CHAPTER : 1   INTRODUCTION

The decrease in rainfall due to world-wide climatic shifts has resulted in the reduction of crop yield in semi-arid areas of the world, which presently affects approximately 3.6 billion ha (25% of upland in the world) in semi-arid and arid areas (United Nations Environment Program 1991). In these areas, desertification and population growth is expected to exacerbate food shortage. Pearl millet [*Pennisetum glaucum* (L.) R. Br.] (Known under synonyms: *P. americanum* (L.) Leeke or *P. typhoides* (Burm) Stapf and C.E. Hubb.), is an important cereal of traditional farming systems in tropical and subtropical Asia and sub-Saharan Africa. It accounts as the sixth most important crop after wheat, rice, maize, barley and sorghum in terms of annual global production (FAO 1992). Pearl millet is the staple food grain with a high nutritive value and is also used as a feed, fodder, construction material. Its utilization as a source of bio fuel is being explored (Wu *et al*., 2006). It is grown on 29 million ha (FAO 2005) in Africa and Indian sub-continent supporting millions of poor rural families mostly in the drought-prone areas where rain fed agriculture is commonly practiced. Pearl millet is the fourth most important cereal crop in India, after rice, wheat and sorghum, which are widely grown in the states of Rajasthan, Maharashtra, Gujarat and Haryana where the food security of the population depends immensely on pearl millet production. The average agricultural area sowed under pearl millet in India is approximately to 9.5 Mha with an average annual grain production of 8.3 M tons (FAO 2005). Pearl millet is known to yield up to 6 t/ha, but due to environmental conditions the common average grain yields are lowered to 800-600 kg/ha (FAO 2005, 2010). Today pearl millet is grown on more than of land 260,000 km² worldwide. The crop is capable of thriving very well in drought areas (Kumar, 1989). Due to highly tolerance to various abiotic stress, pearl millet is be explored (Ferraris, 1973; Maiti *et al.,*1995), as a source of desirable genes for breeding plants suited to excessive drought conditions.

1.1 Importance of Pearl millet

Pearl millet is well adapted to production systems characterized by low rainfall, low soil fertility, and high temperature, and thus can be grown in areas where other cereal crops, such as rice wheat or maize, would not survive. Its rapid growth rate in favorable conditions, high temperature tolerance and ability to extract mineral nutrition and water from even the least fertile soil make it an ideal crop to be grown in even the most hostile agricultural production environments across the world. Information of the genetic constitution for the understanding of different traits of any crop is important for the systematic breeding and long term improvement. Pearl millet is amenable to genetic studies owing to its low chromosome number, availability of an impressive range of variation for several morphological characters, ease of selfing and deliberate crossing, production of both selfed and crossed seed in good quantities, relatively short life cycle and more importantly adaptation to adverse climatic conditions (Vinchon, 1949; Burton and Powell, 1968). Despite this importance, pearl millet is considered as an orphan crop because of its vast untapped potential. As a result, it lags behind sorghum and other major grains in terms of its genetic manipulations. Further, grain yields of pearl millet are limited by the poor inherent fertility and water-holding capacity of soil on which it is grown along with traditional management practices, including little use of fertilizers. Below-optimum levels of limitations are also caused by environments having recurrent droughts, soil salinity, insect pests, diseases, and the root parasites. Another major production constraint is the low yield potential of traditional land race varieties.

1.2 Development of Growth Stages (GS) in Pearl Millet

The growth cycle of pearl millet divided into three major developmental phases: the vegetative phase (GS-1)-from emergence to panicle (floral) initiation of the main stem, the panicle development phase (GS-2)-from panicle initiation to flowering of the main stem and the grain-filling phase (GS-3) from flowering to the end of the grain-filling period (physiological maturity) of the crop (Maiti, and Bidinger, 1981).

GS-1: Vegetative phase: This phase starts with the emergence of the seedlings and continues up to the point of panicle initiation. During this phase, the seedlings, primary

root system (seminal roots) and adventitious roots are produced. All leaves are initiated during GS phase. In early varieties, six or seven leaves (including the embryonic leaves) are fully expanded by the end of this phase. Buds are formed, leaf primordial is initiated, and several tillers emerge by the end of the phase. There is little inter node elongation, however, and the apical meristem remains at or below the soil surface (Maiti and Bidinger, 1981).Dry-matter accumulation is almost entirely confined to leaves and roots. Floral or panicle initiation is marked by the elongation of the apical dome and the formation of a constriction. The size of the apex at floral initiation ranges from as little as 0.5 mm in early varieties to as much as 1.0 mm in late varieties in which floral initiation may not occur until 50-80 days after sowing.

GS-2: Panicle development phase: During this phase all the remaining leaves expand fully and the already extended leaves at the base of the stem begin to senesce. Stem elongation occurs by sequential elongation of internodes beginning at the base of the stem. Tillers undergo floral initiation, leaf expansion, etc., in patterns similar to that of the main stem. The first-formed tillers follow the main stem closely in their development, whereas the development the late tillers frequently ceases due to competition and suppression by the more advanced main stem and early tillers. Dry-matter accumulation takes place in roots, leaves, and stem (Maiti and Bidinger, 1981).

GS-3: Grain-filling phase: This phase begins with the fertilization of florets in the panicle of the main shoot and continues till the maturity of the plant (main stem and tillers).Usually the increase in total plant dry weight during this period is restricted to the grains, however in a few varieties there is also some increase in non-grain components, mainly tiller stems which amass the increase in total dry weight of the plant while the flowers grow afterwards. Senescence of the lower leaves continues and, by the end of the grain-filling phase, normally only two to four leaves remain green towards the top. Some varieties develop small tillers in the upper nodes of the stem, particularly towards the end of the grain-.filling phase. These tillers have a shorter developmental cycle than the basal tillers, producing only a few leaves and a small panicle. The end of the grain-filling phase (physiological maturity) is marked by the development of a small dark layer of tissue in the hailer region of the grain. This occurs in an individual panicle about 20-25 days after

flowering. The grain-filling period for the entire plant (i.e., from flowering of the main shoot to the end of grain filling of the tillers) is longer where tillers flower after the main panicle (Maiti and Bidinger, 1981).
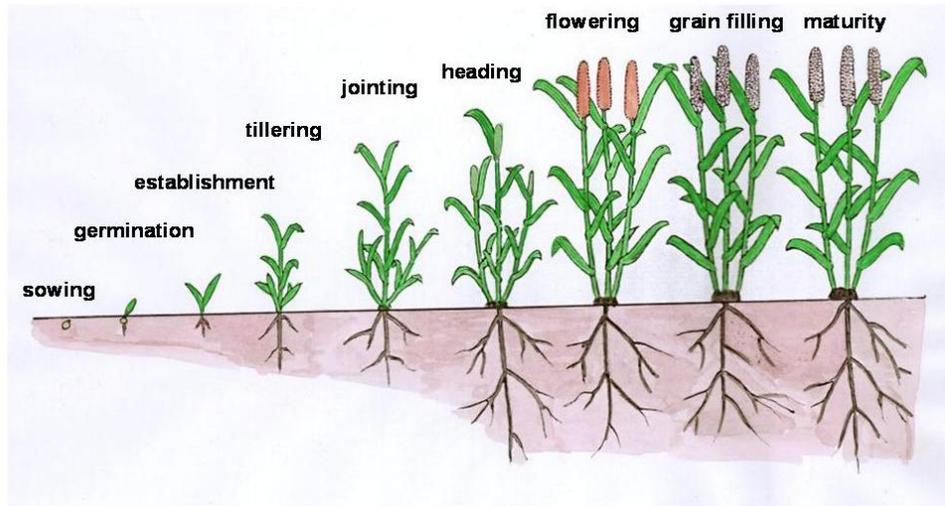

Fig.1.1 Pearl millet development stages

1.3 Classification and Domestication of Pearl millet

Taxonomically, pearl millet belongs to the Family *Poaceae* (grass family), subfamily *Panicoidae*, class monocotyledons, genus *Pennisetum* and species *glaucum*. The genus *Pennisetum* is divided in to five parts *Gymnothrix*, *Eupennisetum*, *Penicillaria*, *Heterostachy a* and *Brevivalvula* (Stapf and Hubbard, 1934). Cultivated pearl millet belongs to the section *Penicillaria*. This genus comprises of over 140 species, with chromosome numbers in multiples of X= 5, 7, 8 and 9 and diploids ranging from diploid to octaploid levels (Brunken, 1977). Sexual, apomictic and facultative apomictic, as well as annual and perennial species are included in this genus. The cultivated crop and its wild progenitors are annual, sexual diploid (2n=14), and its chromosomes are designated as the A genome (Jauhar and Hanna, 1998). Pearl millet possesses seven pairs of large chromosomes and a haploid DNA content of 2.5 pg (Bennet and Smith, 1976). The genome size of pearl millet is about five times larger than that of rice (430 M bp), larger than that of sorghum (750 M bp) and almost equal to that of maize (2400 M bp) (Arumuganathan and Earle, 1991). Cultivated pearl millet is a cross-pollinated annual C4

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crop with a protogynous flowering habit, and can be intercrossed with a large group of wild relatives (Jauhar 1981; Liu *et al*., 1994). One of the closest relative is a Napier grass (*Pennisetum purpureum* Schum.) which is sexual perennial tetraploid ($2n = 4x = 28$) with chromosomes A and B. *P. purpureum* readily hybridizes with cultivated crop species and therefore allows continuous gene flow in to a domesticated gene pools (Harlan and de Wet, 1971; Harlan, 1975).

1.4 Wide Hybridization in Pearl Millet

Wide hybridization of *Pennisetum* species for the development of valuable forage crops is extensive (Dujardin and Hanna, 1983; Sotomayor-Rios *et al*., 1989; Sobrinho, 2005). Pearl millet has been successfully crossed with numerous other *Pennisetum* species such as *P. orientale* L., *P. squamulatum* Fresen, and *P. alopecuroides* (L.).Introgression of genes from these wild progenitors could improve cold tolerance and produce germplasm that reproduce by apomixes (Hanna, 1982). In PMN hybrids pearl millet provides hybrid vigor and high quality forage while Napier grass contributes towards imparting perennial characteristics, high biomass production and photo periodism (Juahar and Hanna, 1998). In nature, both species are predominately cross-pollinated.

1.5 Breeding in Pearl Millet

The floral morphology, breeding behavior and the structure of grain yield in pearl millet make it one of the most flexible and responsive crop species to breed. It appears possible to access genetic variability both from the secondary and tertiary germplasm pools (Hanna 1990; Dujardin and Hanna 1989, 1990).

Pearl millet is a naturally cross-pollinating species, which is achieved through progeny, since all the sessile flowers on each head are perfect (i.e. both male and female fertile). On any one head, all flowers first exert stigmas over a 1 to 3 day period progressing from the mid-top to the bottom of the head. Anthesis occurs one to as many as 4 days later, in the same sequence from the same flowers, and sometimes, later from the pedicel late flowers. Thus, there is a period for each head, when flowers can only be fertilized by external pollen which is freely wind-born. Stigmas wither about 8 h after pollination.

Self-pollination can occur when stigma emergence on later flowering tillers overlaps with the anthesis of earlier heads on the same plant. In random-mating situations (as in landrace cultivar populations or breeder created populations synthetics or composites), the amount of self-pollination (considerations of common parentage and effective population size apart) is influenced by the degree of tillering, relative size and flowering relationships of tillers, and whether all or only primary tillers are harvested. As a generality, about 20% selfing is normal (Burton, 1974; Chirwa, 1991). Heterotic effects in pearl millet are large and most completely expressed in single crosses, though yields from top cross hybrids are similar in all but the highest yielding situations. Top cross hybrids have several advantages including stability and durability of performance and ease of production (Andrews, 1986).

1.6 Molecular mapping in Pearl Millet

Molecular mapping has proved to be a vital tool in the on-going endeavors to improve pearl millet. In 1994, the first RFLP-based genetic map of the pearl millet genome was produced (Liu *et al.,* 1994) and within a year scientists were able to map genes that conferred resistance to downy mildew, which is the most important disease of the crop. Since then, more than 600 molecular markers are developed and mapped, and consequently using these marker-assisted selections, additional diseases resistance and drought tolerance genes have been incorporated into elite hybrid parental lines to strengthen the crops to be naturally resistant to diseases and abiotic stresses. The first product of such marker-assisted selection was "HHB 67 Improved" that was released for cultivation in 2005 (Gazette of India, 2005; Hash *et al.*, 2004).

1.7 Grain quality and Feed value of Pearl Millet

Pearl millet is equivalent to maize and generally superior to sorghum in protein content and quality, protein efficiency ratio (PER) values, and metabolizable energy levels (Hoseney *et al*. 1987; Rooney and McDonough, 1987; Serna-Saldivar, *et al*., 1990; Sullivan *et al*., 1990 and Bramel-Cox *et al*., 1992). Pearl millet does not contain any condensed polyphenols such as tannins in sorghum that can interfere with or slow down

digestibility. Smith *et al*. (1989) reported that pearl millet can replace maize in chick diets without affecting weight gain or feed efficiency. Tribble *et al*., (1986) reported that they were also able to substitute pearl millet for sorghum in sorghum based diets for growing pigs without affecting performance. Calder (1955, 1961) had previously concluded that pearl millet was suitable for pig feeding.

In general, feeding test results support data from biochemical analyses which indicate that pearl millet is similar to maize and superior to sorghum as a feed grain. Pearl millet grain generally has a higher crude protein level by 1 to 2 percentage points relative to sorghum grown with similar cultural practices. Pearl millet is still deficient in essential amino acids, but averages 35% more lysine than sorghum (Rooney and McDonough, 1987). Pearl millet grain has 5 to 6% oil and a lower proportion of the less digestible cross-linked prolamins (Jambunathan and Subramaniam, 1988). These differences can be partly attributed to the different structure of the kernel. The proportion of germ in pearl millet grain (17%) is about double that of sorghum, while the endosperm accounts for 75% as against 82% in sorghum. Works on bio-fortification of pearl millet are also progressing with the aim of meeting the nutritional requirements of poor people especially children for whom pearl millet is the only food grain available (Kodkany *et al*., 2013)

1.8 Cultivation of Pearl Millet

Cultivating, harvesting, and handling a pearl millet crop for grain with existing equipment and in ways similar to current farming practices, will be important for its successful adoption. Hybrid plant types are being bred with this in mind. Pearl millet establishes best when sown slightly shallower than sorghum in well prepared warmer seed beds on well-drained soils. Plant densities should be higher (100,000 to 175,000 plants/ha). The grain is tougher and dense, can be easily combined when well dried using higher cylinder speeds, more air and adjusting the screens for the smaller seed size.

The species' primary and secondary centers of diversity include a diffuse belt stretching from the Saharan plateau region to western Sudan and east India, respectively (ICRISAT, 2011). The International Crop Research Institute for the Semi-Arid Tropics (ICRISAT)

germplasm collection consists of 21,594 accessions of cultivated pearl millet from 50 different countries, and it is the largest collection of pearl millet germplasm in the world. More than 25 million ha of pearl millet are cultivated in Africa and East Asia where it is a staple grain crop. Because of its wide range of adaptation, pearl millet has been labeled the world's toughest crop in arid environments and on marginal soils (ICRISAT, 2011). Because of its wide range of adaptation, pearl millet has been labeled the world's hardiest crop in arid environments and on marginal soils (ICRISAT, 2011). There is a wide range of variability in the ICRISAT collection, and it includes collections from institutions (10,201), farmers' fields (6,537), commercial markets (1,681), farmer co-ops (1,357), and threshing floors (479) (ICRSAT, 2011).

History of the pearl is diverse and difficult to track, but it seems to have originated in China and Africa. Pearl millet probably originated from western tropical Africa more than 3000 years ago and from there spread across Africa and South Asia. However, there is another school of thought regarding origin of millet that believes that pearl millet may have originated in the Abyssinia region (present-day Ethiopia).

1.9 STRESS

Abiotic stresses such as drought, salinity, cold and heat stress pose a major concern for agricultural production. With the changing global climate these environmental stresses are becoming of paramount importance because of their potential adverse impacts on agriculture and food security. The predicted increase of 60 % in cereal production (Rosegrant and Cline, 2003) will certainly not be able to meet the food demand of 8.7 ~ 11.3 billion world population by 2050 (Bengtsson *et al.*, 2006). As the world population grows exponentially, it is becoming the increase agricultural productivity and to expand productive areas of the world into harsher environments (Maestri *et al.,* 2002). Unfortunately, most of the land with agricultural potential is in areas with such environments.

Dehydration stress imparted by drought, salinity and temperature severity is the most prevalent abiotic stress that limits plant growth and productivity. Stress is an altered physiological condition caused by factors that tend to disrupt the equilibrium. Strain is

any physical and chemical change produced by a stress. Plants are sessile organisms. Consequently they have to adapt constantly to fluctuations in the environment. The fluctuations can range between moderate and unfavorable, and the factors can be of biotic or abiotic origin. (Zhu and Coleman, 2001). The environmental factors may reach levels which represent stress for the plant. Stress in plants includes any factors that could lead to the death of the plant. Stress being a constraint or highly unpredictable fluctuations imposed on regular metabolic patterns cause injury, disease or aberrant physiology. Plants are frequently exposed to many stresses such as drought, low temperature, salt, flooding, heat, oxidative stress and heavy metal toxicity, while growing in nature (Vorasoot *et al*., 2003; Jaleel *et al*., 2009; Thakur *et al*., 2010)
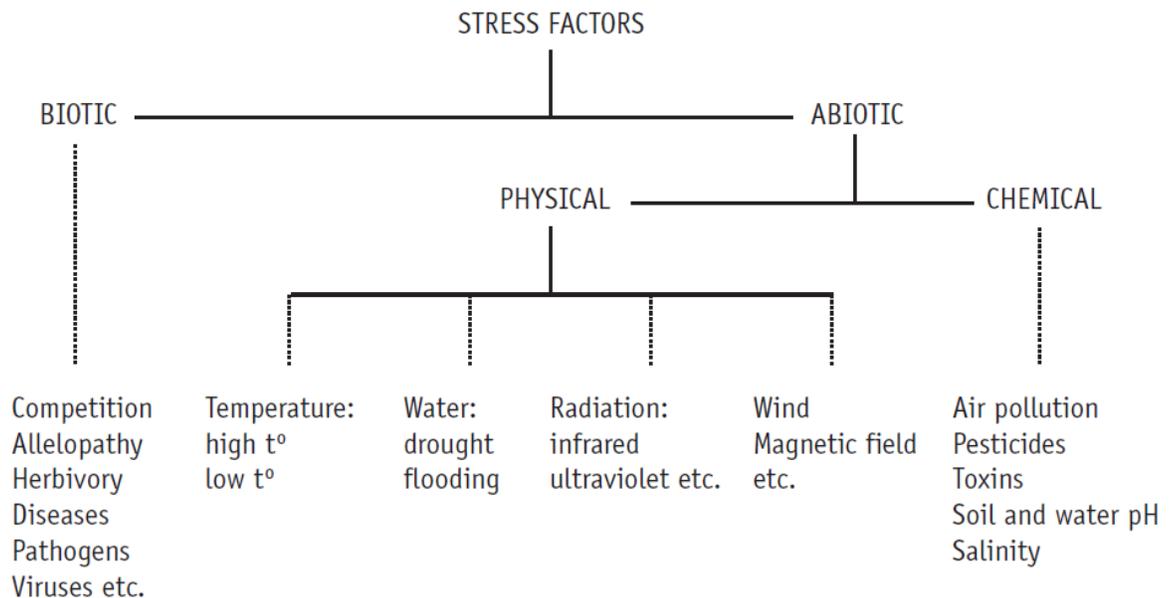

Fig: 1.2 Sources of environmental stress in plants (Schubert; 1985; Nilsen & Ocrutt; 1986)

1.9.1   Water Logging Stress

Soil water logging has long been identified as a major abiotic stress and the constraints it imposes on roots have marked effects on plant growth and development. When such events take place in the spring, they can greatly reduce seed germination and seedling establishment. Thus, soil water logging is an important factor affecting the growth,

development and survival of numerous plant species, not only in natural ecosystems but also in agricultural and horticultural systems (Das*et al.,* 2006). As water saturates the soil pores, gases are displaced, a reduction in gas diffusion occurs and phytotoxic compounds accumulate as anaerobic conditions prevail. All these changes greatly affect the capacity of a plant to survive such conditions. In response, the stomatal resistance increases, photosynthesis and root hydraulic conductivity decline, and the translocation of photo assimilates is reduced (Subbaiah and Sachs, 2003).

- **Physiological changes in plant during Soil water logging**

Soil water logging may not only increase stomatal resistance but also limit water uptake, thus in term leading to internal water deficit.The decrease in Lp may be linked to aquaporin gating by cytosolic pH (Tournaire-Roux *et al.,* 2003). Evidence suggests that the regulation of plasma membrane intrinsic proteins (PIPs) by pH is especially relevant under anoxic conditions (Postaire *et al.*2007), as a conserved histidine residue at position 197 in the intracellular Loop D has been identified to be the major pH sensing site under physiological conditions (Tournaire-Roux *et al*., 2003; Kaldenhoff and Fischer 2006; Secchi *et al.*, 2007). In fact, down regulation of aquaporin genes is commonly associated with a decline in root Lp as aquaporins control radial water movement in the roots (North *et al.*, 2004; Vandeleur *et al.,* 2005). Thus, it seems that the reduced Lp throughout the plant under soil water logging conditions is most probably linked to inhibition of water transport by aquaporins, though in depth studies on the effect of aquaporin on whole plant water regulation during soil water logging are still lacking. Furthermore, the reduction in radial water movement may in part be explained by the presence of cross-sectional oxygen gradients in the root tissue. Indeed, there is clear evidence that in flooded soils, an O_2 gradient exists between the stele, which may be under anoxic conditions, and the cortical cells which may only be under hypoxic conditions (Thomson and Greenway 1991; Colmer, 2003). Thus, these differences in tissue micro environment may also contribute to cross-sectional differences in cellular energy levels and subsequent declines in root Lp. When the stress is prolonged it may lead to the inhibition of photosynthetic activity of the mesophyll (Huang *et al.,* 1994; Pezeshki *et al.,* 1996), as well as reductions in the metabolic activity and the translocation

of photo assimilates (Pezeshki, 1996; Pezeshki, 2001; Sachs and Vartapetian, 2007). The outcome of a decline in photosynthesis on plant growth and development may be dramatic and it may lead to concurrent physiological dysfunctions such as the inhibition of water transport. Indeed, waterlogged soils tend to reduce the translocation of photosynthetic products from "source" leaves to "sink" roots (Barta and Sulc 2002; Yordanova *et a.,* 2004). As a result, the maintenance of photosynthetic activity and accumulation of solublesugars to roots is clearly an important adaptation to flooding (Chen *et al.,* 2005).
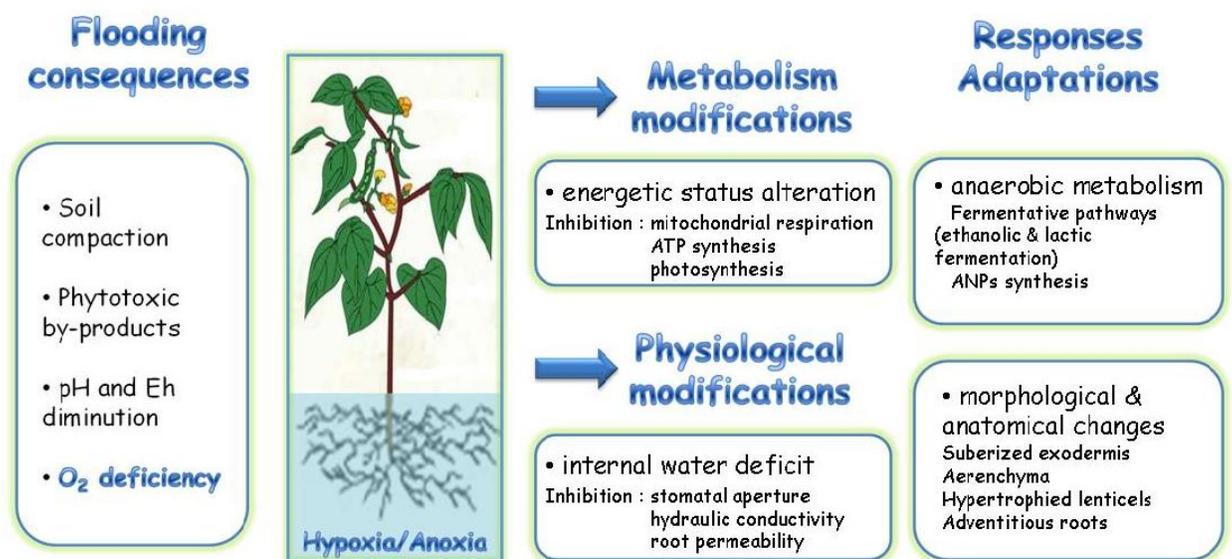

Fig.1.3 Main physio-chemical events taking place in the rhizosphere during soil water logging and the resulting modifications in plant metabolism and physiology followed by the initiation of adaptive responses.

1.9.2 Cold stress

Each plant has its unique set of temperature requirements, which are optimum for its proper growth and development. A set of temperature conditions, which are optimum for one plant may be stressful for another plant. Many plants, especially those, which are native to warm habitat, exhibit symptoms of injury when exposed to low non-freezing temperatures.(Ramankutty *et.al.,* 2008) These plants including maize (*Zea mays*), soybean (*Glycine max*), cotton (*Gossypium hirsutum*), tomato (*Lycopersicon esculentum*) and banana

(*Musa sp*.) are in particular sensitive to temperatures below10–15 °C and exhibit signs of injury. Various phenotypic symptoms in response to chilling stress include reduced leaf expansion, wilting, chlorosis (yellowing of leaves) and may lead to necrosis (death of tissue). Chilling also severely hampers the reproductive development of plants for example exposure of rice plants to chilling temperature at the time of anthesis (floral opening) leads to sterility in flowers. The major malicious effect of freezing is that it induces severe membrane damage. This damage is largely due to the acute dehydration associated with freezing (Thomashow, 1999)

1.9.3 Salinity Stress

Soil salinity is one of the major abiotic stresses that adversely affect crop productivity and quality. The problem of soil salinity is further aggravated through the use of poor quality water for irrigation and inadequate drainage. Soil type and environmental factors, such as vapor pressure deficit, radiation and temperature may also alter salt tolerance (Hussain *et al*., 2008).Two major approaches being used to improve stress tolerance are: (1) Exploitation of natural genetic variations and (2) generation of transgenic plants with novel genes or altered expression levels of the existing genes (Zhang et al., 2004; Zhu, 2001, 2002) recently reviewed signaling and transcriptional control in plants under salt stress.

1.9.4 Drought stress

Under both natural and agricultural conditions plants are often exposed to various environmental Stresses. Drought is one of most important environmental factors inhibiting photosynthesis and decreasing growth and productivity of plants. It is one of the major causes of crop loss worldwide, reducing average yields for most major crop plants by more than 50% (Bray *et al.,* 2000; Wang *et al.,* 2003). Under these stress conditions usually a water deficit in plant tissues develops. In the last years effects of water deficit were studied on different levels from physiology to cell metabolism (Shinozaki and Yamaguchi-Shinogzaki, 1997; Chaves *et al.,* 2003).

In semi-arid countries of the world including India, water scarcity is one of the chief abiotic factors which regulate the crop productivity. Comparatively unpredictable and altering climatic conditions are also responsible for drought stresses, the incidence of which is due to increase severely in the near future owing to escalating global warming and ever-growing industrialization. As a result it has become imperative to develop crop plants with extended adaptive capacities to arid environments in order to build sustainable and equitable food security system (Boutraa and Sanders, 2001). In agriculture, drought resistance refers to the ability of a crop plant to produce its economic product with minimum loss in water deficit environment relative to the water constraint free management production (Rosegrant and Cline, 2003). Drought resistance is the interactive result of different morphological, physiological and biochemical traits. Drought exacerbates the effect of the other stresses to which plants are submitted (abiotic or biotic), where several different abiotic stresses result in water stress (like salt and cold stresses) (Turner, 1986; Ludlow and Muchow, 1990). Being sessile organisms, plants have to cope with drought stress at least once in its life time. They have however evolved mechanisms that allow them to adapt and survive periods of water deficit at some level. According to the type of strategy adopted, plants are said to escape, avoid or tolerate drought stress, although these are not mutually exclusive. The plant drought response will depend on the species inherent "strategy" but also on the duration and severity of the drought period (Bidinger *et al*., 1987).If drought stays for a long time it may cause oxidative damage due to the over production of reactive oxygen species (Araus, 2002). As reactive oxygen species are a common denominator in the detrimental effects of several environmental stresses, therefore the antioxidant genes involved in the detoxification of reactive oxygen species could be a potential source of stress tolerance in crop plants.

There are basically three levels of plant drought tolerance/resistance.

- **Drought escape**: The mean of drought escape is avoiding living in stress full times. Some plants have adapted by having rapid growth, maturation, flowering and fruiting. This keeps tissues from being excessively exposed to dehydration e.g. winter wheat.

- **Drought avoidance**: is the ability of plants to maintain relatively high tissue water potential despite a shortage of soil moisture.
- **Drought tolerance**: is the ability to withstand water deficit with low tissue water potential.

Mechanism for improving water uptake, storing in plant cell and reducing water loss confer drought avoidance. The responses of plants to tissue water deficit determine their level of drought tolerance.

Drought resistance is a complex trait, expression of which depends on action and interaction of different morphological (earliness, reduced leaf area, leaf rolling, wax content, efficient rooting system, awn, stability in yield and reduced tillering), physiological (reduced transpiration, high water-use efficiency, stomatal closure and osmotic adjustment) and biochemical (accumulation of proline, polyamine, trehalose, etc., increased nitrate reductase activity and increased storage of carbohydrate) characteristics (Ozturk, 1998; Monti, 1986). The inheritance pattern and nature of gene action have been reported which are responsible for morphological and physiological traits. Polygenic inheritance of root characters is reported by Ekanayake *et al.,* 1985.The long root and high root numbers are controlled by dominant alleles and thick root tip by recessive alleles. However, leaf rolling and osmotic adjustment (Morgan 1991) has shown monogenic inheritance. Gene *Drt*I in rice is linked with genes for plant height, pigmentation, hull colour and awn, and has pleiotropic effect on the root system.

A variety of genes have been reported to respond to drought condition in various species. Genes induced during drought condition are to function not only in protecting cell from water deficit by the production of important metabolic proteins but also in the regulation of genes for signal transduction in the water stress response (Shinozaki and Shinozaki-Yamaguchi, 1997). Thus these gene products are classified into two groups:

(i) Functional protein
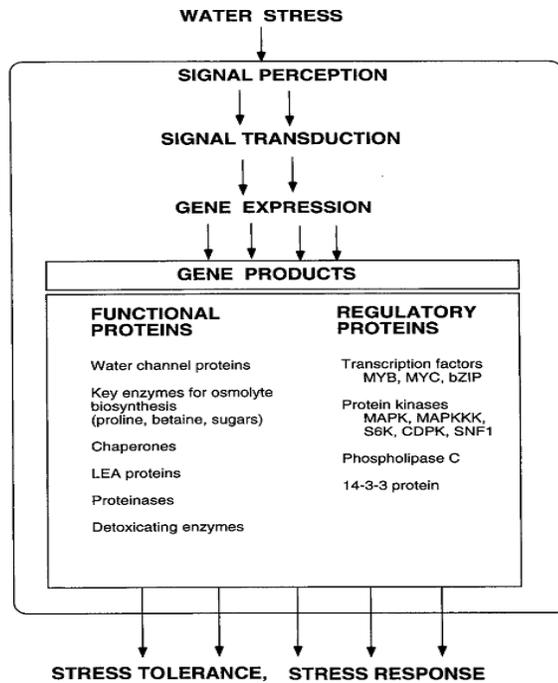
(ii) Regulatory proteins

Fig: 1.4 Regulation of genes for signal transduction in the water stress response and Stress Tolerance.

- **Functional proteins:**

Water channel protein and enzymes mainly constitute the functional proteins that work towards reducing the water stress. Water channel proteins are involved in the movement of water through membrane, whereas enzymes are required for the biosynthesis of various osmoprotectants (Kishor *et al.,* 1995).

Table 1.1: List of some functional protein for drought stress

Gene	Enzyme	Function
TPS1	Trehalose-6-phosphate	Biosynthesis of trehalose synthetase
P5CS	Pyrroline-5-carboxylate synthetase	Proline synthesis
Beta	Choline dehydrogenase	Biosynthesis of glycine betaine
BetB	Betaine aldehyde dehydrogenase	Accumulation of glycine
ADC	Argeninede carboxylase	Polyamine biosynthesis
SAMDC	S-Adenosyl-methionine decarboxylase	Polyamine biosynthesis

- **Regulatory proteins:** This group contains protein factor involved in the further regulation of signal transduction and gene expression that probably function in stress response (protein kinases, transcription factors, *PLC* etc.) Turgor pressure of plant cells is subjected to feedback in response to changes in external osmotic pressure. The cytoplasmic Ca^{++} ions signal transduction pathway is involved in turgor regulation in plant cells. An increase of cytoplasmic Ca^{++} ion serves to stimulate ion transport pathway under hypo osmotic stress. Stomata closure is induced by release of Ca^{++} ions into the cytoplasm. CPK4 and CPK11 are two important positive regulatorsin CDPK/calcium-mediated ABA signaling pathways (Zhu *et al.,* 2007).

1.10 Breeding for drought escape strategies

Drought escape strategy of the crops (in terms of early flowering) has been recognized as a major factor determining relative cultivar performance individual stress environments (Bidinger *et al*., 1987) and is often a major cause of Genotype ×Environment interaction especially in harsh environmental conditions (van Oosterom *et al*., 1996). The breeding for altered life cycle is extremely useful in the environments where the drought periods are highly predictable (Bernier *et al*., 2007). The basic principle of the successful implementation of this strategy into breeding is that shortening of crops life cycle ultimately translates into the relatively extended duration of grain filling stage prior to drought occurs (Bort *et al*., 1998; Richards, 2000). Nevertheless, the crops cultivars possessing the shortened life cycle might not be necessarily considered truly drought tolerant.

1.10.1 Potential of osmolytes in breeding for drought acclimation strategies

As a candidate traits in this category can stand traits related to osmotic adjustment (e.g. accumulation sugars, nitrates, simple amino acids). The primary idea of osmolytes benefit under water deficit conditions was that accumulation of these compounds could decrease the cell osmotic potential and thus maintain water absorption and cell turgor pressure, which might contribute to sustaining physiological processes, such as stomatal opening, photosynthesis and growth (Ludlow and Muchow, 1990; Blum, 1996).Osmolytes have

been emphasized as a selection criterion for yield improvement in dry environments (Zhang *et al*., 1999). Soon after this work on identification of QTLs linked to osmolyte accumulation capacity (Van Deynze *et al*., 1995; Price and Courtis, 1999; Teulat *et al*., 1998) and development of crops through trans-genesis or marker assisted breeding Nguyen *et al*., 1997) with increased levels of osmolytes was initiated. Several stable transgenic events resulting in plants over-expressing osmolytes were documented e.g.manitol (Thomas *et al*., 1995), glycine betaine (Waditee *et al*., 2003) for *A. thaliana* for soybean. Despite producing higher amounts of osmolytes, these plants showed only marginal improvement of drought tolerance. But at least, developed transgenic plants contributed to progress understanding of the osmolytes function in plant tissues.

According to current opinion, traits enhancing osmotic adjustment are of little benefit for yield in the field conditions. In fact these traits are more likely to cause early exhaustion of soil moisture (Sinclair and Serraj, 2002;Kholová*et al*., 2010) and rapid transition of plant to the survival mode where even putative benefits are of little use for growers (Blum, 2005, 2009).

1.10.2 Potential of photosynthetic pigments and anti-oxidative enzymes in breeding for drought acclimation strategies

Drought stress often causes the changes in photosynthetic pigment content and ratio (Anjum *et al*., 2003; Farooq*et al*., 2008; Messacci *et al*., 2008). The magnitude of these changes could in turn negatively influence photosynthesis and so contribute to yield losses. The potential of engineering plants to maintain the pigments content under drought was heavily discussed especially when some experiments revealed their positive correlation with the grain yield (Arun yanark *et al*. 2008). Nevertheless, the physiological constrain of pigment maintenance (especially chlorophylls) in plants facing drought simultaneously give rise to elevated production of reactive oxygen species (ROS) andcan in turn destroy other molecules in photo systems (Schmid, 2008). Therefore, unbalanced photosynthetic pigments content could in fact accelerate the damage of photo systems if ROS production is not regulated further (Maslova and Popova, 1993;Keiper *et al*., 1998; Tardy *et al*., 1998; 2003;Farrant *et al*., 2003). There are basically two detoxification mechanisms plants have developed to avoid excessive ROS production (Scandalios,

1997;Shalata and Tal, 1998; Gomez *et al*., 1999); (i) Non enzymatic radical scavengers, e.g. carotenoids, glutathione, mannitol, ascorbate, tocopherol, flavonoids and some alkaloids; (ii) Enzymatic anti-oxidants of the Hallivel6Asada cycle (Asada, 1994), which involves ROS reactions with superoxide dismutase(SOD), ascorbic peroxidase (APX) and catalase (CAT) and regeneration of substrate for APX assisted by glutathione reductase (GR). There also appeared evidence in some plant species (wheat, mangrove, sesame) tolerance to abiotic stresses could be related to enhanced capacity to scavenge ROS (Sairam and Srivastava, 2001;Parida *et al*., 2004; Fazeli *et al*., 2007). From the other hand, studies on jute, alfalfa and tomato haven't confirmed these results. There appears to be limited effort to transform plants for specific modulation of chlorophylls or anti oxidative enzymes level in order to improve their drought tolerance. However, increased chlorophyll levels were observed as a secondary effect in plants transformed with nicotianamine synthase gene enhancing iron uptake in *Lolium perenne* (Zhang,2008). These plants simultaneously showed improved drought tolerance. Similarly, rice carrying additional gene *"Triticum aestivum* salt tolerance-related gene (*TaSTRG*)" with unknown function had enhanced salt and drought tolerance accompanied by chlorophyll content maintenance (Zhou *et al*., 2008). In tobacco transformed with isopentenyl transferase, which induces cytokinins synthesis and also delay senescence, there were observed enhanced levels of several antioxidative enzymes along with enhanced drought tolerance (Rivero *et al*.,2007). Contrary to chlorophylls and antioxidative enzymes, there has been effort to produce plants with increased levels of carotenoids but more likely in order to meet then nutritional demand in human/livestock diet e.g. "Golden rice" over-expressing ß-carotene (Paine*et al*., 2005) than to increase plant drought tolerance. After all the contribution of photosynthetic pigment contents maintenance, its relation to ROS scavenging systems and finally the link to the yield stability under drought is still not clear and seems to be highly variable depending on species, developmental and metabolic state of plant, and the duration of stress (Smirnoff, 1993; Castillo, 1996).

The exact mechanism of interplay and signaling between the various genes has not yet been clearly understood at the molecular level. Thus the onus on current research is to

discover and elucidate the complex signaling pathways involved in drought (Zhou *et al.*, 2008). By inferring the origins or intersections of such signals, we might be able to discover intrinsic resistance factors that are vital in allowing plants to cope with drought stress. Such applications promise to be particularly useful in the current climatic scenario. We will need an arsenal of drought tolerant genes or better performing alleles of genes known to have role in drought tolerance, which can then be transferred to our food crops, including rice, wheat and other staples to make them "climate ready"(Varshney, *et al.,* 2011)**.**
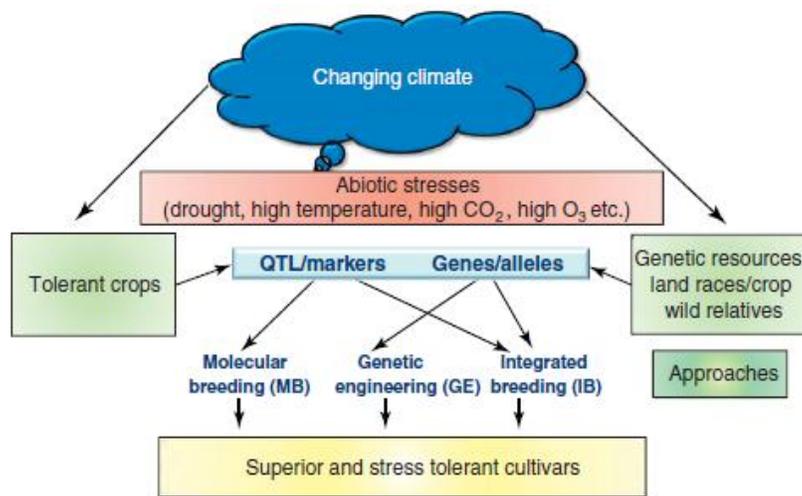

Fig: 1.5 Approaches for Climate Ready crops. (Varshney, *et al.,* 2011)

1.10.3 Potential of plant hormone regulators in breeding for drought acclimation strategies

The discussion about use of plant hormone regulators in breeding programs still persists. The debate focuses mainly on potential role of abscisic acid (ABA), which has been shown to play the role in stomata functioning (Schulze, 1986; Davies and Zhang, 1991; Bray, 2002). However, other hormones like cytokinins are also likely to be involved in the regulation of stomatal aperture, either in isolation or acting in conjunction with ABA (Wilkinson and Davies, 1999, 2002). The regulation through ABA is far from being simple and involves both long-distance transport and modulation of ABA concentration at the guard cells to a given dose of the hormone (Wilkinson and Davies, 2002). Among

the factors implicated in the ABA action are xylem sap and leaf tissue pH, which may increase in condition of high evaporative demand such as high vapor pressure deficit (VPD), high light intensity and high leaf temperature. Large inter/intra-specific variation in ABA levels has been reported (Conti *et al*.,1994; Mugo, 1999;Chandrasekar*et al*., 2000; Li and Wang, 2003; Yin *et al*., 2005;Zhang *et al*., 2005). In wheat and some woody plants, higher ABA level was correlated with drought tolerance (Chandrasekar*et al*., 2000; Li and Wang, 2003; Yin *et al*., 2005;Zhang *et al*., 2005), although no such correlation was reported in maize and sunflower(Conti *et al*., 1994; Mugo, 1999;Cellier *et al*., 1998, 2000) and in phaseolus no ABA increment was detected during drought exposure So, the ABA-tolerance link is, as expected, highly crop and environment specific. There has been also progress in identification of QTLs affecting ABA concentration under drought conditions especially in maize leaves and xylem sap (Lebreton *et al*., 1995;Landi *et al*., 2005;Tuberosa and Salvi, 2007). Some of these results suggest that ABA concentration in plant tissues might be tightly associated with rooting characteristics, especially root internal architecture and relative water content in maize plants. However, the putative effect of these QTLs on the yield under water limited condition spersist questionable (Tuberosa and Salvi, 2007). To study ABA effect to further extend, there have been developed transgenic plants over-expressing ABA under drought conditions in tobacco and A. thaliana (Borel *et al*., 2001; Thompson *et al*., 2007;Iuchi*et al*., 2001). Some of these transgenic events lead, indeed, to the enhanced plants drought tolerance (Borel*et al*., 2001;Iuchi*et al*., 2001) how ever these plants also showed delayed germination with their water management considerably altered. Logically, limitation in water usage in ABA transgenics may in turn restrict plant growth and so can be contra-productive for use in agricultural systems.

Furthermore, importance of ABA independent mechanisms coordinating plants' water use is being emphasized (Cellier *et al*., 1998, 2000; Davies *et al*., 1994; Yamaguci-Shizonaki, 1997). After all, the complexity of plant response to ABA is apparent and the selection for high capacity ABA accumulation has yet to provide conclusive data that could help shape crop breeding for drought conditions (Pekic *et al*., 1995).

1.11 Breeding for drought avoidance improvement

Breeding for drought avoidance improvement holds also putative potential for crops grown under variable environmental drought patterns. The success in drought avoidance crops improvement mostly depends on understanding the complex physiological processes of plants under drought. As a starting point for identification of the crucial mechanisms of drought avoidance in crops there has been often used the simple concept where Yield = T x TE x HI (T- amount of water transpired, TE –transpiration efficiency, HI – harvesting index; Passioura, 1977). According to the component analysis proposed by Passioura (1977, 1996) the traits convenient for breeding selection should be those increasing i) the capacity to capture more water, ii)the efficiency for producing dry matter per unit of absorbed water and iii) the ability to allocate an increase proportion of the biomass into grain. All these mechanisms were thought of as the breeding targets in various crops (e.g. groundnut, sorghum).However, it is also important to take into consideration that this formula over looks possible interactions between the parameters mentioned in the equation. In particular, it overlooks the fact that there may be stages where water utilization (T) might be critical for some other component of the equation (e.g. HI). Therefore, it appears clearer that, at least for certain crops and conditions, the timing of water utilization throughout plants development might be a principal component of drought adaptation, more important than the components of the Passioura's equation (Sinclair *et al*.,2005, Blum, 2009; Kholová *et al*., 2010). Based on recent understanding of the matter, the breeders efforts should be rather focused towards improvement of plants in well-watered conditions which can result in soil water conservation and further in availability of water in soil profile during the prolonged drought (Mortlock and Hammer, 2001; Condon *et al*., 2002; Serraj *et al*., 2004; Kholová *et al*., 2010; Sinclair, 2010). As mentioned above, basic mechanisms of drought avoidance strategies mostly relate to the plant's control of the water usage. By and large plant's use of water principally depends on the balance between the water absorption by the root system, and water, that is released through the leaves by transpiration (which is in the simplistic way the function of plant water conductivity and ambient environment).

Existing variability in root system characteristics could be well utilized in breeding programs. A deeper root system has been shown to allow crops to extract more water from the soil, resulting in higher yield potential under drought (Johansen *et al*., 1997; Kashiwagi *et al*., 2006; Bernier *et al*., 2007). Therefore, efficient regulation of root/shoot growth could be an important characteristic of drought tolerant genotypes. However, if too much carbon is invested in root growth, yield may be affected negatively. Contrarily, if enough growth is not invested in roots, plants can suffer from drought and reduced yields as well. Therefore, a balance between the two is indeed required and the contribution of root depth to drought avoidance is considered highly site specific (Bernier *et al*., 2007). Other root characteristic which can influences plants water use is the root conductivity. There has been demonstrated variability in the ability to extract water from the soil under water limited conditions in maize and some legumes species (Ray and Sinclair, 1997; Bhatnagar-Mathur *et al*., 2007; Hufstetler *et al.,* 2007). But even internal root structure (constitutive or inducible by water deficit) could influence the axial water flow and so might be considered to influence drought tolerance. In this regard, differences in xylem vessels hydraulic properties (xylem diameter and chemical composition) were shown the potential to influence drought tolerance in e.g. agave (Pena-Valdivia and Sanchez-Urdaneta, 2009), rice (Umayal*et al*., 2001) or wheat (Richards and Passioura, 1989). Furthermore, radial transport through plant tissues can also play an important role in the drought resistance. In this regard, the role of root aquaporines in the restriction/enhancement of water absorption during the crucial periods of drought is intensively studied. Aquaporines are trans membrane proteins triggering symplastic (cell-to-cell) movement of water molecules. Substantial inter- and intra-specific variability in aquaporin numbers and types has been examined (Tyerman *et al*., 2002;Javot and Maurel, 2002;Bramley *et al*., 2007).However, exact role of aquaporines in drought resistance is yet to be explored. Another basic mechanism how plants can tune the water usage through the stomata are parameters like stomata density, stomata conductivity and also sensitivity of stomata conductance to soil drying (Muchow and Sinclair, 1989; Henson *et al*., 1983;Masle *et al*., 2005),long term ABA effect on plants morphogenesis (Aasamaa*et al*., 2001,2002) or the mediation of stomata closure by hydraulic signals.

Some plant species (typically C4plants from arid and semi-arid regions) have been found sensitive to high VPD levels, usually above 3-4 kPa where the stomata close to avoid wasteful water loss during the harsh midday conditions (Squire, 1979; Sinclair *et al*., 2007; Kholová *et al*., 2010). Not much attention was paid to possible genetic variations in this strategy, although recent modeling data show that a restriction of daily transpiration rate would indeed contribute to saving water in the soil profile.

1.12 Drought tolerance in Pearl millet

At drought conditions, water potential of soil and plant decreases, at advanced stages of plant growth, turgor pressure decreases, stomata close, leaf growth and photo-synthesis rate reduce (Ozturk, 1998; Monti, 1986). Drought stress is synonymous with water stress, it happens when water lost as vapor (transpiration) is more than water taken (Sade, 2000), so a competition starts between plants for water because of the negative pressure. Drought is one of the main environmental constraints to agricultural productivity worldwide. Many efforts have been made to elucidate the mechanisms of drought tolerance in plants through molecular and genomics approaches, and a number of genes that respond to drought stress at the transcriptional level have been reported.

Pennesitum (Bajra) has ability to tolerate drought because it has some drought resistance mechanism which makes them to grow under drought. Post-flowering drought stress is one of the most important environmental factors reducing the grain yield and yield stability of pearl millet and increasing the incidence of crop failure in dry land production environments (Mahalakshmi *et al*., 1987). Terminal drought stress (flowering through grain filling) is more damaging to pearl millet productivity than stress at the vegetative or pre-flowering reproductive crop growth stages. This is because pearl millet's asynchronous tillering behavior and rapid growth rate allow it to recover rapidly from intermittent drought stress during these earlier stages of plant development, but provide no advantages under unrelieved terminal drought stress (Bidinger *et al*., 1987; Mahalakshmi *et al*., 1987). Improving the adaptation of pearl millet to terminal drought stress environments is, therefore, a major objective for breeding programmers' aimed at

improving both the crop's productivity and its yield stability (Yadav *et al*., 2002; Bidinger and Hash, 2004).

Here our aim to identify genes which are expressed under drought in *Pennesitum* (Bajra) which are not known till date. This study will be very useful from agricultural point of view because some drought tolerant genes can be identified in *Pennisetum* which can help to generate transgenic plants which are drought resistance and can survive under extreme high temperature. Pearl millet is the drought tolerant with an earlier maturity. It also tolerates low soil pH and is the staple cereal in arid and drier semi-arid regions of country. It is the only cereal crop that is capable of producing a reliable yield under the marginal environments and adverse conditions. Its nutritious grain forms the important component of human diet and its leftover forms the principal maintenance ration for ruminant livestock during the dry season.

The large variability in the timing and severity of drought stress and the inadequate understanding of its complexity have made it difficult to characterize the physiological and or phenotypic traits required for screening and improving crop performance under drought stress. This difficulty has been overcome by using Quantitative trait loci (QTL) mapping, biparental crosses, a high-resolution cross (HRC) fine-mapping population, and a germplasm population provides a means to identify gene-based markers [e.g. conserved intron spanning primers (CISPs), expressed sequence tag-single nucleotide polymorphisms (EST-SNPs), conserved orthologous sequences (COS), and EST-simple sequence repeats (SSRs)], and their subsequent association with drought tolerance using targeted genetics and association mapping approaches are also discussed.

As the genome sequence of Pearl Millet is not available, the current focus is directed towards the characterization of transcriptomes of such orphan plant species. The generation of cDNA based EST (Expressed Sequence Tags) libraries proves to be the most convenient method of studying the differential expression of genes in response to stress. The EST sequence information forms a core resource for various genome scale experiments and represents a platform for understanding the complex cellular processes and mechanisms of plant adaptation to a variety of biotic and abiotic factors (Mishra *et al.,* 2007).

A large number of housekeeping genes are perpetually expressed in all the cells whereas genes that are differentially expressed are usually present in low copies. As a consequence, the stress-specific cDNA messages, retrieved from the stressed leaf tissues RNAs, will be further diluted in cDNA libraries. This would therefore result in the repeated sequences of abundant genes which limits overall gene discovery, especially with regard to the representation of less abundant genes (Mishra *et al.,* 2007). Different strategies are used to bring the frequency of each clone within a narrow range in a given cDNA library. This helps to avoid repeated sequences of highly abundant cDNA messages while sequencing ESTs (Reddy *et al.,* 2002). However, to identify the differentially expressed genes, cDNA normalization alone may not suffice and therefore, a variety of alternate methods have been developed and improved. These include low throughput differential display PCR (Cho *et al.,* 2001), cDNA-amplification fragment length polymorphism (cDNA-AFLP) (Vos *et al.,* 1995) PCR-based cDNA-representational difference analysis (cDNA-RDA) (Hubank and Schatz, 1994), suppression subtractive hybridization (Diatchenko *et al.,* 1996; Xiong *et al.,* 2001), PCR-based cDNA subtraction method (Mishra *et al.,* 2005) and microarray based techniques.(Schena *et al.,* 1995; Chee *et al,.* 1996).

Among the various methods enlisted above Suppression Subtractive Hybridization (SSH) proves to be an efficient approach. In this technique differentially expressed genes can be normalized and enriched over 1000-fold in a single round of hybridization (Diatchenko *et al*., 1996). This would substantially increase the chances for identification of rare transcripts involved in stress physiology. SSH has successfully been used to identify genes responsive to various biotic and abiotic stresses in various plant species (Watt, 2003; Zhang *et al.,* 2005; Mishra *et al.,* 2007; Chauhan *et al.,* 2011). This is performed for the generation of subtracted cDNA libraries which means to detect and clone differentially expressed genes during differentiation (Shary and Mukherjee, 2004). In this study differentially expressed genes in Pearl Millet under drought will be studied by suppression subtractive hybridization (SSH).