HEAT AND WATER STRESS IN PLANTS – A REVIEW

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Abstract

Stress is a body's method of reacting to a challenge. Any change in the surrounding environment (i.e. a challenge) may disrupt the steady state of the body in question. Environmental modulation of this steady-state (called homeostasis) is defined as biological stress. Thus, it follows that plant stress implies some adverse effect on the physiology of the plant, induced upon a sudden transition from an optimum condition - where homeostasis is maintained, to a sub-optimal condition where this stability is hampered. Plants cannot locomote and hence need to be more adapted to stressful environments than animals and humans. With increasing temperature and sea level across the globe, heat and water pose a stress to the plants, because anything in excess or deficit is harmful. However, sunlight and water are the two main components of almost all metabolic pathways in a plant and if the plant faces a stressful condition with heat or water, it greatly affects the plant's growth and development adversely. The ability of plants to respond, defend or avoid biotic or abiotic stress depends upon their protein conformation and vigor. Several reviews have reported the tolerance of plants to different abiotic stresses. This review reports a plant's response, avoidance and tolerance mechanism to the increasing heat and water stress.

Key words: Light stress, Heat stress, Heat Shock Proteins (HSPs), Reactive oxygen species (ROS), antioxidants, Water stress.

1. 1. Introduction: Plant Stress

The environment affects an organism in many ways, at any time. To understand the reactions of a particular organism in a certain situation, certain environmental factors are taken into concern, separately, if possible. These factors controlling stress conditions alter the state of equilibrium in the organism and lead to a series of morphological, physiological, biochemical and molecular changes in the organism i.e. plants, which adversely affects their growth, vigor and yield. Thus, plant stress has been defined by Lichtenthaler (1996) as 'any unfavourable condition or substance that affects or blocks a plant's metabolism, growth or development.' [1]. The primary cause of crop loss worldwide, reducing the average yield of major crop plants by 50% is due to abiotic stresses [2]. When a threat is identified or realized, the plant is in a state of 'alarm'. If this stress persists, the organism enters into the 'resistance' phase where it attempts to cope up with mechanisms of protection and defense. In the 'exhaustion' phase, the organism's resources are eventually depleted and the organism is unable to maintain normal function so forth.

1.2. Stress Factors

Environmental factors that induce stress can be biotic or abiotic. Biotic factors result from the interactions with living organisms such as infection by bacteria and fungi or mechanical damage by herbivory. Abiotic factors include non-living factors such as temperature, wind, light intensity, humidity, availability of minerals etc. (Figure 1). The effect of each abiotic factor depends on its quantity. As long as the factor is in optimal quantity or intensity, the plant achieves its "physiological normal type''. Environmental noxae are stress factors which trigger reactions when available in any concentration or intensity, such as ozone, ionizing radiations, heavy metals, xenobiotics etc [2]. Endogenous stress may also occur, for example, by separating an organ from its water supply, as is the case during ripening of seeds and the desiccation of embryo and endosperm.

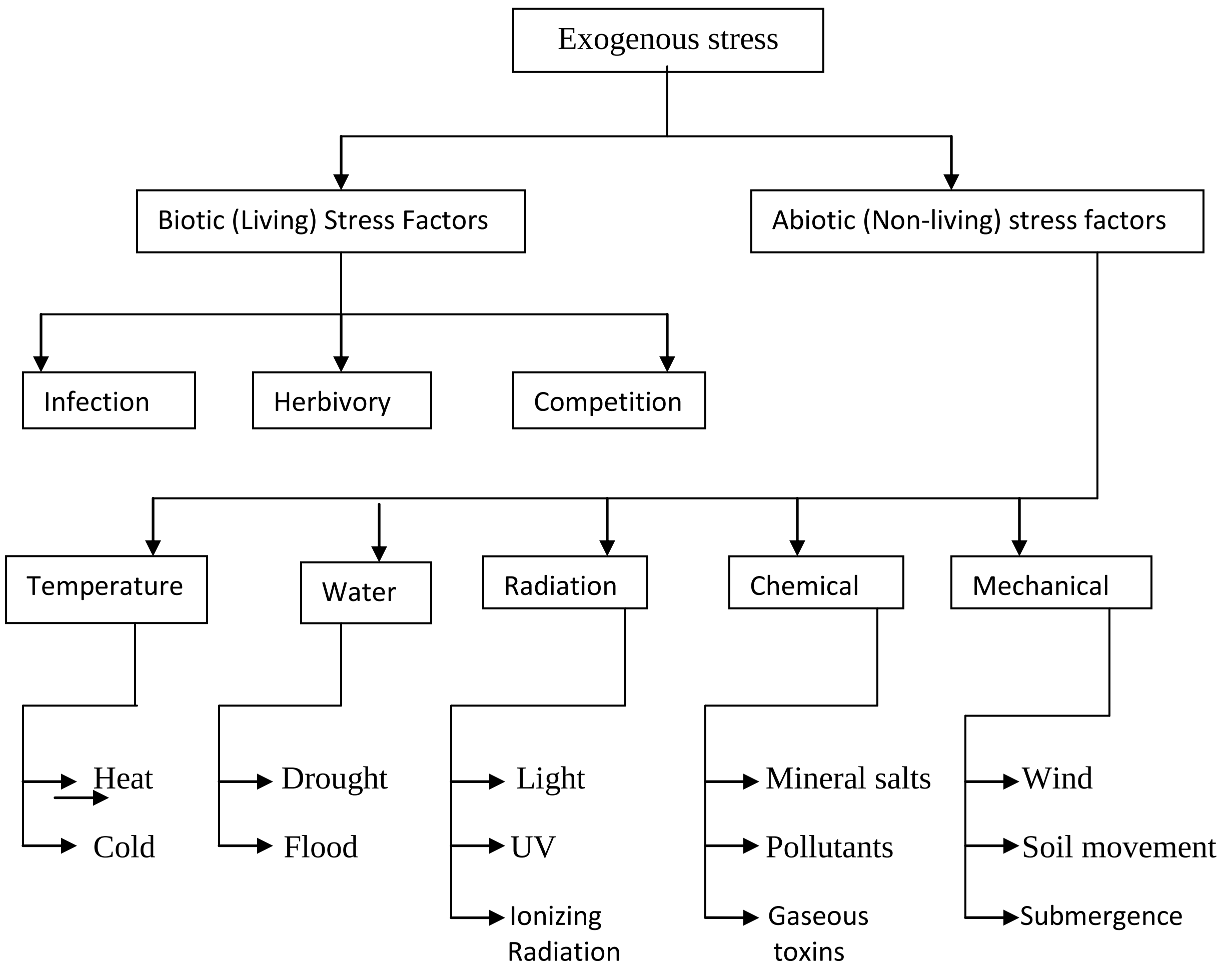

Figure 1:- Biotic and abiotic environmental factors creating stress for plants.

1.3. Basic Concept

There is a theoretical understanding of stress that is applicable to all groups of organisms and is known as the 'physical stress concept'. It posits that, a body is deformed if it is stretched by a force .i.e. stress. This 'deformation' can at first be reversible, but if the force or stress intensifies, deformation continues and the body finally breaks down [3]. However, when this concept is applied to a biological system, like plants, the following important parameters are taken into account:

- **Time factor:** In a biological system, the amount of stress is the product of the intensity of stress and duration of stress [2]. Upon a long duration (persistent) stress, plants may adapt and develop morphological and physiological changes, but if this stress continues and intensifies, the plant responds by change in gene expression (Figure 2).

For example, when temperatures are higher (greater than 30°C), O_2 consumption by plant roots, soil fauna, and microorganisms can totally deplete O_2 from the soil in as little as 24 hours [4].

- **Repair:** Plastic change is not the sure outcome of every stress. In most cases, the organism is able to repair the damage incurred, if it is not too severe.

For example, DNA repair after damage by UV radiation, premature senescence or shredding of damaged plant parts.

- **Avoidance mechanism:** Avoidance mechanism reduces the impact of a stress, though the stress is still present in the environment. For example, many arctic annuals rapidly complete their life cycle during the short arctic summer and survive through winter in the form of seeds. However, 'stress avoidance' should not be confused with 'stress tolerance'. The latter signifies the ability of the organism to endure continuous subjection to stress without adverse reaction.
- **Resistance:** The plants that have a capacity to tolerate a particular stress are considered to be stress resistant. In order to resist a particular stress, the plant needs to adjust itself to the imbalance caused and cope up with the modulation. However, the plants that lack the ability to fight stress and carry on with their normal functions are called susceptible plants.
- **Acclimation:** Acclimation is the process in which an organism adjusts to the gradual change in the environment, allowing itself to maintain performance. Acclimation usually involves the differential expression of genes associated with a particular stress. It is to be noted that, the acclimation process in stress resistant species is usually reversible upon removal of external stress. For example, some plants wilt during the daytime (i.e. when temperature and light intensity is high) to curb the rate of transpiration, and regain turgidity during the night. The process of acclimation to a stress is known as hardening and such plants are called hardy species.
- **Adaptation:** Plants use various non-permanent mechanisms to adapt themselves and respond better to their environment and flourish in the available conditions. For example, xerophytes have adapted themselves to live in an environment with little water by forming spines etc.

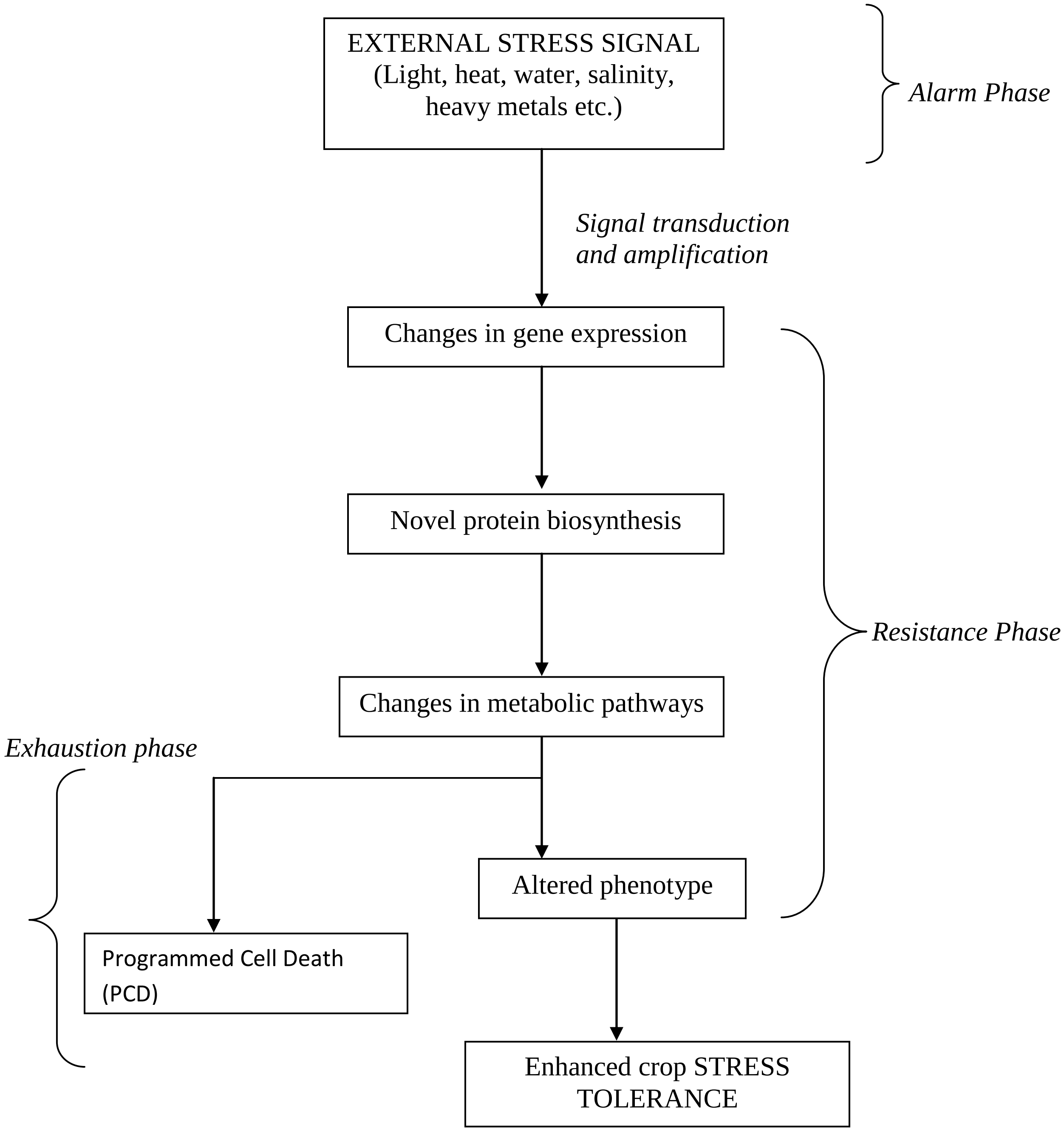

Figure 2:- An overview of stress response in plants.

2.0. High Light Stress

Exposure of plants to irradiances far above the light saturation point of photosynthesis, known as high light stress, induces various responses including light adaptation of the photosynthetic apparatus and chloroplast ultrastructure [5]. Higher energy wavelengths of electromagnetic radiations, especially in the ultraviolet range, can inhibit cell metabolic processes and damage cell membranes, denature proteins and nucleic acids.

2.1. Photoinhibition and Reactive Oxygen Species

Photoinhibition is defined as the light induced reduction in photosynthetic capacity of plants, cyanobacteria and algae, by disruption of chloroplast structure and functioning.

Excess absorption of light energy by photosynthetic pigments also produces excess electrons outpacing the availability of NADP+ to act as an electron sink at PSI [5] (Figure 3). The excess electrons produced by PSI lead to the production of reactive oxygen species (ROS). ROS include free radicals such as superoxide anion ($O_2^{\cdot -}$), hydroxyl radical ($^{\cdot}OH$**)** as well as non-radical molecules such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) and so forth.

Environmental stresses, UV-B radiations as well as pathogen attacks lead to the excess formation of ROS in plants due to disruption of cellular homeostasis (Figure 4). [6]. ROS react with amino acid chains and produce post-transitional modifications (PTMs). When the level of ROS exceeds the defense mechanism, the cell is said to be in an 'oxidative stress'. It causes oxidative damage to proteins, lipids, RNA and DNA therefore leading to programmed cell death (PCD). (Figure 5)

Researchers have found that PSII is more susceptible to damage by exposure to high light. Disruption of Oxygen Evolving Complex (OEC) is caused due to the development of low pH under high light stress. Peroxidation of thylakoid lipids was also more when chloroplasts were photoinhibited by high light intensity [2]

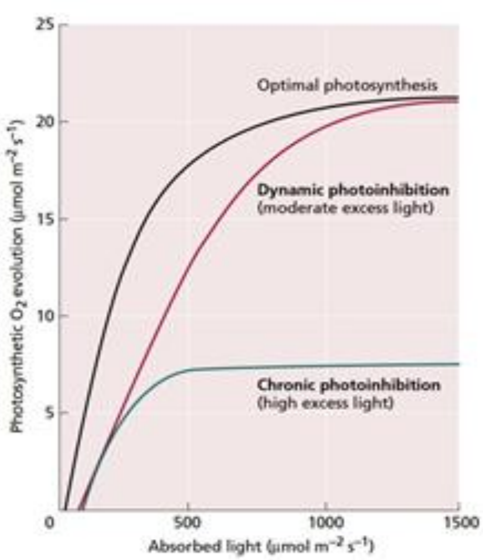

Figure 3:- Changes in the light-response curves of photosynthesis caused by photoinhibition (*source: Taiz L., Zeiger E., 2002*)

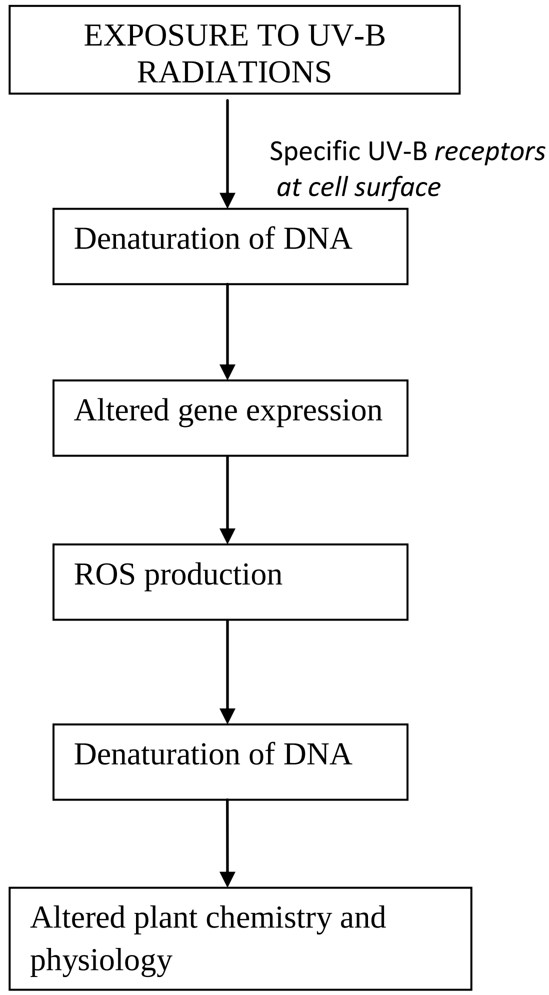

Figure 4: An overview of effect of UV-B radiation on plants

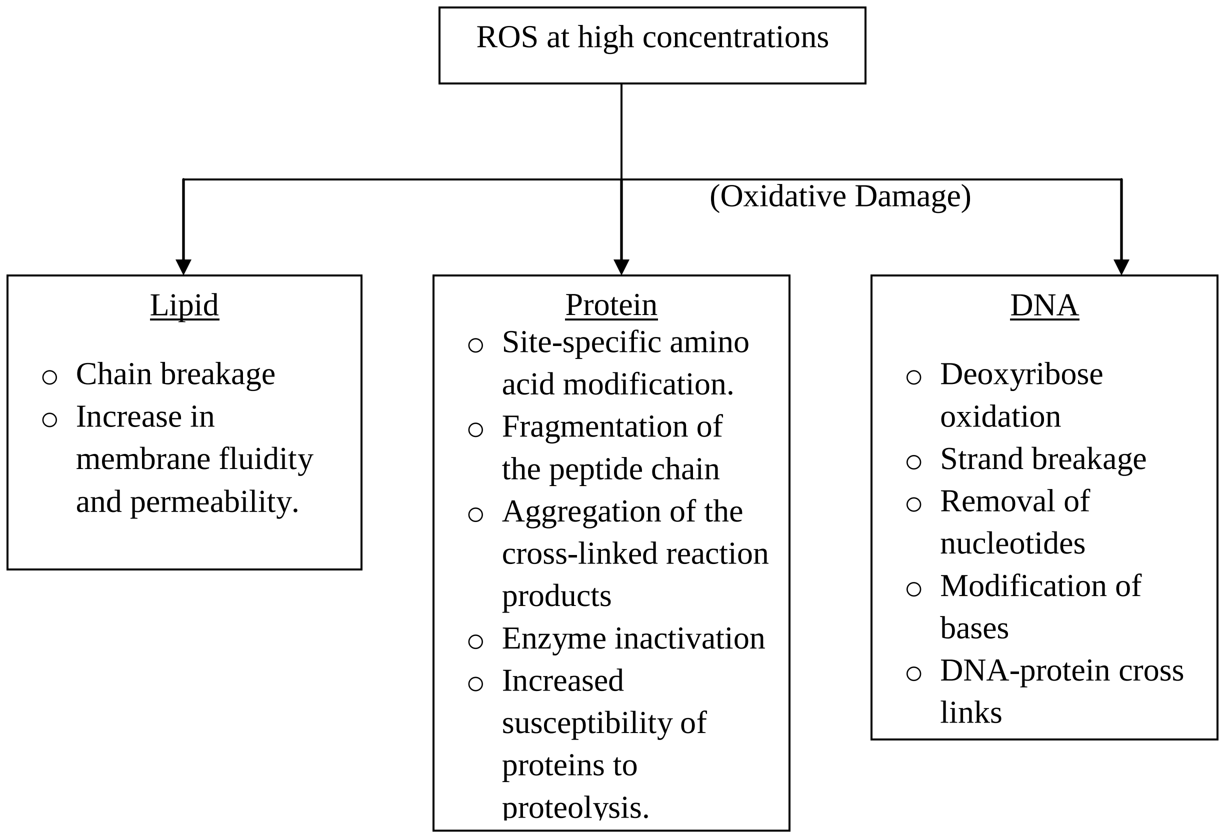

Figure 5:- Effect of increased levels of ROS in plants

2.2. Photoprotection

Photoprotection is a mechanism that helps plants to cope up with the molecular damage caused by high light stress. High light stress leads to the photo-inactivation of reaction centers of PSI and PSII which thereby causes the formation of ROS. Thus, photosynthetic organisms have developed a diverse suite of mechanisms to mitigate these potential threats which become exacerbated under high light exposure, irradiances and fluctuating light conditions.

- **Mechanical photoprotection:** Physical avoidance mechanisms include alteration of leaf blade orientation with respect to direction of light, cuticle and leaf surface waxes that function as sun-screens, formation of leaf hair etc.

- **Biochemical photoprotection:** Accumulation of screening compounds such as anthocyanins and rhodoxanthin can also be a light induced protection mechanism.

- **Antioxidative defence mechanism:** Plants possess complex antioxidative defence mechanism comprising of non-enzymatic and enzymatic components to scavenge ROS. In plant cells, specific ROS producing and scavenging systems are found in different organelles such as mitochondria, chloroplasts and peroxisomes. [6] Non-enzymatic ROS scavengers such as ascorbate (AsA), glutathione, tocopherols, carotenoids and phenolic compounds as well as enzymatic ROS scavengers such as superoxide dismutase (SOD), catalase (CAT), dehydroascorbate reductase (DHAR) etc. may be manipulated or overexpressed under strong light to protect plants from photodamage and photo-oxidative stress (Figure 6).

Rao and his coworkers suggested that UV-B exposure generates activated oxygen species by increasing NADPH-oxidase activity. Plants must adapt to the deleterious effects of UV-B radiation because they are dependent on sunlight for photosynthesis. And therefore, cannot avoid exposure to UV light. [7]. Plants then enhance their antioxidative defence mechanism and release the ROS scavengers to maintain the balance between ROS production and removal. In ***Piceai asperata*** seedlings, although enhanced UV-B (30%) increased the efficiency of antioxidant defense system consisting of UV-B absorbing compounds, carotenoids, and antioxidant enzymes such as CAT and SOD [7], it induced overproduction of ROS and oxidative stress eventually.

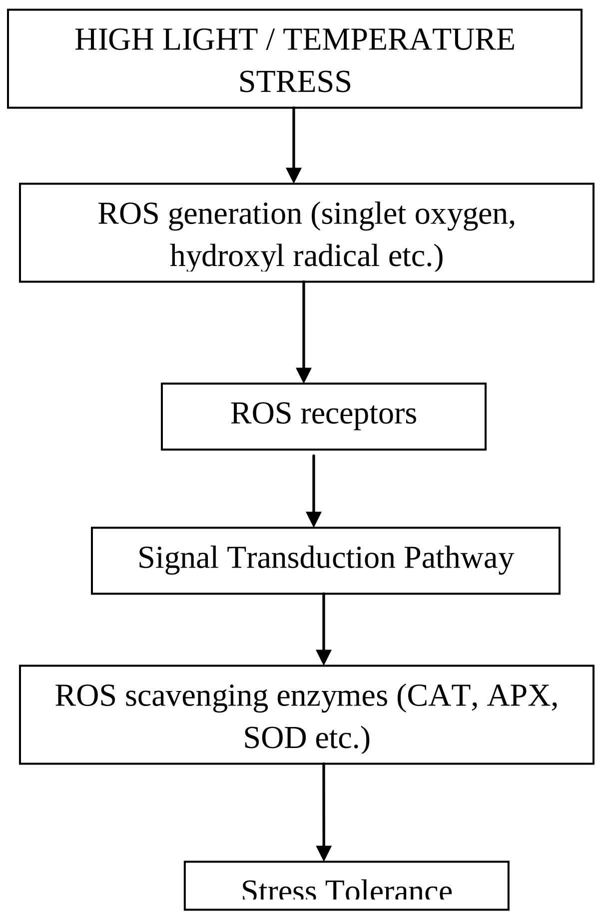

Figure 6:- An overview of ROS generation under high light stress.

- **Non-Photochemical quenching (NPQ):** Non-photochemical quenching (NPQ) is a mechanism employed by plants and algae to protect themselves from the adverse effects of high light intensity. [8] It involves the quenching of singlet excited state chlorophylls (Chl) via enhanced internal conversion to the ground state (non-radiative decay), thus harmlessly dissipating excess excitation energy as heat through molecular vibrations. NPQ occurs in almost all photosynthetic eukaryotes (algae and plants), and helps to regulate and protect photosynthesis in environments where light energy absorption exceeds the capacity for light utilization in photosynthesis.[9]

When a molecule of chlorophyll absorbs light, it is promoted from its ground state to its first singlet excited state. The excited state then has three main fates. Either the energy is:

1. Passed to another chlorophyll molecule. In this way, excitation is gradually passed to the photochemical reaction centers (PSI and PSII) where energy is used in photosynthesis, a process called photochemical quenching.
2. The excited state can return to the ground state by emitting the energy as heat, a process called non-photochemical quenching.
3. The excited state can return to the ground state by emitting a photon (fluorescence). [10] (Fig.7)

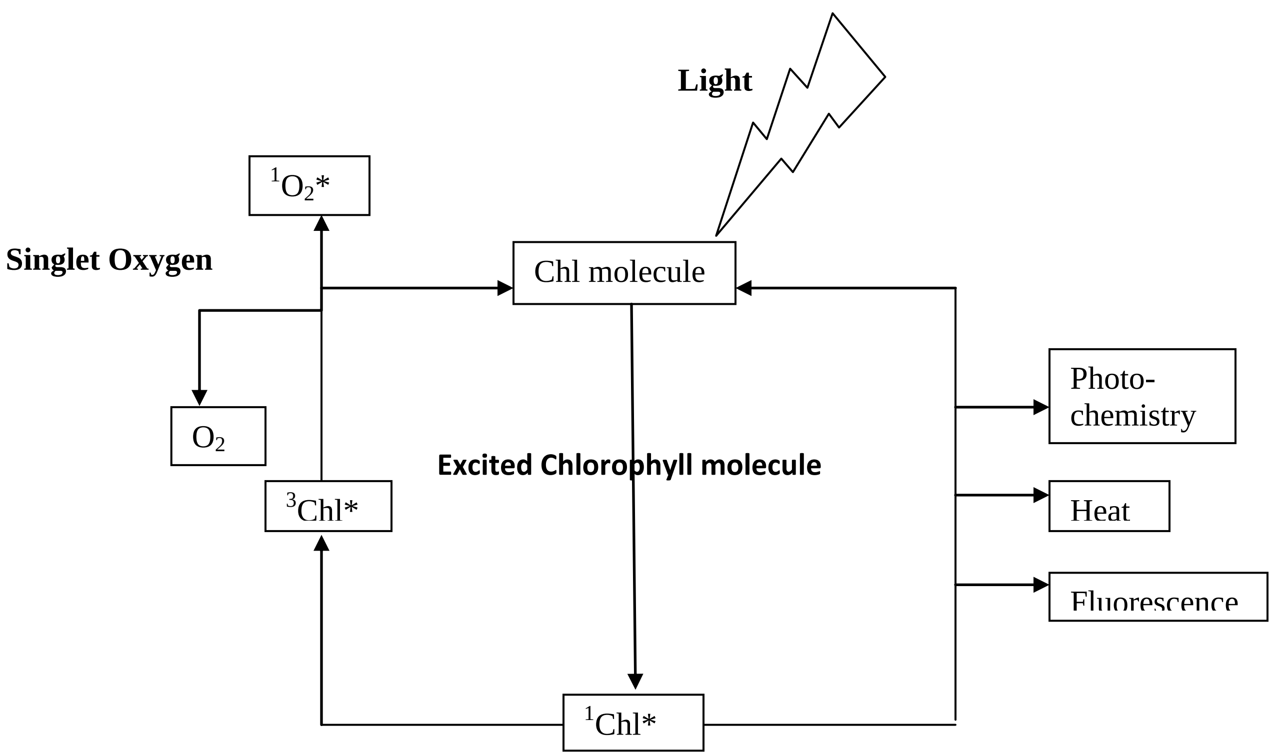

Figure 7:- D.G.M. representation of effect of high light on chlorophyll molecule and production of ROS

3.0. Temperature Stress

The constant rise in ambient environmental temperature is considered one of the most detrimental stresses. The global air temperature is predicted to rise by 0.2°C per decade, indicating 1.8-4.0°C higher temperatures than the existing levels by 2100. Plant growth, developmental processes and yield are adversely affected by such high temperature (HT) stress.

Heat stress has significant effect on protein metabolism, including degradation of proteins, inhibition of protein accumulation and induction of certain protein synthesis. One of the major consequences of HT stress is the excess formation of ROS which leads to oxidative stress. Plants alter their metabolism in various ways in response to HT, particularly by producing compatible solutes that are able to organize proteins and cellular structures, maintain cell turgor by osmotic adjustment and modify the anti-oxidant system to re-establish homeostasis.

Development of new HT tolerant crop cultivars is a major challenge for plant scientists. Plants show dynamic responses to HT. Scientists are trying to investigate how plants can be managed in HT environments. That is being achieved through the developments of transgenic plants by manipulation of target genes.

3.1. Plant Responses

The ability of plants to respond to this stress by maintaining their protein conformation and preventing the accumulation of non-native protein is highly important for the cell survival. At extreme HT, cellular damage or cell death may occur within minutes. Heat stress affects all the major plant processes like germination, growth, metabolic pathways, reproduction and yield. It also alters the stability of various proteins, membranes and RNAs.

- **Growth:** The rate of vegetative growth decreases when the rate of photosynthesis is reduced by heat stress. First of all the seedlings are affected- reduced germination percentage, plant emergence, abnormal seedlings with less vigor, reduced radical and plumule growth of seedlings are major impacts caused by heat stress.[11-13] HT also causes loss of cell water content ultimately reducing growth.[14, 15]. The morphological symptoms of heat stress such as scorching and sunburns of leaves and twigs, leaf senescence and abscission, fruit discolouration and damage, necrosis, damage to leaf tip and margins indirectly affect the plant's growth and development.[14,16]

High temperatures may alter the total phenological duration by reducing the life period. Increases in temperatures 1–2 °C than the optimum result in shorter grain filling periods and negatively affect yield components of cereal. [17,18]

At extreme heat stress plants can show programmed cell death in specific cells or tissues may occur within minutes or even seconds due to denaturation or aggregation of proteins, on the other hand moderately HTs for extended period cause gradual death; both types of injuries or death can lead to the shedding of leaves, abortion of flower and fruit, or even death of the entire plant. [14,19]. (Table 1)

Crops	**Heat Treatment**	**Growth stage**	**Major effects**
Chili pepper (*Capsicum annuum*)	38/30 °C (day/night)	Reproductive, maturity and harvesting stage	Reduced fruit width and fruit weight, increased the proportion of abnormal seeds per fruit.
Rice (*Oryza sativa*)	Above 33 °C, 10 days	Heading stage	Reduced the rates of pollen and spikelet fertility.
Wheat (*Triticum aestivum*)	37/28 °C (day/night), 20 days	Grain filling and maturity stage	Shortened duration of grain filling and maturity, decreases in kernel weight and yield.
Sorghum (*Hordeum vulgare*)	40/30 °C (day/night)	65 DAS to maturity stage	Decreased chlorophyll (chl) content, chl *a* fluorescence, decreased photosystem II (PSII) photochemistry, Pn and antioxidant enzyme activity and increased ROS content, and thylakoid membrane damage, reduced yield.
Maize (*Zea mays*)	35/27 °C (day/night), 14 days	Reproductive stage	Reduced ear expansion, particularly suppression of cob extensibility by impairing hemicellulose and cellulose synthesis through reduction of photosynthate supply.
Soybean (*Glycine max*)	38/28 °C (day/night), 14 days	Flowering stage	Decreased the leaf Pn and stomatal conductance (*gs*), increased thicknesses of the palisade and spongy layers, damaged plasma membrane, chloroplast membrane, and thylakoid membranes, distorted mitochondrial membranes, cristae and matrix.
Tobacco (*Nicotiana tabacum*)	43 °C, 2 h	Early growth	Decrease in net photosynthetic rate (Pn),

		stage	stomatal conductance as well as the apparent quantum yield (AQY) and carboxylation efficiency (CE) of photosynthesis. Reduced the activities of antioxidant enzymes.
Okra (*Abelmoschus esculentus*)	32 and 34 °C	Throughout the growing period	Reduced yield, damages in pod quality parameters such as fibre content and break down of the Ca-pectate.

Table 1:- Effect of high temperature stress in different crop species [20].

- **Photosynthesis:** Photosynthesis and respiration are both inhibited under temperature stress. Photosynthesis, in particular, is highly heat sensitive especially for C_3 plants than C_4. [21]. With increasing temperatures, alterations in chloroplast with respect to morphology i.e. loss of grana stacking and swelling of grana[14, 15]; and photosynthetic pigments are hampered. Again, the PSII activity is greatly reduced or even stopped under HT [22]. Further, stress induced closure of stomata and reduced leaf surface area contribute to impaired photosynthesis [15]. Chloroplast enzymes such as rubisco, rubisco activase, NADP-G3P dehydrogenase, and PEP carboxylase become unstable at high temperatures which means that heat imposes reduced leaf water potential and has a total negative impact on photosynthesis. [23] Ultimately, plant starvation is observed.

- **Reproduction:** The reproductive tissues are the most sensitive to HT. Slight elevation in temperature during flowering can result in the loss of entire grain crop cycles [24]. Heat spells of short duration may initiate flower formation but fruits or seed production is inhibited. The sterility increases due to impaired meiosis in both male and female organs. High temperatures reduce ovule viability, produce anomaly in stigmatic and style production, reduce the number of pollen grains retained by stigma; thereby disturbing the growth and development of the endosperm, pro-embryo and unfertilized embryo. [25] High temperature causes excessive ethylene production. [26].

- **Yield:** Continuous increase in global temperature is raising anxiety regarding crop productivity and food security. As we have seen, a small increase in temperature can have a significant negative impact on crops in every respect. HT affects crop productivity through affecting phonological developmental processes.

Even though cotton originates from warm regions, cotton yields respond negatively to high temperature during the flowering period [27] HT stress not only decreases grain length and grain weight in rice and wheat [26] but also reduces photosynthetic performance and crop quality of barley [28] and reduces fibre content in okra [20].

- **Oxidative stress:** Under temperature stress, ROS production is enhanced in several ways. Inhibition of CO_2 assimilation, coupled with the changes in photosystem activities and reduced photosynthetic transport capacity under HT stress results in accelerated production of ROS via the chloroplast Mehler reaction [29]. The reaction centers of PSI and PSII in chloroplasts are the major sites of ROS production though it is also produced in other organelles viz. peroxisomes and mitochondria. Thermal damage to photosystems causes less absorption of photon. [30]

Among the ROS, $O_2{\cdot}^-$ is dominant and is formed by photo-oxidation reactions. It initiates chain reactions leading to the production of more toxic radical species, which may cause damage far in excess of the initial reaction products. Rice seedlings subjected to HT stress showed increased concentration of $O_2{\cdot}^-$ increased level of lipid peroxidation, chlorophyll bleaching, loss of some antioxidants (AsA, GSH, tocopherols and carotenoids) and thiols. [16]. Singlet oxygen can directly oxidize protein, poly-unsaturated fatty acids, and DNA. [31,32]. Further worse, heat stressed leaf surfaces failed to enhance their antioxidant enzyme activities which caused the accumulation of malondialdehyde (MDA) content in leaves of wheat plant [33].

Under temperature stress one of the real threats to chloroplast is the production of hydroxyl radicals. Hydroxyl radicals are not scavenged and can potentially react with biomolecules like-pigments, proteins, lipids and DNA. Continual heat stress causes ROS accumulation at the plasma membrane outer surface which can cause membrane depolarization. In extreme cases, ROS accumulation in cells can trigger programmed cell death (PCD). [34]

3.2. Avoidance Mechanism

In HT stress, plants cope up with the lethal situation through various mechanisms for survival including long-term phonological and morphological adaptations and short-term avoidance or acclimation mechanisms. The common heat induced avoidance mechanisms are as follows: (Figure 8)

1. *Morphological and anatomical adaptations:* Partial stomatal closure to reduce water loss, increased trichomatous densities and larger xylem vessels. Changing leaf orientations- where the leaf blade often turns parallel to the solar radiations (paraheliotropism), formation of cuticle and other waxy surfaces, reduction in leaf surface area and rolling of leaf blade reduce the solar radiation taken in by the plant from the surface [27].
2. *Crop management practices:* High temperature stress can also be avoided by crop management practices such as selecting proper sowing methods, choice of sowing date, cultivars, irrigation methods, *etc*. For instance, in subtropical zones, cool-season annuals such as lettuce when sown in the late summer may show incomplete germination and emergence due to high soil temperature [35]. Seed priming is another potential solution to this problem which involves placing the seed in an osmotic solution for several days at moderate temperatures and then drying them. In some cases, HT and intense direct solar radiation can cause damage to fruit. This can be avoided if fruit is shaded by foliage. [35]

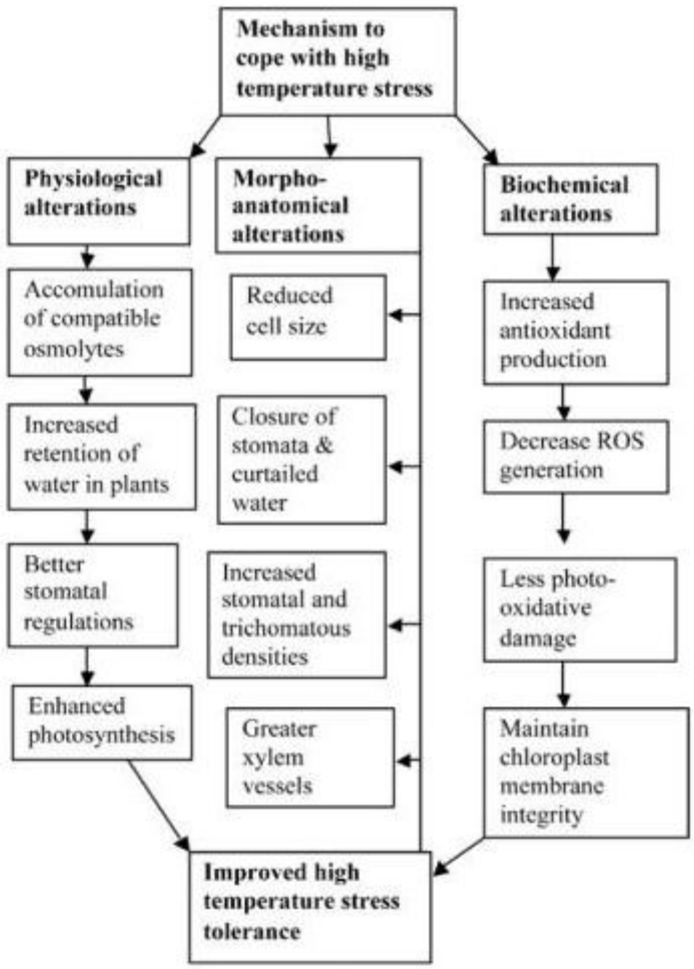

Figure 8:- Schematic representation of morphological, physiological and biochemical alterations of plants to cope up with high temperature stress.

3.3. Tolerance to Temperature Stress

Heat tolerance is generally defined as the ability of plants to grow and produce economic yield under high temperature stress. This is a highly specific trait which can vary among closely related species and even differ from organ to organ of the same plant. Over the years, with increasing global temperatures, plants have evolved various mechanisms to thrive under the stressful prevailing temperature; which can be either short term (avoidance) or long term (evolutionary adaptations). Some major tolerance mechanisms, including ion transporters, late embryogenesis abundant (LEA) proteins, osmoprotectants, antioxidant defense, and factors involved in signaling cascades and transcriptional control are essentially significant to counteract the stress effects. [14,36]. In case of sudden heat stress, short term mechanisms such as alterations in leaf orientation, leaf size and shape etc. can be an alternative to thrive through the heat stress. Early maturation in summer can give smaller yield but is a way to escape the sudden heat spell. The stress responsive mechanism is established by an initial stress signal that may be in the form of ionic and osmotic effect or changes in membrane fluidity. This helps to re-establish homeostasis and to protect and repair damaged proteins and membranes.

3.4. Antioxidant Defence System of Plants

Plants can protect themselves from heat induced oxidative stress for their survival under HT. Tolerant plants entail a tendency of protection against the damaging effects of ROS with the synthesis of various enzymatic and non-enzymatic ROS scavenging and detoxification systems [37]. The enzymatic antioxidants like superoxide dismutase, peroxidases, catalases and thioredoxin show an initial decrease in activity before declining at 50°C. Total antioxidant activity is seen to be maximum at 30-40°C in tolerant species and at 30°C in susceptible ones. [38].

Heat acclimated plants produce lower levels of ROS due to enhanced synthesis of ascorbate (AsA) and glutathione (GSH). The imposed stress is sensed by the system and through some signaling molecules they cause an increase in the antioxidant capacity of the cells to 'scavenge' the ROS.

3.4.1. Signal Transduction

Up regulation of many genes has been reported to help the plant deal with high temperature stress and adapt to the changing environment. Upon stress plants perceive the external and internal signals through different independent or interlinked pathways which are used to regulate various responses for its tolerance development [39]. (Figure 9). Plant responses to stress, governed by signal transduction, are complex integrated circuits. To generate a response in specific cellular compartments or tissues against a certain stimuli, interaction of cofactors and signaling molecules are required. Signaling molecules are involved in activation of stress responsive genes and there are various signal transduction molecules involved in stress responsive gene activation depending upon the plant type and stress incurred [40]. Some broad groups of these are: Ca dependent protein kinases (CDPKs), mitogen activated protein kinase (MAPKs), NO, and phytohormones. These molecules together with transcriptional factors activate stress responsive genes. [42] Once the stress responsive genes activate, these help to detoxify the ROS (by activating detoxifying enzymes, free radical scavengers); reactivate the essential enzymes and structural proteins [26] and all the above stated processes help to maintain the cellular homeostasis (Figure 9). This can be said as a typical model through which heat resistance or tolerance is developed within the plant.

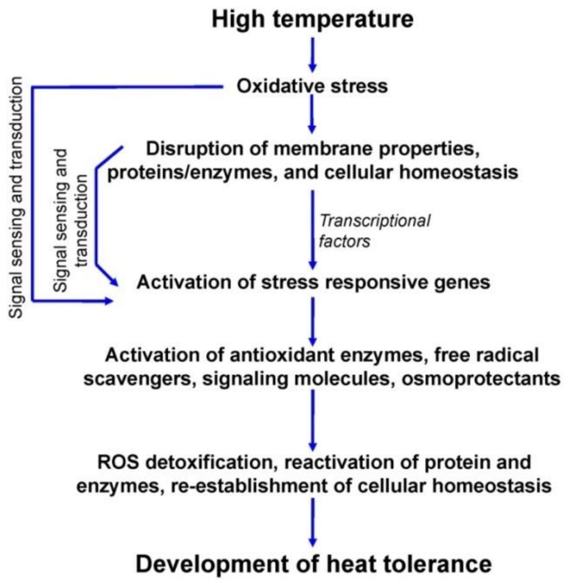

Figure 9:- Schematic illustration of heat induced signal transduction mechanism and development of heat tolerance in plants.

3.4.2. Heat Shock Proteins (HSPs)

Definition: Heat shock proteins (HSPs) or stress proteins are highly conserved and present in all plants and animals [41]. These proteins are very much needed for the survival of organisms under lethal HT, and are encoded by the up-regulation of certain genes called "heat shock genes" (HSGs). Some HSPs also referred to as molecular chaperones, play an important role in protein stabilization such as assembling of multi-protein complexes, folding or unfolding of proteins, transport or sorting of proteins into correct departments at sub-cellular level, control of cell cycle and signaling as well as cell protection against stress or apoptosis [41]. The HSPs are extremely heterogeneous in nature and have been widely studied in the fruit fly, *Drosophila melanogaster* where the idea was first identified.

Diversity: Several heat shock proteins have been described in eukaryotes, including plants. They are designated by their approximate molecular weight in kDa as HSP110, HSP90, HSP70, HSP60 and low molecular weight HSPs are 15-30kDa [43]. (Table 2). There is a difference in the number of HSPs between organisms and even within an organism among the different cell types. To whatever degree, all organisms show the expression of HSPsthat belong to the family HSP70 and the molecular weight of this family falls between 98-78 kDa [44, 45]. This family of HSPs has a strong correlation with resistance to heat, either permanent or transient [42,46]. The response of many organisms to elevated temperatures has been described as "heat shock response (HSR)" and is primarily studied in soya bean, maize and cotton [45].

HSP Class	Size (kDa)	Probable Function	Cellular Location
HSP 100	100-114	Not known	Cytosol, mitochondria and chloroplasts
HSP 90	80-94	Protection of protein receptors	Cytosol, ER
HSP 70	69-71	As molecular chaperone, preventing proteins from denaturation or aggregation	Cytosol, mitochondria, chloroplasts and nucleus.
HSP 60	57-60	As molecular chaperone, directing the protein assembly of multi- sub. unit proteins.	Mitochondria and chloroplasts
Low mol. Wt. HSPs	15-30	Function largely unknown.	Cytosol, mitochondria, chloroplasts and ER.

Table 2: Types of HSPs and their probable function.

Induction of HSPs: Heat shock- the stressor that has been studied greatly, together with a wide range of metabolic stressors is known to cause the synthesis of heat responsive proteins. The HSR causes the enhanced expression of heat stress genes and multi-gene families encoding molecular chaperones [47]. However, HSP expression is reduced at 47°C and ceased at 49°C due to the inability of cells to survive at such high temperature [48].

The expression of HSPs is restricted to certain developmental stages of the plant like seed germination, embryogenesis, microsporogenesis and fruit maturation. HSP70 is an ideal candidate for cellular signaling system which can sense as well as recognize conformations of abnormally elevated levels of non-native proteins and conveying this information to the HSFs. When a cell is stressed it accumulates abnormal amounts of non-native proteins. The proteins compete with HSF for binding of HSP70. The flux of non-native proteins leads to phosphorylation and timerisation of HSF. The trimers translocate to the nucleus and bind to the promoter region of HSP genes and mediate HSP gene transcription, followed by translation. HSP70 gene adheres to the surface of non-native proteins and thus prevents them from denaturation.

If the stresses are removed, HSPs continue to form and non-native proteins either revert to their native conformation or may be degraded by proteolytic enzymes. In due course, the available HSP70s present in cellular system bind with HSF to reform HSF-HSP70 heterodimers.

Physiological function: HSPs may bind with proteins that are sensitive to heat in a cell under heat shock to prevent them from degradation as well as preventing any havoc following precipitation and permanently influencing the viability of the cells [49, 50]. We know that the function of any protein is determined by its formation and folding into three dimensional structures that requires 50% of principle amino acid sequence [51]. Hence, the role of HSPs in the folding of proteins is important. [47] Indicated that HSPs protect cells from injury and facilitate recovery and survival after a return to normal growth condition. It has been observed that upon heat stress, the role of HSPs as "molecular chaperones" is without doubt [52-54]. They prevent the irreversible accumulation of other proteins and are involved in the refolding of proteins under stressful conditions [55]. (Figure 10)

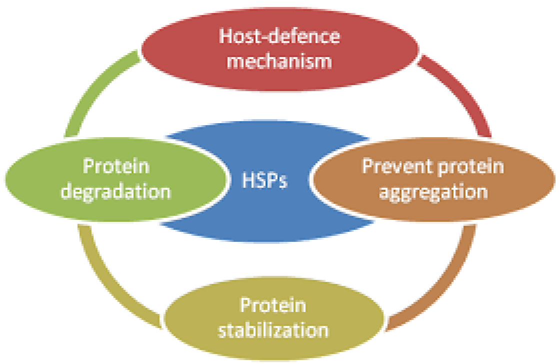

Figure 10:- Function of HSPs under stress

4.0. Water Stress in Plants

Water deficit is one of the major abiotic stresses which adversely affects crop growth and yield and plants are often subjected to periods of soil and atmospheric water deficit during their life cycle. The frequency of this phenomenon is increasing today, with increase in water scarcity. Plants are put to water stress either when the water supply to the roots becomes limiting or when the transpiration rate becomes high [2]. Thus, water stress is primarily caused due to drought or high soil salinity. Even in the conditions like flooding (where water is very much available) and low soil temperature, in spite of water being present in the soil; plants cannot uptake it- a situation commonly known as "physiological drought".

Plant responses to water scarcity are complex, involving deleterious and/or adaptive changes. Since the beginning of agriculture, mild to severe drought has been one of the major factors limiting production. With the increasing mouths to feed, the ability of plants to withstand such stress is of great economic importance. Therefore, the biochemical and physiological mechanisms associated with water stress tolerance and water-use efficiency has been extensively studied.

4.1. Plant Responses

Water in excess or in deficit poses a stress to the plant and affects it at various levels of organization. In fact, under prolonged drought plants may dehydrate and die. Drought is the meteorological term for a period of insufficient precipitation that results in plant water deficit. This water stress in plants reduces the plant cell's water potential and turgor, which elevate the solute's concentrations in the cytosol and extracellular matrices. As a result, the cells become flaccid leading to inhibition of growth and reproductive failure [56]. Although plant growth rates are generally reduced when soil water supply is limited, shoot growth is often more inhibited than root growth; and in some cases, the absolute root biomass of plants in drying soil may increase the water use efficiency (WUE) [57]. (Figure 11)

This is followed by the accumulation of abscisic acid (ABA) and compatible osmolytes like proline, which cause wilting [56]. When soil is water-saturated, the water potential (Ψw) of the soil solution may approach zero, but drying can reduce the soil Ψw to below -1.5 MPa, the point at which permanent wilting can occur. By altering the chemical and physical appearance of tissues, water deficits also modify various aspects of plant quality, such as- the taste of fruits and density of wood [58]. At this stage, over production of reactive oxygen species (ROS) and formation of free radical scavenging compounds such ascorbate and glutathione further aggravate the adverse influence [56].

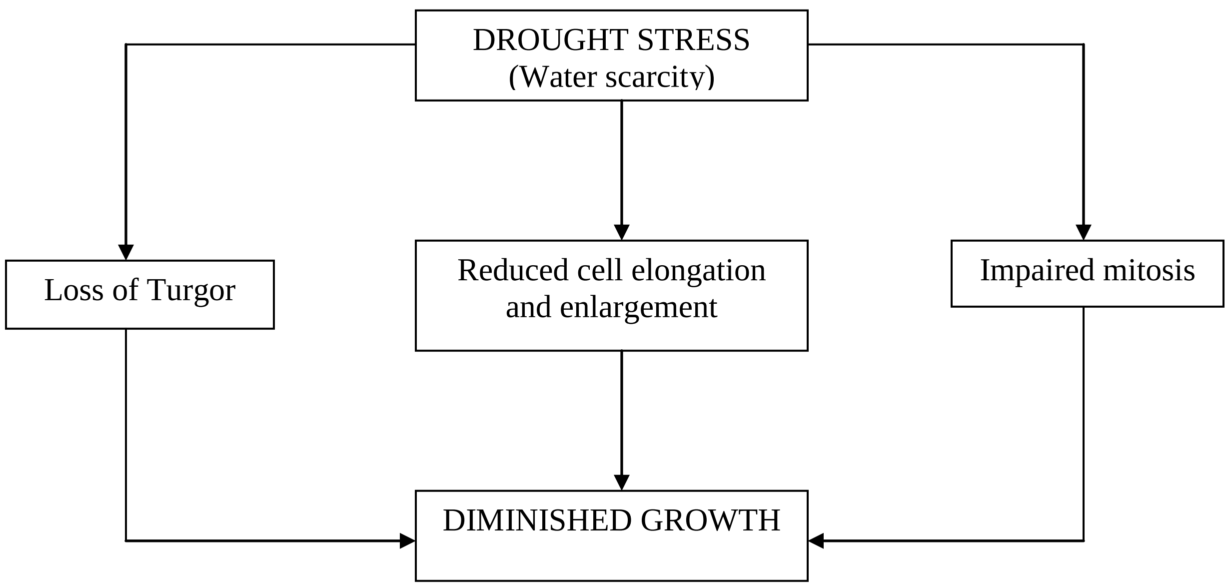

Figure 11:- Effect of drought stress on vegetative growth of plant.

Photosynthesis:

Since water stress alters the morphological features of the plant, by decreasing leaf area; it directly reduces the rate of photosynthesis per unit leaf area (Figure 12). Photosynthesis by crops is severely inhibited and may cease altogether as water deficits increase. The decrease in leaf growth or an increase in leaf senescence under drought conditions may also inhibit photosynthesis in the existing leaves [59].

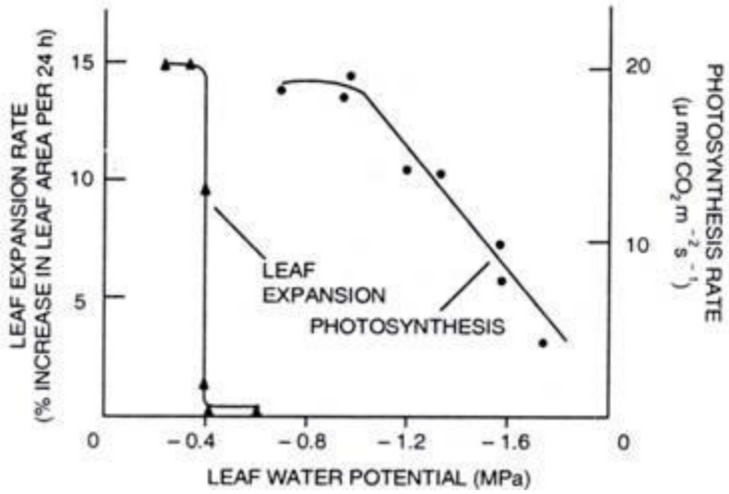

Figure 12:- Effects of water stress on leaf expansion and rate of photosynthesis in sunflower plant. Leaf expansion is more sensitive to water stress than rate of photosynthesis (After Boyer, 1970).

Decreasing water content is accompanied by loss of turgor and wilting, cessation of cell enlargement, closure of stomata- thereby reduction in photosynthesis, and interference with many other basic metabolic processes [60]. Thus, low CO_2 uptake due to partial stomatal closure and resistance leads to poor assimilation rates in photosynthetic leaves (Figure 13). Further, the drought stress produced changes the chlorophyll 'a' and 'b' and carotenoid ratio [61]. Additionally, reduced photosynthetic metabolites and enzyme activity lead to low carboxylation efficiency and inhibition of chloroplast activity at low water potential [58].

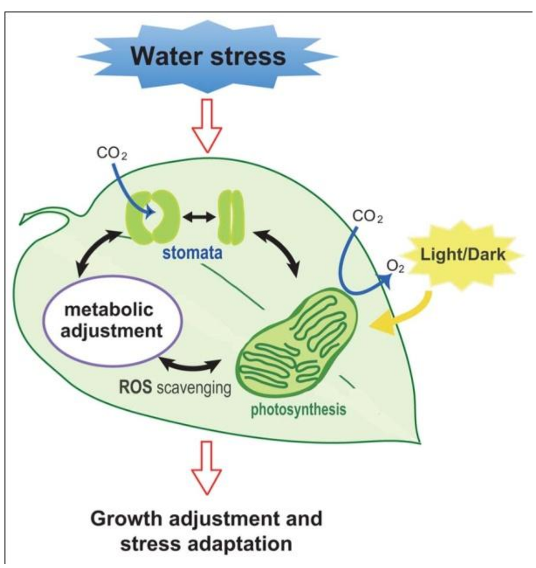

Figure 13:- Effect of water stress on photosynthesis.

Again, the damage of the photosynthetic apparatus through the production of ROS such as superoxide and hydroxyl radicals during drought is considerate. Low chloroplastic ratio CO_2/O_2 also favours photorespiration leading to increased production of ROS such as H_2O_2 [62]. ROS disrupt normal metabolism through lipid peroxidation, denaturing proteins and nucleic acids in several plant species [62]. However, unlike chlorophyll, increase in xanthophylls pigments such as zeaxanthin and antheraxanthin in plants under water stress have been reported; that play a protective role on plants under stress.

Under the conditions of water stress, a rapid decrease in the amount of Rubisco takes place in most plants which in turn leads to lower enzymatic activity [58]. Moreover, drought stress conditions acidify the chloroplast stroma causing inhibition of Rubisco activity. This leads to reduction in the activity of ATP synthase and degeneration of plastids. Both the photosystems are affected by water deficiency.

Protein synthesis: Drought conditions bring about qualitative and quantitative changes in plant proteins. Quite a number of chloroplast proteins and photosynthesis proteins respond to different abiotic stresses. The main proteins that synthesize in response to water stress are LEA, desiccation stress protein, proteins that respond to ABA, dehydrins, cold regulation proteins, and detoxification enzymes (SOD, CAT etc) [63]. These proteins are linked to hydrophobic interactions needed for macromolecular stabilization.

Drought stress is a major factor influencing plant chloroplast. The chloroplast protein response to drought stress is dependent on the genotype of the plant. Changes in the chloroplast proteome of tall fescue (*Festuca arundinacea)* with different levels of drought stress tolerance have been studied [64]. All the identified proteins were involved either directly in photosynthetic reactions (including ATP synthase and oxygen envolving enhancer protein) or in the protection of photosynthetic apparatus against different components of drought stress.

Lipids: Cell membranes are a major target of environmental stresses. Along with proteins, lipids are important membrane components and changes in their composition may help to maintain membrane integrity and preserve cell compartmentation under water stress conditions [65]. Strong water deficit leads to a disturbance of the association between membrane lipids and proteins as well as to a decrease in the enzyme activity and transport capacity of the bilayer.

Drought stress provoked considerable changes in the lipid metabolism in *Brasica napris* [66]. The decline in leaf polar lipid was mainly due to a decrease in MGDG (monogalactosyldiacylglycerol) content. It was suggested that the pathway leading to MGDG synthesis was strongly affected by water stress.

Drought also results in the variation of fatty acid composition, for example, an increase in fatty acids having less than 16 carbons in chloroplasts. Lipid peroxidation is also a well known effect of drought.

ABA accumulation: The phytohormone- abscisic acid (ABA) plays a regulatory role in many physiological processes in plants. Different stress conditions of drought, temperature, and light result in increased amount of ABA. Thus, it is also referred to as a “stress hormone”. It regulates several aspects of plant growth and development. Recent studies have demonstrated a pivotal role for ABA in modulation at the gene level of adaptive responses for plants in adverse environmental conditions [67]. ABA is also involved in several other physiological processes such as stomatal closure (Figure 14), embryo morphogenesis, development of seeds, and synthesis of storage proteins and lipids, germination, leaf senescence and defense against pathogens [68-71].

During vegetative growth the roots of many angiosperms synthesize ABA and transport it into the shoots under water stress. ABA is an essential mediator in triggering plant responses to adverse environmental stimuli [70]. This is known to occur in a number of crop plants such as rice, barley, soyabean, tomato, cotton and alfalfa. Substantial evidence suggests that increased ABA levels limit water loss by reducing stomatal aperture [72]. The amount of ABA in xylem sap increases substantially under water scarcity in the soil.

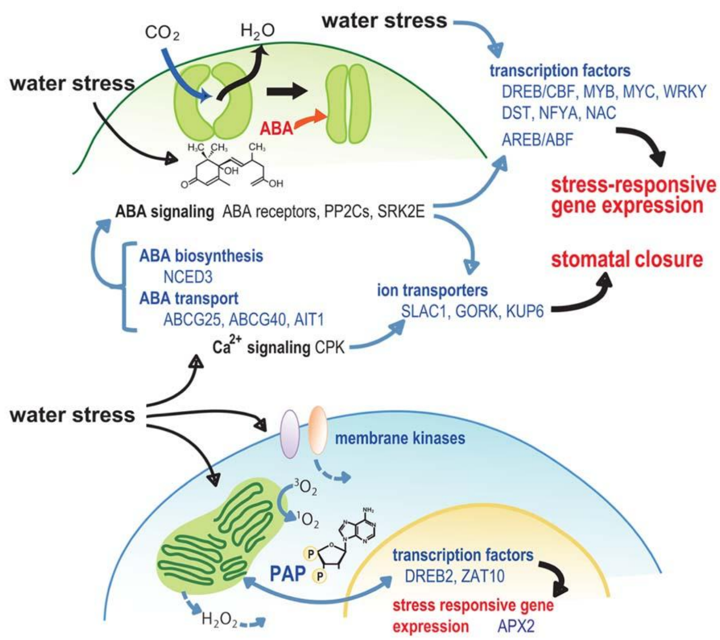

Figure 14:- Plant responses to water stress

Mineral nutrition: Environmental factors such as drought may cause nutrient deficiencies, even in fertilized fields, as the physiochemical properties of the soil can lead to reduced mobility and absorbance of individual nutrients [73]. Mineral nutrients are essential chemical elements for plant growth and reproduction, acquired primarily in the form of inorganic ions from the soil [74]. Apart from K and Ca, all macronutrients are integrated into important organic compounds such as amino acids and proteins (N and S), nucleic acids (N and P), phospholipids (P) and chlorophyll (Mg) [73].

However, most mineral nutrients are dependent on soil moisture to move through soil matrix and be taken up by plants [74]. Under conditions of water stress, roots are unable to take up many nutrients from the soil due to a lack of root activity as well as slow ion diffusion and water movement rates [75]. Moreover, the mineralization process depends on micro-organisms and enzyme activity, which may be affected by drought.

Drought also causes stomatal closure that reduces transpiration. Thus, nutrient transport from the roots to the shoot is limited by the decrease in transpiration rate, imbalance in active transport and membrane permeability resulting in a reduced absorption power in roots. Thus, drought stress causes low nutrient availability in the soil and low nutrient transport in plants [76].

Oxidative stress: As discussed earlier, oxidative stress is accompanied with many abiotic stresses like high temperature, salinity or water stress and causes serious adverse effects on plants. Under environmental stresses such as drought, a rapid ROS accumulation including singlet oxygen, superoxide, hydroxyl radical and hydrogen peroxide may occur leading to negative impact on antioxidant metabolism and consequently cell peroxidation damage [77-79]. They damage membranes and macromolecules, affect cellular metabolism and play a crucial role in causing cellular damage under drought stress.

4.2. Avoidance Mechanism

Drought is a highly complex issue to tackle. Irrigation is only method that provides a complete solution to the problem of drought. However, the adverse effects of drought can be avoided by morphological changes in plants, such as reduced stomatal conductance, decreased leaf area, development of extensive root systems and increased root/shoot ratio.

Many desert annuals escape drought by completing their life cycle before severe water stress develops. Their seeds remain dormant during the dry season. The seeds germinate, grow and flower within a few weeks after rain has considerably wet the soil.

Some plants such as palm, growing at an oasis, develop roots that grow deep down to the water table or mesquite tree (*Prosopis glandulosa*) and alfalfa (*Medicago sativa*) that have roots which extend up to 7 to 10 metres down to the water table, aggressively consume water and avoid drought. Such plants are known as water spenders and in-fact they never face extremely negative water potentials (or water deficits). A combination of potentially deep rooting species and soil conditions favourable for deep rooting is advantageous for water spenders to avoid drought.

Under moderate water stress, some succulent plants such as cacti, century plant (*Agave americana*) and other CAM plants resist drought by storing water in their succulent tissues. Such plants which use water conservatively are known as water collectors. Because of thick cuticle and stomatal closure during the day, the loss of water is lesser in such plants so that they can survive longer dry periods. Some succulent plants which are subjected to periodic drought are known to exhibit facultative CAM. Under water stress conditions, they show CAM, but they switch over to $C\text{-}_3$ mode of photosynthesis when enough water is available.

As a result of increasing water stress in many xerophytes, certain organic compounds such as amino acid pro-line and sugar alcohol sorbitol etc., accumulate in the cytoplasm of cells. These substances (compatible solutes) lower the osmotic potential and also the water potential of cells without damaging enzyme functions. This drop in osmotic potential which helps in maintaining plant water balance has been called as osmotic adjustment or osmoregulation by Morgan (1984).

4.3. Tolerance to Water Stress

Drought tolerance is a plant's ability to maintain its normal functions even at low tissue water potential. Drought tolerance is achieved by cell and tissue specific physiological, biochemical and molecular mechanisms which include specific gene expression and the accumulation of specific proteins.

One of the most common stress tolerance strategies in plants is the overproduction of different types of compatible organic solutes [80]. Compatible solutes are low molecular weight, highly soluble compounds that are usually non-toxic, even at high concentrations. Generally, they protect plants from stress through different means such as contribution towards osmotic adjustment, detoxification of ROS, stabilization of membranes, and protection of enzyme activity and nature of proteins. Osmotic adjustment is a mechanism to maintain water relations under stress. It involves the accumulation of a large number of osmotically active molecules/ions including sugars, proline, organic acids, calcium, potassium, and chloride ions. Under water deficit and as a result of solute accumulation, the osmotic potential of the cell is lowered, which attracts water into the cell and helps with the maintenance of turgor. By means of osmotic adjustment, the organelles and cytoplasmic activities take place at about a normal pace and help plants to perform better in terms of growth, photosynthesis and assimilate partitioning to grain filling [81, 82].

Under conditions of plant cellular water deficit, changes in gene regulation take place. Various genes are induced in response to drought at the transcriptional level, and these gene products are thought to function in tolerance to drought [82]. Chemical signals, e.g., reactive oxygen species, calcium and plant hormones are involved in inducing stress tolerance by acting via transduction cascades and activate genomic re-programing. Calcium has been established as a ubiquitous intracellular second messenger in plants. . More recently, it is reported that calcium can improve water stress tolerance in *Zoysia japonica* by reducing the proline oxidase activities [83].

5. Conclusion

The natural environment for plants is composed of a complex set of biotic and abiotic stresses. This review is intended to provoke thought about the increasing environmental stress factors for the plants today. Increasing global temperature and constant need for water is creating a hype, not only amongst us, but the natural world too. In this race of "survival of the fittest", plants have generated morphological, physiological and biochemical adaptations and mechanisms to cope up with the prevailing environmental stress conditions. One such major mechanism is the "antioxidant defense mechanism" that detoxifies the plant under stress by curbing the reactive oxygen species (ROS). This system, comprising of various enzymatic (e.g. superoxide dismutase, catalase) and non-enzymatic (ascorbate, vitamin E) 'cleansers' protects the proteins from denaturing and lipids from oxidizing. Thus, preventing the overall integrity of the cell and helping it to move strongly on its path to achieve homeostasis by successfully regulating its metabolic pathways.

Mutants or transgenic plants exhibiting differential capabilities for ROS formation and elimination could be useful to elucidate this fundamental point. Molecular knowledge of response and tolerance mechanisms is likely to pave the way for engineering plants that can withstand and give satisfactory economic yield under stress.

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