Chapter II

REVIEW OF LITERATURE
2.1 Impact of water stress:

About 63% of India’s total cultivable land is rainfed, which contributes nearly 44% food production, supporting 40% of the human and 60% of livestock population (Singh, 2003). Abiotic stresses like drought limit crop productivity in every season and in every crop worldwide, yet the nature of tolerance is not well understood, hence understanding of its mechanisms will help to improve the crop productivity (Singh and Chaudhary 1998; Joshi et al., 1998; Singh, 1998; Boyer, 1996). In general, tolerance to abiotic stresses is associated with the many morphological and physiological traits; such as root morphology and depth, plant architecture, variation in leaf cuticle thickness, stomatal regulation, osmotic adjustment, antioxidant capacity, hormonal regulation, desiccation tolerance (membrane and protein stability), maintenance of photosynthesis, and the timing of events during reproduction (Shinozaki and Shinozaki, 1996; Bray, 1997). The crop transpiration, WUE and HI are major determinants of grain yield in water deficit environment. The response of plants to drought stress is highly complex because under stress conditions they have to tolerate significant variations in soil composition, temperature, water potential etc. One of the most challenging traits for which various biochemical, physiological and biotechnological approaches are followed is drought tolerance, because the selected materials need to perform well under both water-limited and well watered condition, establishing optimal environments for improved performance under drought. Loss due to drought in the tropics alone is thought to exceed 20 million tons of grains per year, or around 17% of well watered production (Edmeades, et al. 1992), reaching up to 60% in severally affected regions such as South Africa in 1991-92.

Plant response to water stress causes reductions in growth and yield along with drastic metabolic changes (Munns, 2002). Drought is the single most important weather related natural disaster, often aggravated by human action. It affects very large area, for months and years and thus has a serious impact on regional food production, often reducing life expectancy of entire populations and economic performance of several countries (Chetti, 2002). Because of its genetic complexity, drought tolerance is probably the most difficult trait to improve through conventional plant breeding. The challenge is even greater for developing drought tolerant plants for water-limited environments where the occurrence, timing, and severity of drought may fluctuate from year to year (Ribaut et al. 2002).
differ in their ability to withstand abiotic stresses, both between species and within species. The onset of drought adversely affects seed germination, seedling growth, flowering, grain filling and yield. It induces drastic changes in almost all the physiological, biochemical and enzymological processes in stressed plants. The adaptations to drought stress involves accumulation of osmolytes, maintenance of turgor pressure along with favorable changes in morphology and anatomy of plants.

The availability of soil water for plant growth, development and reproduction is taken as the amount of water retained in soil between field capacity (upper limit) and wilting percentage (lower limit). FC of soil is the water content after the drainage of gravitational water and then the water content becomes relatively stable. The soil water potential of wilting is approximately -10 to -20 bars with a mean value of -15 bars. However, the influence of water stress on various physiological, biochemical, enzymological and molecular processes depends on genotypic as well as phenotypic nature of the plant, soil water conductivity, meteorological conditions and the osmotic potential of the plant. The brief review given below regarding influence of water stress on various parameters mentioned above is with reference to sorghum and other cereals.

2.2 Effect of water stress on seed germination, seedling growth and physiology:

Water absorption is prerequisite to initiate a series of metabolic changes in emergence of embryo. Gill et al. (2002); Joshi and Jadhav (1992) noted that in sorghum cultivars the seed germination, plumule and radicle length were progressively decreased with increase in osmotic tension created by manitol. Meena et al. (2003) reported that in sorghum and wheat seedlings under PEG-6000 induced water stress the root and shoot length, as well as root: shoot ratio was reduced with increased level of water stress. Salunke (1966) and Lad (1986) also reported similar results in two different cultivars of sorghum i.e. M 35-1 and PJ-7R. Increase in moisture tension is known to delay and affect the seed germination. Scarascia et al. (1979) observed that germination percentage was decreased with increasing concentration of PEG, in eleven different sorghum cultivars. Sajjan et al. (2004) reported that seed germination; seedling length and vigour index was reduced with increased levels of water stress in sorghum cultivars.

Gill et al. (2002) also noted decrease in fresh and dry weight of seedlings with increase in water stress level. Gill and Singh (1985) reported that germination, growth, respiration and other related processes can be affected in the seeds that are subjected to environmental stresses. The water stress imposed on germinating seeds might be affecting
metabolic activities and particularly carbohydrate metabolism, which plays an important role in seed germination and seedling development in sorghum and other cereals.

Maiti and Huerta, (1990) conducted experiment to evaluate 25 genotypes of sorghum for resistance to drought at the seedling stage and noted significant differences in seedling height, root length, leaf number and root number. They claimed that these parameters can be considered to evaluate drought tolerance. Vardhini and Rao (2003) reported the enhanced level of soluble proteins and proline in sorghum during seed germination.

Bewley and Black (1994) clearly explained that such types of metabolic alterations are helpful for inducing seed germination under stressful environment. Bolarin et al. (1995) noted that increase in carbohydrate along with increased stress level in germinating seeds, play an important role in osmotic regulation of cell during germination. Further they have claimed that increased accumulation of sugars in such seeds regulate the expression of some genes involved in seed germination under stress condition.

Many workers like Reynolds and Smith (1995), Yu et al. (1996), Hoekstra et al. (2001) confirmed that accumulation of sugars/carbohydrates act as central dogma in the development of desiccation tolerance. Earlier study by several workers on carbohydrate accumulation during various abiotic stresses in cereals are in conformity with the above finding (Gill et al., 2001; Prado et al. 2000; Wang et al. 1996; Siddique et al. 2000). Masoud et al. (2004) studied the effect of water deficits on seedlings in forage sorghum and reported accumulation of osmolytes like sugars and proline at different levels of PEG induced water stress. They further indicated that proline concentration increased with the increasing level of water stress.

Many workers like Bhalerao (2003), Borse (2004) reported reduction in protein content in sorghum and Solanum khasianum respectively during seed germination. Ismail (2003) showed that in germinating seeds of sorghum soluble proteins were reduced under salt stress. Zayed and Zeid (1998) reported decrease in soluble proteins due to PEG induced water stress in mungbean seedlings.

Among the twenty protein amino acids, proline occupies a special position in plant metabolism due to its involvement in response of plants to variety of environmental constraints (Pawar, 2004). According to Yoshiba et al., (1997) in plants during water stress amino acids and ammonia are formed by break down of protein. As ammonia is toxic to plants, proline is used as a sink for soluble nitrogen. The role of proline in osmoregulation and cytoplasmic osmoticum in maintenance of turgor pressure is recognized in several plant

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species. In many plants proline is widely distributed osmolyte which does not interfere with normal biochemical reactions and acts as osmoprotectant under the condition of drought.

Jha and Singh (1997) reported that in all the rice cultivars the seed germination percentage was reduced due to external water potential created by PEG-6000 from control to -10.0 atm. Singh (2000) noted that in five wheat genotype under PEG-6000 induced water stress seed germination percentage decreased with reduction in external water potential.

Turner et al. (2000) suggested that more emphasis may be laid on biochemical and physiological processes, which result into rapid accumulation of starch and protein contents under the harsh environmental conditions. Panda and Khan (2003) found that due to water stress proline content was enhanced in cotyledons of Cicer arietinum. The reduction in germination might be due to the less availability of free water to the seeds during early hours of imbibition, thus leaving the hydrolytic enzymes inactive (Hadas, 1976). Inhibition of germination at higher osmotic potential may possibly be attributed to moisture deficit in the seed below the threshold requirement of germination (Everiff, 1983). Therefore it seems possible that water potential exerts major influence on seed germination by retarding water imbibition. The reduction in germination was attributed to less availability of free water to the seed during early hours of imbibition and this was resulted in to inactivation of hydrolytic enzymes.

Singh et al. (1996) and Prado et al. (2000) explained that the reduction in seed germination under water stress condition was due to enforced dormancy in the seeds. They further considered it as an adaptive strategy for seeds to prevent germination under stressful environment, ensuring proper establishment of seedlings. Similarly under osmotic stress a significant reduction in water potential and tissue water content in the germinating seeds may be responsible for inhibition of seed germination under stress. Kameli and Losel (1996) reported linear correlation between water stress and dry matter accumulation in Triticum sps.

Munjal and Dahiya (2004) had screened forty barly genotypes for drought tolerance under PEG-induced water stress and reported that the drought tolerant cultivars showed higher percentage of seed germination, coleoptile length, root length, shoot length. Mulge et al. (2005) studied the effect of moisture stress on germination of onion seeds and reported that moisture stress had significant influence on percent germination, shoot weight, shoot length and vigor index.

2.3 Influence of water stress on morphological and yield parameters:

Like seed germination and seedling growth the morphological as well as yield parameters are also significantly influenced by the water stress. Osvald and Osvald (1991)
noted that under rainfed condition (drought stress) in grain sorghum growth was very poor. Garrity et al. (1984) noted that grain and DM yield reduced by 36 to 37% respectively under water stress. They further claimed that reduced LA in stressed plants was responsible for this. Gonzalez (1984) reported that water stress inhibited reduced functional LA, delayed panicle expansion and reduced grain yield and number of seed head in hybrid sorghum. Berenguer and Faci (1999) reported that plant height, dry matter production, LA, peduncle etc. were reduced under different levels of water stress in sorghum. Lamani et al. (1997) observed that plant height, LA, LAI, growth rate were decreased under water stress in sorghum. Similar was the observation noted by Sharma and Kumari (1996) in sorghum cultivars. Ibrahim and Aldesuquy (2003) reported distinctly reduced yield components of sorghum under water stress.

Munamava and Riddoch (2001) reported that in sorghum cultivars at vegetative, booting and flowering stage under water stress condition total leaf area, effective green leaf area, and specific leaf weight was decreased and grain yield was significantly decreased with stress. Similarly Berenguer and Faci. (2001) noted that seasonal evapotranspiration, total dry matter, grain yield and harvest index was decreased in sorghum cultivars under water stress. Rao (1997; 1999) reported that leaf area index; crop growth rate and net assimilation rate were decreased in glossy (light green) and non glossy (dark green) lines of sorghum. He further noted that glossy lines produced more grain yield than non glossy lines under water stress. Younis et al. (2000) noted that in three sorghum cultivars under drought stress total green leaf area, shoot dry weight and relative growth was decreased. Abeytunge (1983) observed that the responses of sorghum to summer irrigation in terms of grain yield, DM, leaf area, root growth, evapotranspiration and water use efficiency was altered due to delayed irrigation. Zaifnejad et al., (1997) reported that in sorghum cultivars increased water stress affected the shoot and root dry matter, leaf area, main axis root length.

Bharambe and Joshi, (1993) reported decrease in grain yield per plant under water stress in sorghum. Leport et al. (1999) they also concluded that water shortage reduced yield by 50 to 80% due to reduction in seed number and seed size. The grain yield per plant of drought tolerant inbreeds was higher in rainfed condition than in irrigated condition (Varade et al. 2003). Jirali et al. (2003) noted that in fourteen sorghum genotypes under water stress conditions the grain yield was reduced along with reduced LAI, TDM, HI, grain number per panicle and 1000 grain weight. Rao et al. (2003) noted that in grain legumes yield and yield components viz. number of pods and number of seed pod, 100 seed weight etc. were drastically reduced under stress conditions. Yadav et al. (2003) reported that grain yield and dry matter content decreased in response to water deficit in sorghum. The reduction was
related to reduction in grain number, when the stress was imposed at anthesis and the reduction in grain size when the stress was imposed at grain filling stage. According to Singh (2003) various morpho physiological traits like root length (rooting depth) and density, canopy structure, HI, osmotic adjustments, net photosynthetic, WUE, LWP, RWC allocation of assimilates to shoot are most important determinants in sorghum under dry land conditions for improving the yield. Prabhakar and Raut (2004) had performed an experiment for evaluation of 183 accessions of rabi sorghum germplasm for the response towards terminal drought on the basis of various physiological traits associated with drought adaptation. They have reported that higher grain yield, HI, panicle mass and biomass exhibited positive correlation with grain yield and drought tolerance.

The most significant and adverse impact of drought is noted on various reproductive and yield parameters. The flowering, anthesis, grain filling as well as ear head length, number of grains and 100 grain weight etc. are negatively influenced by water stress. The source sink relation and storage mechanisms are disturbed by water stress in sorghum and other cereal crops (Chetti and Hiremath 2002).


Gu et al. (1989) reported that in maize drought stress at different stages markedly reduced the morphological parameters like plant height, leaf growth etc. Srinivasa Rao (2005) used the same parameter for screening onion cultivars in response to water stress tolerance with reference to bulb and yield. Batt et al. (2005) studied the differential sensitivity to water stress in onion on the basis of morpho physiological traits like RWC, photosynthetic rate, stomatal conductance, LA, DM accumulation and yield. Similarly the differential sensitivity to water stress in all the four promising cultivars of sorghum was evaluated on the basis of morpho physiological characters. Burman et al. (2005) noted that
under different intensities of water stress there was significant reduction in growth and seed yield in clusterbean at pre-flowering stage.

2.4 Influence of water stress on physiological and biochemical parameters:-

Photosynthetic pigments and photosynthetic rate:-

The main damages caused to the photosynthetic pigments under water stress includes structural changes distortion of lamellae, vesiculation and hence the appearance of lipid droplets in chlorophyll (Paleg and Aspinall, 1981). Gujrathi et al., (1981) reported that total chlorophyll content declined significantly during water stress condition in sorghum. Satbhai et al. (1997) recorded same results in sorghum under PEG-6000 induced water stress. Jagtap et al. (1998) reported 20- 30 % chlorophyll losses in five sorghum cultivars under water stress. Deshmukh and Dhumal (2000), Deshmukh et al. (2001) and Deshmukh et al. (2004) reported that in sorghum cultivars under PEG-6000 induced water stress total chlorophyll, chlorophyll-a and b content was decreased with increase in moisture stress.

Deshmukh and Dhumal (2005) reported that the chlorophyll content was decreased under water deficit condition at anthesis stage in promising cultivars of sorghum. Rao et al. (2003) reported that in twenty two sorghum genotypes the chlorophyll contents were decreased at boot and at maturity stage. Younis et al. (2000) noted that in three sorghum cultivars under drought stress net assimilation rate and chlorophyll content was decreased. Similarly Gujrathi et al. (1984) reported that chlorophyll content in sorghum and ground nut cultivars were decreased under water stress. Cechin (1998) observed slight decrease in the efficiency of chlorophyll-a under water stress. Ibrahim and Aldeesuquy (2003) reported reduced chlorophyll-a and chlorophyll-b content of sorghum under water stress.

The decline in chlorophyll pigments in different crop plants due to water stress has been documented by many workers in maize (Alberte et al., 1977 ; Sharma et al., 1990), groundnut (Krishnamohan and Rajeshwar Rao, 1989), barley (Bhardwaj and Singhal, 1981), sunflower (Quartacci et al., 1992), tomato (Natrajan, 1990) and sorghum (Maiti and Rios, 1990).

Alberte et al., (1975) reported that water potentials lower than – 0.5 Mpa retard the development of chlorophyll by reducing the rate of formation of chlorophyll –a and b. Similarly Bhardwaj and Singhal (1981) found that chlorophyll destruction was hastened by the water stress.

A loss of chlorophyll may also arise in situations where desiccation causes leaf senescence. Boyer (1976) reported that stomatal closure is accompanied with the inhibition in chloroplast activity. The reduction in nitrogen content and activity of NR or nitrogenase
also induce degradation in chlorophyll contents (Morilla et al., 1993; Ergin and Sprent, 1973).

Sharma et al., (1990) reported that both photosynthetic rate and chlorophyll content decreased with increasing drought stress. Drought stress was responsible for the gradual shift in carbon flow from the C₄ carbon fixation pathway to an alternate (glycollate) pathway in maize. Drought stress reduced the net and gross photosynthesis and caused the decrease in chlorophyll content of some plants (Boyer, 1976 and Gupta, 1978). Garcia et al., (1992) found decreased chlorophyll content in maize seedlings due to PEG induced stress. Sairam et al. (1997) reported that chlorophyll was decreased under moisture and temperature stress in three wheat genotypes as compared to control. They also showed that there was a decrease in chlorophyll a/b ratio, both under moisture and temperature stress. Sairam et al. (1998) reported that in leaves of wheat under water stress conditions total chlorophyll showed a decrease. Sairam and Saxena (2000) reported that in wheat the chlorophyll content decreased with increasing water stress in all the cultivars.

Colom and Vazzana (2002) noted significant reductions in chlorophyll contents in water stressed plants. Sepehri and Sanavy (2003) reported decreased chlorophyll content by 8-20% in two different cultivars of maize. Kraus et al. (1995) and Pastori and Trippi (1992) have also reported a decrease in chlorophyll content upon exposure to oxidative stress and a comparatively higher chlorophyll content in tolerant wheat and maize genotypes under stress conditions than in susceptible ones. The workers like Dwivedi et al., (1979) observed rapid changes in the chlorophyll content in water-stressed leaves of rice.

Burman et al. (2005) reported significant decrease in total chlorophylls under different intensities of water stress in clusterbean. Joshi et al. (2005) reported that under PEG-6000 induced water stress total chlorophyll content was decreased with increasing water stress in wheat genotypes at germination stage. Drought stress-induced damage to the photosynthetic apparatus was reported by Van Rensburg and Krueger (1993). Similarly Kadam and Tambe (2003) also reported that in grape chlorophyll-a and b were decreased with increase in water stress.

**Carotenoids:-**

These accessory pigments are effective as antioxidants and play a unique role in protecting photochemical processes (Burton and Ingold, 1984). Carotenoids protect the photo systems by reacting with lipid peroxidation products to terminate chain reactions, by scavenging singlet oxygen and dissipating the energy as heat, by reacting with excited chlorophyll molecules to prevent formation of singlet oxygen or by the dissipating the excess excitation energy through the xanthophyll cycles (Demmig et al. 1988). The main protective
role of β-carotene in photosynthetic tissue may be through direct quenching of triplet chlorophyll, which prevents the generation of singlet oxygen and therefore avoids oxidative stress completely.


2.5 Effect of water stress on macromolecule content:

Carbohydrates:

In cereals like sorghum changes in carbohydrate content under water stress has great significance, because it will determine its tolerance or susceptibility to moisture stress and will predict its yield potential. Deshmukh and Dhumal (2000), Deshmukh et al. (2001), Deshmukh and Dhumal (2001) and Deshmukh et al. (2004) reported that in sorghum cultivars under PEG-6000 induced water stress reducing sugars concentration was increased with the increase in moisture stress. However, starch and non-reducing sugars concentrations were decreased. Similar was the trend noted by Yadav et al. (2003; 2005) in sorghum cultivars. Ibrahim and Aldesuquy (2003) reported significant increase in total soluble sugars of sorghum under water stress.

White and Newton (1982) recorded higher levels of glucose, sucrose and fructose in stressed sorghum plant than non-stressed plants. Wang et al., (1991) reported that in sorghum seedlings, accumulation of reducing sugars was increased with increased water stress. Satbhai et al., (1997) found that the water stress induced by PEG-6000 in sorghum seedlings enhanced the content of reducing sugars with increased water stress. Koster (1991) also noted the accumulation of sucrose and oligosaccharides consistently in drought tolerant plants. Yadava et al. (1991) reported that at 50 % anthesis in various genotypes of sorghum sugar contents were accumulated under drought condition.

Pandey and Agarwal (2003) noted that in rice under different water stress treatments free sugars were increased with a decline in starch content. Dwivedi et al., (1979) reported that in Oryza sativa, water stress accelerated the changes in the levels of starch and non-reducing sugars along with retardation in soluble sugars and total carbohydrates. Similarly Jha and Singh (1997) also noted that in rice seedlings the total sugars in shoot axis were increased with increase in moisture stress. Gautam et al. (2005) noted that the total free
sugar content was increased under PEG-6000 imposed water stress in rice. Sing and Sing (1981) observed that total soluble sugar content increased but starch content decreased in water stressed seedlings of maize. Similarly Kameli and Losel (1996) observed that sugars particularly glucose made the largest contribution to osmotic adjustment in wheat leaves under water stress.

Khapke et al. (2004) recorded that under PEG-6000 induced water stress reducing sugars concentration was increased along with the increased moisture stress, in soybean. Burman et al. (2005) reported there was significant decrease in starch content under different intensities of water stress and phosphorus application in clusterbean. It has been known that, water deficit often causes a decrease in starch content and some times an increase in sugar. The reduction in photosynthesis leads to decreased supply of carbohydrates (Hopkins, 1995).

The concentration of soluble sugars in leaf tissue increases under water stress due to number of reasons, such as increased starch hydrolysis, synthesis of sugars by other pathways or their decreased conversion to other products. Maranville and Paulsen (1970) have shown an increase in the amylase activity in water stressed leaves causing degradation of starch in to sugars. Similarly Vyas et al. (1985) observed a significant decline in the starch content under mild stress with a concomitant increase in the reducing sugar content. It was further reported that this could arise from increased starch hydrolysis coupled with increased utilization of sugars under the stress condition the direct effect of stress on translocation of sugars and noted the accumulation of sugars. The accelerated starch loss was accompanied by an increase in free sugars.

Proteins:

Proteins are the important constituents determining the growth and yield of plant. The water stress induces many changes in the types and amount of proteins and alters the metabolism (Chandra et al. 2003). Protein synthesis and breakdown which is intimately connected with the activities of the nucleic acids is much affected by drought. The stress induced proteins allow plants to make biochemical and structural adjustments that enable them to cope with the stress. Hence, understanding the biochemical and molecular changes in proteins will be helpful in developing the strategies for improving drought tolerance (Chandra et al. 2003).

Osvald and Osvald (1991) noted poor growth and pollination in sorghum under drought and concluded that the decrease in protein concentration is a typical symptom of oxidative stress. Deshmukh and Dhumal (2000); Deshmukh et al. (2003; 2004) and Khapake et al., (2004) reported significant decrease in content of proteins in sorghum and soybean exposed to PEG-6000 induced water stress.
Singer (1962) observed that moisture stress degrades proteins by changing their surroundings, which is essential for the membrane stabilization. Frota and Tucker (1978) observed a significant decrease in protein content of plants subjected to water stress. Singh et al. (1996) explained that decrease in protein content under water stress could be due to reduced availability of free amino acids.

Sharma et al., (1990) noted decreased protein contents in leaves of 15 days old seedlings of groundnut under water stress. Quartacci and Navari Izzo (1992) reported about 17% less soluble protein in water stressed sunflower seedlings than the control. Sarkar et al., (1989) reported reduction in protein content in pea seedlings treated with PEG –6000 (-1 Mpa) for 72 hours. Similarly Jha and Singh (1997) noted that stress tolerant rice varieties have more proteins than the susceptible.

Chakraborty et al. (2001) studied the drought induced biochemical change in young tea leaves and reported decreased protein content. Bergmen et al. (1994) also reported accumulation of stress induced proteins in barley under water stress conditions. Burman et al. (2005) noted that under different intensities of water stress and phosphorus application there was significant decrease in soluble protein content in clusterbean at pre-flowering stage. However, Agarwal and Pandey (2003) reported that in Cassia angustifolia the protein contents increased significantly under water stress. Datta et al. (2004) claimed that LEA proteins are generally synthesized in the seeds exposed to water stress, which provide the stress tolerance. They have reported that the expression of these genes was induced by ABA and stress signals in the seedling of barley.

2.6 Effect of water stress on cellular water status and membrane injury:-

Relative water content (RWC):-

The genotypes, which can retain higher amount of water under conditions of soil moisture deficit, was considered as more tolerant to water stress. Tissue water status or RWC is regulated by stomatal conductivity, rate of transpiration, ABA content, soluble sugars, free amino acids (especially proline) and inorganic ions like potassium. It also regulates the root morphology under stress conditions.

Deshmukh and Dhumal (2005) reported that the RWC was decreased under water deficit condition at anthesis stage in four promising cultivars of sorghum. Ibrahim and Aldesuquy (2003) reported reduced relative water content in sorghum under water stress. Sivramakrishnan et al., (1988) and Wang et al., (1991) reported decreased RWC of cell, with increased water stress in sorghum. Varade et al. (2003) reported that under limited water condition the RWC was higher in drought tolerant inbreds of maize. Kushwaha et al. (2003)
noted that high RWC cause a relatively less damage to the membrane system resulting into the production of higher biomass. Due to imposed stress there is a gradual lowering of osmotic potential, as the solute concentration increases resulting into solvent water which cause the decrease in RWC. Many workers like Munns and Cramer, (1996), Maldonado et al., (1997), Flores- Ninedez et al., (1990), Uprey et al., (1998), recorded decrease in water and, osmotic potential as well as relative water content, along with increase in ABA and proline contents in different crop plants under drought stress. Similar results were also observed in rice (Krishnayya and Murty, 2005) and barley (Argandona and Pahlich, 1991). Sairam et al. (1998); Sairam and Saxena (2000); Shangguan and Chen (1991) also reported decreased RWC in wheat under drought stress.

Ming et al. (2003) noted reduction in RWC in liquorice when exposed to 10% PEG. Kushwaha et al. (2003) noted that in chickpea under rainout shelter conditions RWC was decreased as compared to rainfed. Dubey and Chandra (2003) reported decrease in RWC up to 35% after four days of water stress in Cenchrus. The reduction in RWC after withholding of water has been reported in many species by Phutela et al. (2000); Rao et al. (2003). Yadav et al., (2003; 2005) reported decreased RWC in stressed plants of sorghum in all growth stages. Burman et al. (2005) noted that at pre-flowering stage there was significant decrease in RWC under different intensities of water stress and phosphorus application in clusterbean.

Membrane stability index:-

The decrease in membrane stability index, estimated by taking comparative ion leakage, is an indicator of membrane damage as a result of membrane (lipid) peroxidation caused by active oxygen species many workers like Dhindsa, (1991); Upadhaya et al. (1990). Premchandra et al. (1990) proposed that cell membrane stability acts as an indicator of drought tolerance. Deshmukh and Dhumal (2005) reported that the MSI was decreased under water deficit condition at anthesis stage in four different promising cultivars of sorghum.

Premchandra and Shimada (1988); Sairam et al. (1997, 1998); Sairam and Saxena (2000) reported decreased MSI values with increasing as water stress in all genotypes of wheat.

Pastori and Trippi, (1992); Kraus et al. (1995); Baisak et al. (1994); Menconi et al. (1995); Kushwaha et al. (2003); Rao et al. (2003); Thippeswamy et al. (2005) reported significant decrease in MSI under water stress in various crops.

Lipid peroxidation:-

The values of LPO also indicate the drought tolerant and susceptible nature of the plants. It is generally enhanced as the degree of water stress increases. Jiang et al. (1991)
revealed that in rice cultivars PEG induced water stress caused increased LPO. Sairam et al. (1998); Sairam and Saxena (2000) noted increased lipid peroxidation (LPO) in leaves of wheat genotypes under water stress. Moran et al. (1994); Jagtap and Bhargava, (1995) reported increased values of LPO in sorghum under stress conditions. Ming et al. (2003) reported increased MDA content under stress conditions in liquorice.

Chander et al., (2003) noted about 50% increase in MDA content when water stress was imposed at different stages to chilly. Similarly Thippeswamy et al. (2005) reported that during water stress i.e. 100, 75, 50 and 20% FC level the lipid peroxidation was increased in safflower.

2.7 Effect of water stress on osmolyte accumulation:

The turgor maintenance through osmotic adjustment is positively associated with yield (Grover, 2004). The cells isolated from drought resistant varieties showed higher resistance to osmotic stress in vitro as compared to those from susceptible varieties. A wide variety of organic solutes accumulate in plant tissues during water stress and contribute to osmotic adjustment (Datta et al., 2004). Osmotic adjustment (OA) allows more water to be extracted by improving water extraction efficiency and helps in maintaining stomatal conductance and photosynthesis (Datta et al., 2004). It is positively associated with yield under drought environments in wheat (Morgan et al. 1986), barley (Blum, 1989) and sorghum (Morgan, 1984). The osmotic adjustment positively correlate with growth and yield under stress.

Proline:-

Kemble and Macpherson, (1954) first noted proline accumulation in wilted plant tissue. It mainly ameliorates the effects of environmental stresses. The accumulation of free proline has been reported in many plant species subjected to hyper osmotic stress conditions, since last five decades. The accumulation of proline under abiotic stress conditions is depending on the species and extent of stress (Delauney and Verma., 1993; Bohnert and Jensen., 1996). As stated by Delauney and Verma (1993) the accumulation of cellular proline is mainly due to increased synthesis and decreased degradation under a variety of stress conditions. Hare (1998) also supported this view. Although proline is known to confer osmotic tolerance during stress conditions, it also plays a key role in stabilization of proteins, membranes and sub cellular structures (Vanrensburg et al. 1993), and protects the cellular functions by scavenging reactive oxygen species (Bohnert and Shen., 1999; Verma, 1999).

Asada, (1984) indicated that the accumulation of compatible solutes like proline under stress condition protects the plants from damage by reactive oxygen species. Matysik
et al. (2002) explained the molecular mechanism of the protective role of proline under stress.

The concentration of proline increases in a variety of plants under stress, up to 100 times more than the normal level (Aziz et al. 1998; Lee and Chang, 1999).

According to Kavi Kishore et al. (1995; 2003) the dramatic accumulation of proline under stress is due to increased synthesis and or decreased degradation.

Deshmukh and Dhumal (2005) reported that the proline content was increased under water deficit condition at anthesis stage in promising cultivars of sorghum. Deshmukh and Dhumal (2000); Deshmukh et al. (2003; 2004) reported increased proline in sorghum seedlings under PEG-6000 induced water stress.

Ibrahim and Aldesuquy (2003) reported accumulation of proline in sorghum under water stress. Jadhav et al. (2001) reported that in sorghum cultivar Sel-3 content of leaf proline was increased by (63%) with increased water stress. Sivaramakrishnan et al., (1988) noted that the resistant lines of sorghum were accumulating high proline, compared to susceptible lines. According to them proline accumulation might be contributing to immediate recovery of plants from drought. Zaifnejad et al. (1997) reported extensive increase in proline in water deficit shoots of sorghum. Bharambe and Joshi, (1993) also reported increase in proline content with increase in water stress in 8-days old seedlings of sorghum cv. CSH- 8R. Satbhai et al., (1997) also observed enhanced level of proline in water stressed cultivars of sorghum. The concentration of proline increased significantly with the increased intensity of stress, however, the percentage increase over control was higher in Lakadi than SPV-504.

Yadav et al. (2003; 2005) reported proline accumulation in response to water deficit in sorghum cultivars at vegetative, anthesis and grain filling stage.

Singh et al. (1972) reported a strong correlation between proline accumulation in seedlings of barley and grain yield stability index. Argandona and Pahllich (1991) also noted similar trend in barley seedlings. Pandey and Agarwal (2003) noted increased proline concentration in rice under water stress. Gautam et al. (2005) noted that the proline content was increased under PEG-6000 imposed water stress in rice. They further reported that the activity of protease was increased at the same time indicating the role of protease activity in proline generation for osmotic adjustment. Wang et al. (1989) noted that in several wheat cultivars leaf proline increased with duration and intensity of water stress, but there was no correlation between the increase of proline content and drought resistance. Gharti-Chhetri and Lales (1990) showed that in wheat cultivars leaf proline increased as drought progressed. Bao et al., (1991) studied accumulation of proline in three maize cultivars imposed to water
stress during grain filling stage and claimed that proline accumulation was very rapid during leaf elongation. They claimed that proline accumulation was related to growth stage and degree of water stress. Garcia, et al. (1992) noted increased proline with increasing water stress in maize. Sharma et al., (1990) proposed that proline accumulation could be used as an indication of water stress tolerance.

**Glycine betaine (GB):**

GB helps to maintain cellular proteins, enzymes, cell organelles and membranes against injury due to water loss. Many cereal crops accumulate GB except some like rice. The concentration of GB acts as compatible osmotic solute. As suggested by Rathinasabapathi et al. (1997) glycine betaine plays an important role in adaptation of plants to saline and arid environments. Hanson and Scott (1980) proposed that GB is synthesized in plants from serine via ethanol-amine, choline, and betaine aldehyde and the activities of various enzymes involved in this synthesis and stimulated by osmotic stress.

Deshmukh and Dhumal (2005) reported that the content of GB was increased under water deficit condition at anthesis stage in promising cultivars of sorghum. The studies of Yang et al. (1996, 2003) revealed that five promising sorghum genotypes, sampled at seedling and 50% anthesis stage, were containing very high GB under water stress and the level of GB was ten times more than maize. According to the Girousse et al. (1996) prolong periods of drought leads to accumulation of glycine betaine which is an adaptive response to several abiotic environmental stresses. Chander et al., (2003) reported significant accumulation of glycine betaine in stressed plants of chilly.

**Free amino acids:**

In plants during stress conditions, the free amino acid content increase and hence helps to indicate the metabolic status of stressed plants. The increase in FAA could be due to stimulated synthesis, inhibited oxidation, impaired protein synthesis or enhanced protein hydrolysis Chen et al. (2002).

In three rabi sorghum cultivars a significant increase in the level of free amino acids due to water stress was observed by Satbhai et al. (1997). Yadav et al. (2003; 2005) also reported increased AA in sorghum cultivars under water stress at all the growth stages while Pandey and Agarwal and Pandey (2003) noted altered concentration of AA in rice under different water stress treatment.

An increase in amino acid pool size induced by water deficit was reported in maize (Thakur and Rai, 1981; Ranieri et al., 1989 ), Rice (Jha and Singh, 1997), maize and sunflower (Navari-Izzo et al., 1990), mungbean (Kumar and Singh, 1991), pearl millet (Kumar and Bharti, 1991), groundnut (Sharma et al., 1990) and in chillies (Chander et al.,
According to Venekamp and Koot (1988) the increased AA content during stress served as a source for accumulation of proline and asparagines which provides stress tolerance. The tolerant genotypes had comparatively higher TFA content as compared to susceptible ones. Ranieri et al., (1989) claimed that modification in protein turnover affect the free amino acid accumulation, which in turn contribute to osmotic adjustment.

Phenols: -

Phenols offer resistance to various biotic and biotic stresses in plants Subhashini and Reddy, (1990). The increase in polyphenols under stress may be due to triggered metabolism of shikimic acid pathway (Madhukar and Reddy, 1991). Deshmukh and Dhumal (2000) and Deshmukh et al. (2004) reported that in sorghum cultivars under PEG-6000 induced water stress total phenols were increased with increase in moisture stress.

Chakraborty et al. (2001) reported enhanced phenolic contents under water stress conditions in tea leaves. Bergmen et al. (1994) also reported accumulation of phenolics in barley under water stress conditions. Chander et al., (2003) noted significant increase in total phenols in stressed seedlings of chilly.

2.8 Effect of water stress on $\Delta^{13}$C discrimination: -

According to Udaykumar and Prasad (2003) $\delta^{13}$C values could be a reflection of CO$_2$ diffusive process of water and carboxylation reactions and hence to be used to assess differences in water use efficiency (WUE) and drought stress tolerance. They further reported that differences in discrimination between C$_3$ and C$_4$ plants shows variations. The discrimination of PEP Case is relatively lower than Rubisco hence discrimination is always lower in C$_4$ plants like sorghum than in C$_3$ plants.

Deshmukh and Dhumal (2005 b) reported that under water deficit conditions $\Delta^{13}$C values were associated with photosynthesis and consequently with yield in sorghum. Williams et al. (2001) studied the carbon isotope discrimination in Sorghum bicolor under CO$_2$ enrichment and drought. According to Farquehar (1983) the discrimination against $^{13}$C in C$_4$ plants is highly complex. Condon et al. (2003) reported that in different plants genotypic variation exists regarding carbon isotope discrimination. They further claimed that carbon isotope discrimination can be used as a selection criterion for improved transpiration efficiency in wheat and other C$_3$ crops.

Apel et al. (1993) studied the relationship between carbon isotope discrimination and grain yield in spring barley under drought conditions. Farquehar et al. (1982) indicated close relationship between WUE and $\delta^{13}$C values. They have correlated the values of carbon isotope discrimination and grain yield.
2.9 Effect of water stress on mineral constituents:

In rainfed crops like sorghum, millets etc the poor soil physical and fertility conditions are the major constraints, limiting crop productivity. As stated by Lahiri (2001) the availability of nutrients in the soil is not only a prerequisite for optimum crop growth, but also help plants to ameliorate the adverse effect of water stress. N, P, and K are the major plant nutrients involved in the synthesis of chlorophylls, proteins, carbohydrates, nucleic acids and photosynthesis. Singh (2003) reviewed the putative drought tolerance traits and management of the dry land crops like sorghum to sustain high productivity through the management of nutrient level. According to him proper combination of water and fertilizers will be the best option to improve crop productivity in sorghum under water stress conditions.

**Nitrogen (N):**

It is the constituent of proteins, N.A., nucleoproteins, chlorophylls etc. It regulates the photosynthetic efficiency, water use efficiency, NR activity, carbohydrate metabolism and biosynthesis of chlorophyll pigments as well as several secondary metabolites. According to Kalita et al. (1995), Patra et al. (1995), Vyas et al. (1999), Bhalerao (2003) the availability of nitrogen to crop governs the growth and canopy development, LA, DM accumulation and crop productivity. The content of nitrogen in plants greatly influence all the vegetative and reproductive parameters along with yield (Katiyar et al. 1999; Balakrishnan, 1999; Bhalerao, 2003). The nitrogen content has direct impact on RGR, CGR, LAI and LAD (Patra et al. 1995).

Bataglia et al. (1985) reported that nitrogen has a great impact on osmotic adjustment in maize and sorghum. Eck and Musick (1979) reported that plant water stress reduced N content in sorghum. The impact of N under water stress in two different cultivars of sorghum was studied by Utzurrum et al. (1998) and proposed that N content was closely related with osmotic adjustment and DM accumulation, LA, and grain filling.

Cechin (1998) reported that the rate of photosynthesis, chlorophyll content, stomatal conductance, transpirational rate were affected by low nitrogen content under water stress in two different hybrids of sorghum. They further proposed that leaf water potential, water use efficiency, water relations and mineral nutrition in plants under water stress had shown close correlation. Garg et al. (2004) studied the interaction of moisture and N level in mustard and reported that N application significantly increased net photosynthesis, stomatal conductance; RWC under water stress conditions. Bhyresappa et al. (2005) reported increase in growth and yield due to N application in ajowan, similarly Qureshi and Lawande (2005), Amin and
Patel (2005) reported improved yield in onion, garlic, cumin and fennel respectively with N applications.

**Phosphorus (P):**

It participates in many metabolic processes leading to healthy and vigorous growth as well as higher yields and plays an important role in energy metabolism of plants. Its demand is associated with the rate of plant growth and level of metabolic activities (Teiz and Zeiger, 1998). According to Hare (1966) and Johri (1975) increase or decrease in P content is closely related with carbohydrate and starch content.

Ogata et al. (1985) noted that leaf water potential and nutrient absorption particularly N and P were decreased with increased water stress in sorghum and other crop plants. Eck and Musick (1979) reported that plant water stress reduced P content in leaves of sorghum. Nasir-Ud-Din (1972) reported that in sorghum under PEG induced water stress N and P contents were comparatively low. The content of phosphorus being a constituent of membranes has important role in lipid peroxidation. The decrease in P might be affecting membrane damage under water stress conditions. The decrease in P might be having a great impact on general metabolic status of plants along with grain filling and grain maturity. As P is closely related with carbohydrate metabolism, it has more relevance during grain filling in sorghum the high starch containing crop.

Pandey et al. (2005) indicated that P is one of the major limiting nutrient next to N in crop production, hence its deficiency seriously limit crop production. Patel et al. (2004) reported that moisture stress affects P distribution in plants, which then affects the nutrient dynamics in wheat. Padhiyar et al. (2005), Bhyresappa et al. (2005) reported the influence of P supply on growth and yield in various crops.

**Potassium (K):**

Mengel and Kirkby (1980) reported the involvement of K in various physiological, biochemical and enzymological processes. It improves the WUE through its influence on maintenance of turgor potential, osmotic potential, stomatal physiology and root growth (Hsiao and Lauchli, 1986). According to Singh et al. (1997) and Vyas et al. (2001) K has many beneficial effects on plants under soil water deficits. As sorghum is generally grown under rainfed conditions, experiences water stress of varying degree and duration at different growth stages, the relevance of K nutrient under such conditions assumes great importance. Vyas et al. (2001), Sharma and Kuhad (1999), Umar et al. (1993) reported that K level maintains the RWC in plants. They further explained that K has key role in osmoregulation and in the maintenance of osmotic potential and water uptake in plants. Chow et al. (1990) reported that the plants exposed to drought had higher K requirement. According to Maniver.
et al. (1995), Vyas et al. (2001) K content under water stress conditions is responsible for increasing FAA and of proline contents which directly or indirectly help in stress tolerance. Many workers like Singh et al. (1997), Sharma et al. (1992), Umar et al. (1993), Garg et al (1998) reported yield improvement even under water stress indifferent crops due to application of K. Yadav et al. (2005) reported that K concentration marginally increased in response to water stress at anthesis stage in sorghum hybrid CSH-14 which was correlated with drought tolerant ability of this cultivar.

Lavania and Jain (1998); Patra et al. (1995) and Mitra et al. (1998) explained the vital role of K in carbon assimilation, sucrose synthesis and its translocation, opening and closing of stomata, activities of several enzymes involved in photosynthesis, respiration, dry matter production, LA, LAD, CGR etc.

Rao et al. (2001) noted that K has a great role in increasing the grain yield and biomass even under water stress in rabi sorghum. Arjomand et al. (2000) reported that potassium helps to maintain leaf water potential and turgor potential in sorghum under drought conditions. Nunez (1984) recorded correlation between accumulation of various osmotic solutes like sucrose, glucose, proline and K content under mild water stress in sorghumSimilarly Tang et al. (1985) noted that accumulation of proline under water stress condition in sorghum seedlings was associated with K content. Sharma and Kumari (1996) level the that with increased K content plant height, LAI, LAD, CGR and total dry matter production were increased significantly in various sorghum cultivars. Srivastava and Singh (2004) noted that increased K under water stress had positive impact on plant development and growth, number of leaves per plant, LA and yield attributes in wheat. Further they have also recorded increase in rate of photosynthesis, RWC, protein and carbohydrate contents under water stress condition by application of K.

Gavade et al. (2004) reported alleviation of water stress by applying K in mustard, the application of K along with micronutrients improved the seed yield, biological yield, HI and RWC over control under stress condition. Similar finding was reported by Mandavakar et al. (2004) in horse gram. They have reported increase in LA, chlorophyll contents, RWC and drought tolerance ability due to the treatment of K. Singh and Kuhad (2004); Bhyresappa et al. (2005) reported similar results in many other crops.

Calcium(Ca):-

Calcium is important in mitotic spindle formation during cell division, controls growth, involved in protein metabolism, activation of enzymes of carbohydrate metabolism, required for physical integrity and normal functioning of cell membrane, maintains the ionic balance, cellular organizations and regulates auxin transport, absorption and translocation of...

Reiss and Herth (1998) reported that calcium was positively influencing the yield, quality, growth, dry matter, chlorophyll contents, sugars, starch, phenols, protein and nitrogen contents. Cousins et al. (2002) reported that in sorghum grown under drought condition, Ca enhances by the photochemical light energy usage.

Nasir-Ud-Din (1972) reported that Ca content was increased in sorghum by PEG induced water stress. They further noted that in water stressed sorghum N and P were decreased but Ca and Mg highly accumulated.

Magnesium(Mg):-

The effect of magnesium nutrition on chlorophyll content was studied by Bogorad (1966) and Ananthanarayana and Rao (1980). Magnesium acts as cofactor in biosynthesis of sucrose and activates RuBPCase and PEPCase enhancing the net assimilation rate (NAR). Nason (1956) reported that Mg is involved in biosynthesis of nucleic acids, protein, and carbohydrates. It also regulates the uptake of other nutrients (Willstates, 1960).

Eck and Musick (1979) reported decrease in Mg content which was correlated with reduced dry matter production and grain yield in sorghum under water stress. Nasir-Ud-Din (1972) reported that in water stressed sorghum the content of Ca and Mg were accumulated. As Mg plays a key role in chlorophyll synthesis, its decrease under water stress will affect negatively the chlorophyll content (Willstates., 1960).

Chetti et al. (2003) studied the influence of Mg nutrition and moisture regimes on photosynthesis, yield and quality in groundnut. They have concluded that with higher Mg level even under drought stress the photosynthetic rate, stomatal conductance, RWC, NR activity and chlorophyll contents were increased. Further they concluded that Mg is having positive role in overcoming the effect of water stress.

2.10 Influence of water stress on enzymatic activities and protein profile:-

Antioxidant enzymes:-

In response to stress plants produce reactive oxygen species (ROS) that cause serve damage to the tissues and/or signal the start of physiological defense responses (Dat et al. 2000). These ROS like superoxide radical, hydrogen peroxide, hydroxyl radical and others together constitute oxidative stress (Rao et al. 1996, Dai et al. 1997, Mackerness et al. 2001). To get rid from this stressed plants develop enzymatic defense which includes superoxide dismutase, peroxidase, polyphenol oxidase etc. while the non-enzymatic defense includes antioxidants such as glutathione, ascorbate, α-tocopherol, carotenoids, proline etc.
Superoxide dismutase (SOD) is a major scavenger of superoxide \( \text{O}_2^- \) and its enzymatic action results in the formation of hydrogen peroxide \( \text{H}_2\text{O}_2 \) and oxygen \( \text{O}_2 \). The \( \text{H}_2\text{O}_2 \) produced is then scavenged by ascorbate peroxidase (APOX). The peroxidase and polyphenol oxidase (PPO) utilize \( \text{H}_2\text{O}_2 \) to produce lignin compounds for cell wall. Peroxidase and polyphenol oxidase are oxidoreductive enzymes and their role in plant defense has been attributed to their ability to oxidize phenols in plants. Water stress and UV-B radiation induced changes in antioxidant contents have been reported by various workers (Sairam et al. 1998a, Bartoli et al. 1999, Santos et al. 1999, Costa et al. 2002, Agarwal and Pandey 2003).

Lipid peroxidation is often used as an index of oxidative stress. Hydrogen peroxide, a toxic compound produced as a result of scavenging of superoxide radical, is injurious to cell/plant resulting in lipid peroxidation and membrane injury.

The capacity and activity of the antioxidative defense system are important in limiting oxidative damage and in destroying active oxygen species (Arora et al. 2002).

**Superoxide dismutase [EC 1.15.1.1]:**

All the activated oxygen species are extremely reactive and cytotoxic in all organisms. Peroxidation damage the plasma membrane leading to leakage of cellular contents, rapid desiccation, cell death, affect respiratory activity in mitochondria, cause pigment breakdown, and loss of carbon fixing ability (Scandalios, 1993).

Deshmukh and Dhumal (2005) reported that under water deficit condition SOD activity at anthesis stage was increased in promising cultivars of sorghum. Durai et al. (2004) reported that the activity of SOD was located in bundle sheath cells of sorghum under water stress. Jiang et al. (1991) and Ming et al. (2003) reported stimulated activity of SOD in rice and liquorice. Thippeswamy et al. (2005) reported that during water stress i.e. 100, 75, 50 and 20% FC level the activity of SOD was increased in safflower.

However, the depression in SOD activity was reported by Zhang and Kirkham (1994); Dubey and Chandra (2003) and Quartacci and Navari Izzo (1992) and Agarwal and Pandey (2003). Water stress did not influence SOD activity under moderate stress in sorghum (Zhang and Kirkham 1996).

**Peroxidase (EC 1.11.1.7):**

Peroxidase catalyses the dehydrogenation of a large number of organic compounds such as phenols, aromatic amines, hydroquinones etc. Deshmukh and Dhumal (2005) reported that under water deficit condition PER activity at anthesis stage was increased in promising cultivars of sorghum. Similarly Deshmukh, (2000); Deshmukh and Dhumal (2005a) also reported that under PEG induced water stress PER activity was increased in
fifteen days old seedlings of sorghum. According to Durai et al. (2004) the activity of peroxidase is located in bundle sheath cells of sorghum under water stress. Sarkar et al., (1989) reported that the polyethylene glycol-6000, -1 Mpa treatment up to 72 hours increased the activity of peroxidase. The increased activities of peroxidase were directly proportional to the duration of water stress. Li et al., (1994) studied the effects of PEG induced osmotic stress, on protective enzyme activities and their relationship to drought resistance in maize. They further noted that the activities of superoxide dismutase (SOD), catalase (CAT) and peroxidase increased slowly under severe osmotic stress and indicated higher activities of protective enzymes for providing the mechanisms of drought resistance in hybrid maize. Zhang and Kirkham, (1994) recorded increased activity of peroxidase under drought stress in hexaploid species of wheat. Similar observation was noted by Ashraf et al., (1994) in wheat under drought condition. Jha and Singh, (1997); Jiang et al. (1991) reported significant increase in peroxidase activity with increased moisture stress in rice seedlings. Garcia et al. (1992) in maize seedlings noted that both water stress and salt stress had induced increase in peroxidase activity.

Chakraborty et al. (2001) reported significant stimulation in PER activity in tea leaves under stress condition. The levels of stimulation of enzymatic activity was correlated with drought tolerance or susceptible nature of the cultivar.

According to Allen (1995) modulation in the activity of peroxidase is important in the resistance of a plant to environmental stresses because tolerant genotypes must have highly active system to guard against oxidative injury. Sairam et al., (1997); Sairam and Saxena (2000); Zhang and Kirkham (1996) noted increased peroxidase activity in wheat and sorghum genotypes. Kraus et al. (1995); Jagtap and Bhargava, (1995) claimed that PER activity is correlated with relative tolerance/susceptibility of the crop.

Dubey and Chandra (2003); Agarwal and Pandey (2003) noted increased peroxidase activity under stress in Cenchrus and Cassia respectively. Rao et al. (2003) and Ming et al. (2003) reported increased PER activity during water stress in grain legumes and liquorice.

Similarly Joshi et al. (2005); Chander et al., (2003); Thippeswamy et al. (2005) reported stimulated activity of peroxidase with increasing water stress in wheat, chilly and safflower.
**Polyphenol oxidase** *(EC 1.14.18.1):-*

It catalyzes the aerobic oxidation of certain phenolic substrates to quinons, which are autooxidized to dark brown pigments generally known as melanins. The polyphenol oxidase (PPO) comprises of catecol oxidase and laccase which are important in plant defense.

Deshmukh, (2000); Deshmukh and Dhumal (2005a) reported that under PEG induced water stress PPO activity was increased in fifteen days old seedlings of sorghum. Chakraborty *et al.* (2001) reported significant stimulation in PPO activities in tea leaves under water stress and the increased activities were correlated with drought tolerance or susceptible nature.

Significant increase in polyphenol oxidase activity was reported in rice subjected to various environmental stresses (Subhashini and Reddy, 1990). Similarly Agarwal and Pandey (2003) also reported that in *Cassia angustifolia* the polyphenol oxidase activity was increased significantly under water stress.

**Enzyme of nitrogen metabolism:**

**Nitrate reductase enzyme** *(NADH – Nitrate Oxidoreductase- EC 1.6.6.1):-*

The NADH-dependent nitrate reductase is most prevalent in plants. The reduced transpirational pull and water potential during the water stress caused decline in NR activity in wheat (Sairam *et al.*, 1989) and sorghum (Sivaramakrishnan *et al.*, 1988).

Deshmukh, (2000); Deshmukh and Dhumal (2005a) reported that under PEG induced water stress NR activity was decreased in fifteen days old seedlings of sorghum. Similarly Satbhai *et al.* (1997); Jadhav *et al.* (2001); Bharambe and Joshi, (1993) reported decreased nitrate reductase activity in different cultivars of sorghum under water stress.

Foyer *et al.* (1998); Garcia *et al.* (1992) reported decreased nitrate reductase activity under water stress in wheat and maize genotypes. Many workers like Sharma *et al.*, (1990); Gharti-Chhetri and Lales (1990) noted that in groundnut and wheat the nitrate reductase activity was reduced with drought stress respectively. Recently Burman *et al.* (2005) reported significant decrease in NR activity under different intensities of water stress in clusterbean at pre-flowering stage.

**Photosynthetic enzyme:**

**PEPCase (EC 4.1.1.31):**

It is the most important carboxylating enzyme in C4 plants. Its activity is adversely affected by all types of abiotic stresses. Harmut and Babani, (2000); Earl and Tollenaar, (1999); Jagtap *et al.* (1998) and Garrity *et al.* (1984) reported decreased PEP Case activity under water stress in all sorghum varieties they have studied. Gujrathi *et al.* (1984) reported decreased activities of PEPCase and RuBPCase in sorghum and ground nut cultivars under...
water stress. However, Kumar and Gupta (1986) reported that PEPCase activity was increased and RuBp Case activity was decreased with decreasing leaf water potential in sorghum.

Bhatt and Rao (2005) noted that in okra water stress caused significant decrease in PEP Case activity. Similar was the observation of Sepehri and Sanavy, (2003) in maize.

2.11 Protein profile:-
Jadhav et al. (2001) reported that due to water stress in rabi sorghum, the soluble protein showed 10 to 11 new bands of different molecular weight ranging from 6 to 68 KD. They further noted that the extra band of new protein subunits (33 KD) was due to drought stress, similarly Ahire et al. (2005) noted a newly synthesized protein band of ≈17.78 KD and ≈36.3 KD size in drought tolerant cultivar of chick pea. Tyagi et al. (1995) also reported a new polypeptide band ≈22 KD in Lathyrus sativus during drought stress. Similarly Close et al. (1993) identified a dehydrin protein of ≈17 KD size in drought tolerant maize. Lee et al. (2002) also reported a 18 KD band as a major protein band induced by drought stress in white clover.

According to Bewley et al .(1983) the quantitative differences with some proteins exhibiting more intense synthesis under stress and some with reduced synthesis. Similarly Shinozaki and Yamaguchi- shinozaki (1997) also explained the genes induced during drought stress function not only in protecting the cell from water deficit by the production of important metabolite but also regulate the genes for signal transduction in drought stress response. Dure et al. (1989) postulated that such type of newly synthesized proteins may be belonging to a family of hydrophilic proteins, which play a role in the acquisition of desiccation tolerance or water stress tolerance.

Thus the overall review on different aspects in sorghum under water stress conditions indicate that till today very scanty work has been done on this cereal crop of the world. There are many biochemical, physiological, enzymological and morpho- physiological markers available for screening towards water stress tolerance in various crops including sorghum, but still today biotechnological work is not yet sufficient and hence it should be focused in future.