed during radiation exposure and also inhibited formation of more reactive pro-oxidants. Radiation is a well known factor that affects antioxidant status and increases free oxygen radical generation. However, it was evident that significant increase in the activity of SOD exposed to ionizing radiation was noticed. These findings indicated increased antioxidant activity (Sreedhar *et al.*, 2013).

Effect of temperature on peroxidase activities of litchi pericarp was studied where its activity was highest at 70°C and remained active for a period of 120 min at 70°C and 80°C whereas it became completely inactive when maintained at 90°C for 10 min or 1 min at 100°C (Rodrigo *et al.*, 1996). With increase in temperature, peroxidase activity increased slowly, at temperatures above 30°C the rate of activity increase was higher and at 70°C the activity reached its peak. After 120 min at 70°C and 80°C the peroxidase activity in the extract was reduced by 58.8% and 67.6%, respectively. These results confirm that litchi is highly heat-resistant (Rodrigo *et al.*, 1996), suggesting that extensive heat treatments are needed to inactivate litchi peroxidase (Mizobuts *et al.*, 2010). Similarly, the low temperature induced physiological responses of *Avena nuda* was studied (Liu, 2013). The activities of
SOD, POD, and CAT increased under low temperature. The increased POD activities under low temperature had improved cold tolerance in some degree. POD activities decreased greatly in later days, indicating that low temperature had affected POD enzyme. It may be due to low temperature affected RNA transcription and translation, reducing the synthesis of POD. At the same time proline content also increased under low temperature, which can degrade peroxidase. It can also decrease the POD activity (Liu et al., 2013). A study by Carolina et al. (2012) was made to determine influence of light on peroxidase activity on tomato (Lycopersicon esculentum Mill. cultivar Heinz) where paper electrophoresis scans of peroxidase isozymes showed a bimodal pattern of a conspicuous increase in staining intensity reflecting a high level of enzymatic activity with high light. Furthermore, treatment with various wavelengths of light produced the most marked effects on enzyme activity in the later rather than in the earlier stages of development (Benedict, 1971).

Light and temperature variations are important during the vegetative stage of the Chinese red radish where it was found that peroxidase (POD) and catalase activities and the content of pelargonidin decreased by treatments of short of light period and low temperatures, while the content of hydrogen peroxide increased. The results suggested that POD expression was both time dependent and tissue-specific and that light and temperature conditions influenced the growth and antioxidant activity of the radish (Wang et al., 2009) in Beta vulgaris L. (Panagopoulos, 1990) and in light-grown Spinacia oleracea (Penel and Greppin, 1979). CAT activity was higher under the cold treatment than normal temperature in Avena nuda. Increased CAT activity contributed to cold tolerance of naked oats under cold stress (Liu et al., 2013). Catalase activity is also associated with the scavenging of H₂O₂ and an increase in its activity is related with increase in stress tolerance (Almeselmani et al., 2006). In Eupatorium adenophorum, the coordinated increase of the activities of antioxidant enzymes was effective in protecting the plant from the accumulation of active oxygen species (AOS) at low temperature, but the activities of catalase (CAT), guaiacol peroxidase (POD), ascorbate peroxidase (APX), glutathione reductase (GR) and monodehydroascorbate reductase (MDAR) were not accompanied by the increase of superoxide dismutase (SOD) during the heat treatments (Lu et al., 2008). Catalase eliminates H₂O₂ by breaking it down directly to form water and oxygen. In leaves of E. adenophorum, CAT activity was depressed by high temperature and increased to a high level in the low temperature treatment. In leaves
of *Eupatorium odoratum*, however, the response pattern of CAT activity was reversed, CAT activity was enhanced by high temperature and the plants were unable to maintain normal activity when subjected to low temperature, such as 10°C and 5°C. An increase in SOD activity with a decrease in CAT activity had been reported when the plants were subjected to abiotic stress (Saruyama and Tanida 1995; Fu and Huang 2001; Jung 2003). Others have found that CAT activity is photoinactivated under low and high temperature stresses and CAT activity varies with plant species (Feierabend *et al.*, 1992). At long light period the substantial increase in the activities of SOD, CAT and POD increased rapidly but then decreased drastically with the extension of treatment duration in lettuce (*Lactuca sativa* L.) (Fu *et al.*, 2012). Effects of light intensity on scavenging enzymes during acclimatization of micropropagated *Calathea* was studied which showed that under low light catalase activity increased but under high light intensity it decreased (Vanhuyslenbroeck *et al.*, 1997).

Antioxidant enzymes play a crucial role in detoxification of ROS and generation of antioxidants in response to prevailing stress. Accumulation of the ROS, H$_2$O$_2$ induced by various environmental stresses result in the combined activity of Catalase and peroxidase. Severe deactivation of catalase accompanied by increased H$_2$O$_2$ during temperature suggested the operation of a similar antioxidant mechanism in French bean as has been observed in many species (Blokhina *et al.*, 2003). Similar results were reported in heat stressed mustard (Dat *et al.*, 1998) and drought stressed pea (Moran *et al.*, 1994) which exhibited a significant increase in endogenous H$_2$O$_2$ and peroxidase, and marked decline in catalase. Contradictory to decrease under temperature stress, the CAT activity was elevated under cold stress. Increased activity of catalase and peroxidase had been suggested as an adaptive mechanism to reduce the H$_2$O$_2$ and offer protection against oxidative damage (Agarwal and Pandey, 2004).

A total decline of catalase under temperature and over expression of all the isozymes under temperature stress, further strengthened the view of divergent response to light and temperature stresses. Another antioxidant enzyme, glutamate reductae was drastically reduced, implying an elevated GSH (Babu and Devaraj, 2008). Our observations are in contrast to those patterns reported during low temperature stress in pea and maize, wherein a rise in the GR activity and reduction in GSH was found (Edwards *et al.* 1997).
An experiment was conducted to study the effect of high temperature stress on the antioxidant enzyme activity in five wheat genotypes viz., PBW 343, PBW 175, HDR-77, HD 2815 and HD 2865 where almost all the genotypes showed an increase in ascorbic acid content. HD 2815, HDR-77 showed relatively higher ascorbic acid activity than others PBW 343, PBW 175 and HD 2865 variants (Almeselmani et al., 2006). The low temperature increased GST specific activity and glutathione (GSH) pool size in resistant and susceptible *Alopecurus myosuroides* biotypes. Findings demonstrated differences in GST activity between resistant and susceptible populations, which are transient at lower growth temperatures (Milner et al., 2007). The coordinate function of antioxidant enzymes such as SOD, APX, catalase and GR helps in processing of ROS and regeneration of redox ascorbate and glutathione metabolites. The oxidative damage to cellular components is limited under normal growing conditions due to efficient processing of ROS through a well-coordinated and rapidly responsive antioxidant system consisting of several enzymes and redox metabolites. However, under various abiotic stresses the extent of ROS production exceeds the antioxidant defense capability of the cell resulting in cellular damages. Tolerance to high temperature stress in crop plants has been reported to be associated with an increase in antioxidant enzymes activity. According to Babu et al. (2008) temperature stressed seedlings suggested involvement of distinct biochemical components. ROS scavengers GSH and ascorbic acid, which accumulated in response to oxidative stress, are part of a well-established ascorbate cycle. Remarkable increase in ascorbic acid levels indicated the induction of antioxidant mechanism such as ascorbate cycle in French bean, as reported for a number of plants (Halliwell and Gutteringe, 1989; Koca et al., 2007). In *Eupatorium adenophorum*, the coordinated increase of the activities of antioxidant enzymes was effective in protecting the plant from the accumulation of active oxygen species (AOS) at low temperature, ascorbate peroxidase (APX) were not accompanied by an increase of superoxide dismutase (SOD) during the heat treatments (Lu et al., 2008).

Effects of light intensities on antioxidant enzymes during short-term acclimatization on micropropagated *Phalaenopsis* plantlet was also noticed (Ali et al., 2004). There was an increase in ascorbate peroxidase activity in leaves of about 50% at high light compared to *in vitro* grown plantlets whereas no changes in roots were observed. Significant enhancement of ascorbic acid in all three light treated leaves compared to *in vitro* grown plantlets indicated...
the oxidation by molecular oxygen of ascorbic acid to dehydroascorbate with the formation of H$_2$O (Loewus, 1980; Esaka et al., 1992). Though the exact function of ascorbic acid is still not clear but induced activity under light stress may modify the ascorbic acid levels and therefore maintains the redox cellular balance, which provides additional support to the plants against the oxidative stress (Potters et al., 2000).

2.7 Secondary Metabolites in Plants

Plant secondary metabolites are often referred to as compounds that have no fundamental role in the maintenance of life processes in the plants, but they are important for the plant to interact with its environment for adaptation and defense. Accumulation of such metabolites often occurs in plants subjected to stresses including various elicitors or signal molecules. The secondary metabolites are defined as bioactive molecules, which provide the plant with defense mechanisms to survive from herbivores, environmental stresses, diseases, or competition and may affect the growth and development of other organisms (Seigler, 1996). In higher plants a wide variety of secondary metabolites are synthesized from primary metabolites (e.g., carbohydrates, lipids and amino acids). The production of these compounds is often low (less than 1% dry weight) and depends greatly on the physiological and developmental stage of the plant (Namdeo, 2007).

It is evident that abiotic stress factors influence growth and physiology in plants. Oxidative stress induced by abiotic stress triggers signaling pathways that affect production of specific plant metabolites. In particular, reactive oxygen species (ROS), generated during abiotic stress, may cause lipid peroxidation that stimulates formation of highly active signaling compounds capable of triggering production of bioactive compounds (secondary metabolites) that enhances the medicinal value of the plant (Gill and Tuteja, 2010).

In 2000, a new concept for the production of transplants in a controlled environment was introduced (Kozai et al., 2000). In this system, artificial light is the sole light source for plant growth; photosynthetic photon flux (PPF), CO$_2$ concentration, air temperature, relative humidity, and air speed are well controlled for optimizing plant productivity and quality. Environmental factors also have remarkable effects on secondary metabolite biosynthesis. Quality of medicinal plants is determined by their superior genetic characteristics and
biomass with high and consistent secondary metabolite content (Kozai, 2005). High-quality medicinal plants can be produced only under carefully controlled environments (Afreen et al., 2005). Therefore, growing plants under a controlled environment can be considered an alternative way for medicinal plant production to ensure safety and efficacy. A number of earlier investigations have reported that environmental factors such as light intensity can significantly improve growth and alter the metabolite concentrations. For example, the increasing light intensity at a PPF of 100 μmol m$^{-2}$s$^{-1}$ significantly improved the growth and photosynthetic capability of in vitro Momordica grosvenori plantlets (Zhang et al., 2009). Briskin and Gawienowski (2001) reported that growing St. John's wort plants at a PPF of 400 μmol m$^{-2}$s$^{-1}$ significantly increased the hypericin concentration with enhanced photosynthetic activity. Light is also involved in regulating antioxidant enzymes and secondary metabolites. Mohammad et al. (2005) found that micropropagated Phalaenopsis plantlets had higher superoxide dismutase (SOD) content to adapt the increasing light intensity. Zhong et al. (1991) successfully increased anthocyanin production in cell culture of Perilla frutescens by increasing light intensity. Secondary metabolites play a major role in the adaptation of plants to the environment and in overcoming stress conditions. Environmental factors viz. temperature, humidity, light intensity, the supply of water, minerals, and CO$_2$ influence the growth of a plant and secondary metabolite production (Ramakrishna and Ravishankar, 2011). Physiological significance of glucosinolates and their hydrolysis products in the plant response to different abiotic stresses in Brassica was found by Martinez-Ballesta et al. (2013). Abiotic stresses affect the biosynthesis, concentration, transport and storage of primary and secondary metabolites. Metabolic adjustments in response to abiotic stressors involve fine adjustments in amino acid, carbohydrate, and amine metabolic pathways (Velazquez and Hernandez, 2013).

2.7.1 Withanolides

Withania somnifera is chemically rich and contains more than 80 compounds derived from it (Mwitari et al., 2013). The biologically active chemical constituents are the alkaloids and steroidal compounds which includes the ergostane type steroidal lactones, withanolide A, withasominiferin-A, withaferin A, withasominiferols A-C, withasomidienone, withanone etc. The medicinal properties of plant are attributed to group
of compounds called withanolides which are present in a large number in its roots and leaves. Withanolides are a group of compounds characterized by a C-28 basic skeleton with a nine carbon atom side chain in which C-22 and C-26 are appropriately oxidized to form a six membered ‘δ’ – lactone ring CS - 8 (Lavie et al., 1974). A 1 – keto - Δ2 – system, found in ring A, has been observed to be a general feature of the molecule (Ray and Gupta, 1994). From the biogenetic point of view, the withanolides can be considered to have a cholestane type structure with an extra methyl group at C-24 and with various oxygenated groups or double bonds placed at different sites of the skeleton (Singh and Kumar, 1998).

The first scientific investigation by Power and Salway (1911) on South African populations of the species, revealed the presence of withanolin in the roots and somnirol and somnitol in the leaves. Kurup (1956) isolated the first unsaturated lactone from the leaves of plants growing in Jamnagar (India) which was subsequently identified as withaferin A by Lavie et al. (1965) and its remarkable antibacterial properties were investigated first by Kurup in 1958. Since then many workers have identified other withanolides of the series from plants growing in India, Egypt, Pakistan, Israel and South Africa. Withaferin A represents the first natural lactone of the withanolide series isolated from W. somnifera shoots (Nigam and Kandalkar, 1995). According to Heble (1985) total withanolide (withanolide A, withanone, withanolide G, withanolide I, withanolide E) contents in W. somnifera shoot cultures, represented 0.32 % of dry weight of the biomass. Ganzera et al. (2003) described an improved method for quantitative HPLC analysis of presence of marker withanolides in different plant parts of W. somnifera. Till date, approximately more than 12 alkaloids, 40 withanolides and several other sitoindosides have been isolated from the aerial parts, roots and berries of Withania (Power and Salway, 1911; Majumdar, 1933, 1955; Kurup, 1956, 1958; Dhalla et al., 1961; Khanna, 1963; Sastry and Singh, 1982; Ray and Gupta, 1994).

Incidentally, the withanolide profile in the shoot cultures was different from that of the foliar tissues. Withaferin A, the major withanolide component reported from the leaves of naturally growing plants could not be detected in shoot cultures (Jain et al., 2012). In another study, Roja et al. (1991) compared withanolide synthesizing potential of PGR induced callus, cell suspension and multiple shoot cultures. The callus failed to synthesize withanolides
whereas multiple shoots synthesized withanolides along with trace amounts of withanolide D. Withanolides and alkaloids are the major secondary metabolite groups of medicinal interest isolated and characterized from *W. somnifera* (Tripathi *et al.*, 1996; Ray and Gupta, 1997). In all, 13 Dragendorff positive biochemically heterogeneous alkaloids were isolated and characterized from its root extracts. Furmanova *et al.* (2001) compared withanolide contents in leaves of *in vitro* and greenhouse-grown tissue cultured plants and highlighted the importance of prolonged incubation environmental conditions on withaferin A production. In another study, Ray and Jha (2001) established multiple shoot cultures from single shoot-tip explants and reported production of withaferin A and withanolide D. Recently Ray and Jha (2002) reported the presence of withaferin A in the shoots and withanolide D in the roots of tissue culture regenerated plants. Taya *et al.* (1989) monitored the cell growth by conductometry in *W. somnifera* whereas four peroxidases were isolated from the roots of the plant, purified and characterized with a view to explore their role in secondary metabolism (Johri *et al.*, 2005). Few reports are available on the toxicity of some withanolides (Budhiraja *et al.*, 2000). Jayaprakasam *et al.* (2003) isolated 4 novel glycosides, a new withanolide and known withanolides from the methanolic leaf extracts of *W. somnifera* on the basis of 1D-2DNMR and MS spectral data and assayed. A study reported higher production of withanolide A, withanone and withaferin A from the elicited-hairy roots of *W. somnifera* under optimal inoculum mass, harvest time, and elicitor exposure time in root culture which could be useful for biochemical and bioprocess engineering for the viable production of withanolides (Sivanandhan, 2013).

A study for the quantification of Withanolide A from the dried root was done using HPLC and TLC (Sumitradevi *et al.*, 2011). Quantification of bioactive ingredient-withanolide A from roots of different climatic zone of Madhya Pradesh which concluded that withanolide A and withaferin A content depend on root sources (Awasthi *et al.*, 2011). Enhanced biosynthesis of withanolides was found by elicitation and precursor feeding in cell suspension culture of *W. somnifera* in shake-flask culture and bioreactor (Sivanandhan, 2014). Withanolides quantification and production was analysed from *W. somnifera* by development of cellular technology (Sangwan *et al.*, 2014).
2.8 Herbicidal Effect

Herbicides are chemicals used to manipulate or control undesirable vegetation. The most frequent application of herbicides occurs in row-crop farming, where they are applied before or during planting to maximize crop productivity by minimizing other vegetation. Herbicides are used in forest management to prepare logged areas for replanting; the total applied volume and area covered is greater but the frequency of application is much less than for farming (Shepard et al., 2004). Herbicides are applied to water bodies to control aquatic weeds that impede irrigation withdrawals or interfere with recreational and industrial uses of water (Folmar et al., 1979).

The potential effects of herbicides are strongly influenced by their toxic mode of action and their method of application. The molecular site of action is challenging to predict because structural associations have not been identified (Duke, 1990), but modes of action are well-established. Herbicides can act by inhibiting cell division, photosynthesis, or amino acid production or by mimicking natural auxin hormones, which regulate plant growth, and causing deformities in new growth (Ross and Childs, 1996). Methods of application include spraying onto foliage, applying to soils, and applying directly to aquatic systems. Chemicals that impose allelopathic influences are called allelochemicals or allelochemics. These allelochemicals could be extracted out from the plants and applied or spayed over target weeds. In a review of the potential use of allelochemicals as herbicides, Putnam (1988) listed allelochemicals namely alkaloids, benzoazinones, cyanogenic compounds, flavonoids which had been isolated from over 30 families of terrestrial and aquatic plants. All these chemicals possess actual or potential phytotoxicity. According to Rice (1984) tens of thousands of secondary substances out of several hundreds of low molecular weight compounds of primary metabolism are known today, but only a limited number has been recognized as allelochemicals.

Herbicidal effect of aqueous extracts of different part of *Eclipta alba* (L.) Hassk on some crop and weed plants was studied (Gulzar and Siddiqui, 2013). It was found that aqueous extract of leaves showed the maximum inhibition followed by root and stem in *Eclipta alba*. Assessment of some medicinal plants for their allelopathic potential was
done against *Amaranthus retroflexus* (Nekonam *et al*., 2014). The study was conducted to determine the allelopathic effects of *Crocus sativus* L., *Ricinus communis* L., *Nicotiana tabacum* L., *Datura inoxia* Mill., *Nerium oleander* L., and *Sorghum vulgare* L. on the germination and growth of *Amaranthus retroflexus* (redroot pigweed). It was found that the powder and extracts of the tested species have an herbicidal potential against redroot pigweed and could be used as natural herbicides and mulches. Herbicidal effects of aqueous extracts of *Eucalyptus occidentalis*, *Acacia ampliceps* and *Prosopis juliflora* on the germination of three cultivated species (*Hordium vulgare*, *Medicago sativa* and *Corchorus olitorius*). The aqueous extracts decreased the germination rate of seeds of three plants tested *H. vulgare*, *M. sativa* and *C. olitorius*. The decrease in species was more significant for leaf extract in the studied three species (Saadaoui *et al*., 2014).

Crude extracts, plants residues and purified compounds of allelopathic plants (crops, grasses, broad-leaf weeds and trees) have shown their herbicidal activity against germination and growth of *Parthenium* (Javaid, 2010).

Norsworthy and Meehan (2005) tested isothiocyanates for control of *Amaranthus palmeri* S. Wats., *Ipomoea lacunose* L. and *Cyperus esculentus* L. and found several that suppressed emergence of weeds, reducing even yellow nutsedge emergence 95%. In other studies, barley, oat, wheat, and cereal rye residues reduced total weed biomass and the weight of several indicator species (Putnam and DeFrank, 1985). Although much is known about herbicides, future research is needed on the mechanisms of allelochemical selectivity, the modes of release, and the environmental and fertility effects on activity, persistence, and potential for synthesizing bioactive products as herbicides. Allelopathic crops open the potential for development of higher levels of weed suppression through conventional breeding or new methods (Singh *et al*., 2003). More than 200 active ingredients are registered as herbicides around the world, this estimate does not include compounds that are used exclusively as crop growth regulators or crop desiccants and 29 major mechanisms of herbicide action are known (WSSA, 2010).