CHAPTER-I

INTRODUCTION

1.1. Climate change

Climate change is referred as any statistically significant change in climate over prolonged time duration. It could be due to natural variability or attributed (directly/ indirectly) to anthropogenic activities that alter the composition of global atmosphere and land use (Parry et al., 2007). Global climate change is largely attributed to natural variability, which is related to interactions among the atmosphere, ocean, and land, as well as changes in the amount of solar radiation reaching the earth. Almost 41 to 64% of pre-anthropogenic (pre-1850AD) decadal-scale temperature variations were predicted due to changes in solar irradiance, volcanic activity and the Earth's orbit around the Sun (Crowley, 2000). Volcanic eruptions are episodic and have relatively short-term effects on climate. While, large scale climate change is observed in the past due to changes in solar irradiance as evident from the geological records (Petit et al., 1999). Ice core data from the Polar Regions showed large variations in Earth's climate accompanied by naturally caused changes in the atmospheric concentrations of carbon dioxide (CO_2) and methane (CH_4). Cores from Greenland provided the first evidence for fast and drastic climate changes during the last glacial epoch, including the transition to the present interglacial epoch (the Holocene, for the past 10,000 years) in the Northern Hemisphere. Records from Greenland and Antarctica cores lead to various proposals showing mechanisms for the cause of rise in temperatures. New Vostok records covering four transitions from glacial to warm epochs support the idea that changes in the orbital parameters of the Earth (eccentricity, obliquity and precession of axis) causes variations in the intensity and distribution of solar radiation, which in turn trigger natural climate changes (Stauffer., 1999).

The climate change witnessed in 21st century is far beyond the natural variability. Many strong indications for climate change induced by anthropogenic activities are coming from the research activities of the past ~50 years. Anthropogenic activities are changing the global climate dramatically since the beginning of Industrialization. As evident from the outcomes of various climatic models, it can be considered that natural variability plays only a subsidiary role in the 20th century warming and that the most parsimonious explanation for the majority of the warming is due to the anthropogenic activities (Crowley, 2000; Rosenzweig et al., 2008). Human activities alter the climate by causing change in the atmospheric composition of gases [such as CO_2, CH_4, NOx (Nitrous oxide), CFCs (chlorofluorocarbons), hydro fluorocarbons

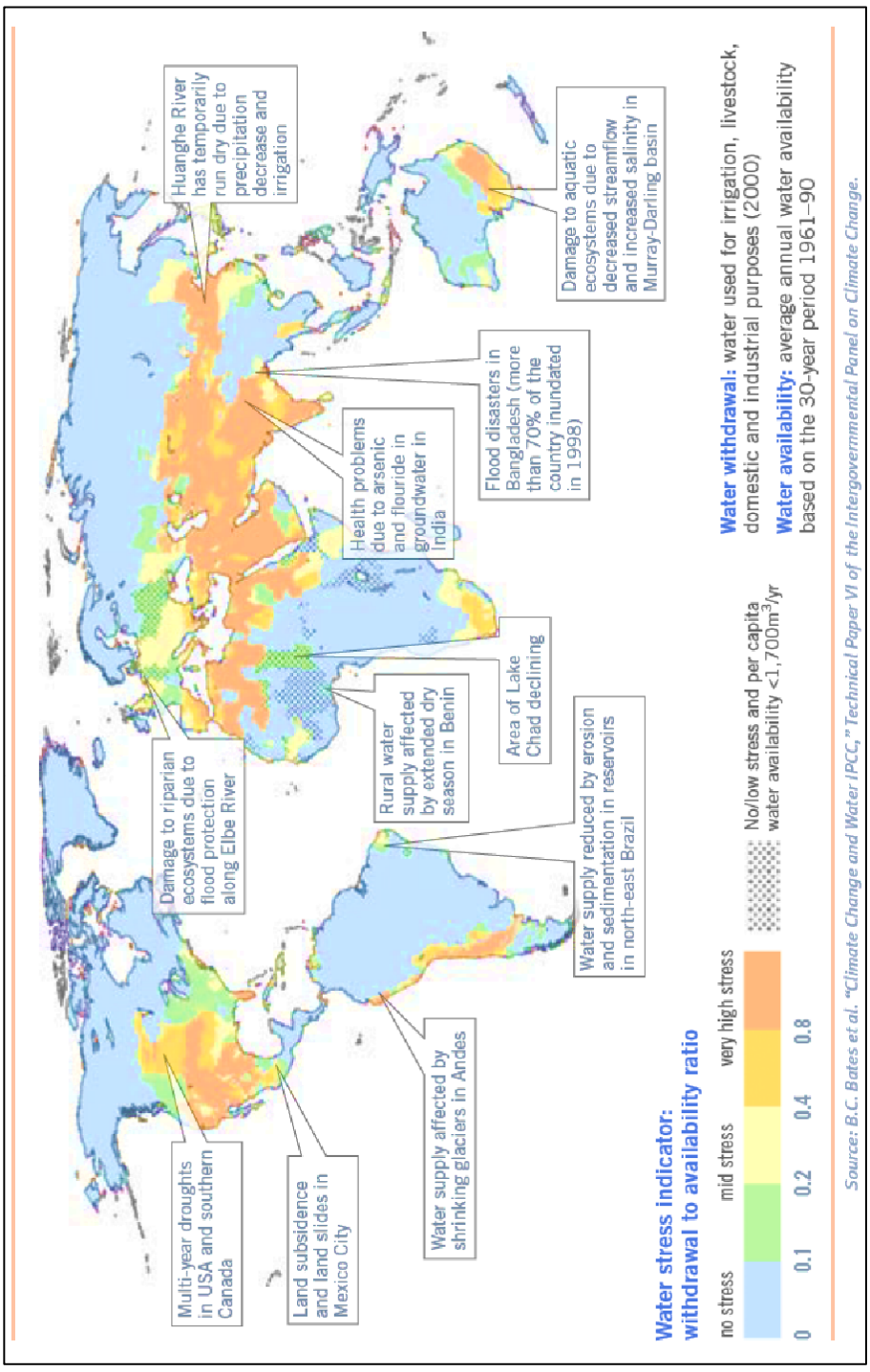
(HFCs), perfluorocarbons (PFCs)] and aerosols (small particles). One of the major human activities causing large scale climate change includes burning of fossil fuels (like coal and oil) thereby increasing the concentrations of CO_2, which has increased 31% since preindustrial times (Vitousek et al., 1997; Karl and Trenberth, 2003). Other activities like rearing the livestock, cultivation, biomass burning, landfills, coal mining, wood combustion, nitrogenous fertilizers, coolants in refrigerators and air conditioners, foam insulation, deforestation, clearing of land for agriculture and industry have also increased the concentrations of atmospheric gases like CH_4, NO_x, CFCs, HFCs and PFCs (Vitousek et al., 1997). Current estimated atmospheric concentration of CO_2 are 385 parts per million by volume (ppmv) and these levels are speculated to rise to 450-600 ppmv by the end of this century (Solomon et al., 2009). Atmospheric concentrations of CH_4 are currently > 1,774 parts per billion (ppb) which is much higher than the levels seen at the beginning of industrialization (~320-790ppb). Concentration of halogen containing gases (like CFCs) which were absent in the atmosphere before the industrial era have also increased. Tropospheric ozone levels have risen by 38% since pre-industrial times (Solomon et al., 2007). Atmospheric gases are collectively termed as Greenhouse gases (GHGs) (with few exceptions) as they cause global warming through the phenomenon called as greenhouse effect. Greenhouse effect warms the surface of the earth by affecting the incoming solar radiation (one third of it is reflected back to space by earth's atmosphere) which is absorbed by land and ocean; and the rest is radiated back in the form of thermal radiations. This natural warming is very important as it keeps the surface of the planet warm which otherwise would be very cold (temperature below 0°C) at night. The rise in concentration of GHGs gases traps high quantities of outgoing radiation from the Earth to space, creating a warming of the planet (Karl and Trenberth, 2003). Human activities are bringing in faster and unforeseen changes to climate. Some of these unprecedented events are likely to have unexpected impact on natural processes.

1.1.1. Consequences of climate change - water deficit

GHGs and land use change (particularly deforestation) cause alterations in global mean temperature which under prevailing conditions is projected to rise by 2-4°C by the year 2100 (Houghton, et al., 2001; New et al., 2011; Hillel and Rosenzweig,

2011). Increase in both global and regional temperatures causes rise in the amount of water vapor which causes land surface drying (Karl and Trenberth, 2003). It increases the potential solar incidence and severity of droughts, which has been observed at many places worldwide. Increase in water vapor (because of higher evapotranspiration) can also lead to erratic precipitation rates with total annual precipitation remaining constant or reduced slightly (Solomon et al., 2007). These conditions therefore increase the risk of both drought and flooding at different places and times (Solomon et al., 2007). For example, in Europe, during the summers of 2002 heavy rains caused floods at many places, which was followed by record-breaking heat waves and drought in the following year (i.e. 2003). Recent reports by Intergovernmental Panel on Climate Change (IPCC) states that all the freshwater resources will change under global warming and conclude that "water and its availability and quality will be the main pressure on, and issues for, societies and the environment under climate change" (Bates et al., 2008). Figure 1 depicts the risk of water availability and related challenges due to climate change in different parts of the world. Decrease in the amount of precipitation and drying is observed at many regions throughout the world such as Sahel, the Mediterranean, southern Africa and parts of southern Asia (Alley et al., 2007; Solomon et al., 2009). Such occurrence and severity of drought spells mediated by decrease in precipitation at different regions of the world are speculated to rise further with change in climate (Yinpeng et al., 2009).

Figure 1 Climate change and risk for water availability at different parts of the world



1.1.2. Response of plants to water deficit

Plants, unlike animals cannot escape from environmental stresses like water deficit/drought owing to their sessile nature. Water deficit is a condition where the water availability to plants undergoes change due to deprivation of soil water content. Water deficit is estimated to be the prime factor suppressing net primary production of the vegetation of the earth (Larcher, 2003). Drought is a principle abiotic stress and adversely affects both wild and cultivated plant communities. Plants of Amazonian rain forest are speculated to reduce by 70% by the end of 21st century due to drought and modification of the seasonal cycles (Cook and Vizy, 2008). Drought, as witnessed in current times causes loss in yield of major crop plants; raising concern for future food security (Lobell et al., 2008). Plant growth and development are rigorously affected by drought. Plants have evolved two major mechanisms to cope with water deficit; stress avoidance and stress tolerance (Ramanjulu and Bartels, 2002; Akashi et al., 2008). In stress avoidance mechanism, plants keep away from stress by the formation of seeds (before drought conditions prevail) and by specialized adaptations in the plant architecture. While in stress tolerance mechanism, plants cope with water stress by coordination of physiological and biochemical alterations at the cellular and molecular level (Ramanjulu and Bartels, 2002). These physiological and molecular alterations aid in developing adaptive traits (Urano et al., 2010; Reddy et al., 2004). Tolerance to water deficit is known to vary between the plant species (Cortes et al., 2013; Arvin and Donnelly, 2008; Onemli and Gucer, 2010; Gao et al., 2008). Studying species specific response to stress signal is important not only with respect to basic understanding but also has great potential for improving stress tolerance in crop plants. One of the strategies for improving drought tolerance in crops is to identify the natural mechanisms of drought tolerance in wild species. This can be used to test for the betterment of crop plants (Tuberosa and Salvi, 2006; Ashraf, 2010).

Wild species of crop plants are known to have higher tolerance to environmental stresses (Crawford, 1989). Major reason for higher tolerance of wild plants can be attributed to their continuous exposure to environmental stresses like drought. It can lead to the development of stress adaptive traits that help in their survival and growth. In nature, some wild plant populations are observed to grow in wide range of geographical conditions which may indicate their tolerance to environmental stresses. Wild plants growing in natural populations (especially arid and semi-arid regions) are

observed to have higher tolerance to drought than most of their relative cultivated plant species (Akashi et al., 2008). On the contrary, cultivated plants have been selected by humans for increased yield in a relatively benign environment, where nutrient and water resources are often supplemented (Mayrose et al., 2011). Such a domestication of plants helps in maximizing growth and yield (Diamond, 2002; Gepts, 2004; Hancock, 2005). Increase in yield implies that plants are investing more in particular structures that are desirable to human needs (Heiser, 1988; Richards, 2000; Zohary, 2004; Purugganan and Fuller, 2009). However increase in yield is not combined with other critical investments- including resistance to stresses such as drought and diseases, leading to trade off against one another (Donald, 1968; Halpin, 2005).

Several reports indicate higher tolerance of wild relatives of cultivated species to drought. For example, wild populations of common bean (*Phaseolus vulgaris)* are found growing in mesic to very dry soils, while crop species of common bean are highly sensitive to water deficit (Cortes et al., 2013). Similarly, wild species of *Helianthus* (wild sunflower), *Solanum* (wild potato), *Aegilops geniculata* (wild wheat), *Oryza rufipogon* (wild rice), *Citrullus lanatus* (wild watermelon) were reported to show higher tolerance to drought as compared to their cultivated varieties (Xiao et al., 1998; Kawasaki et al., 2000; Arvin and Donnelly, 2008; Mayrose et al., 2011; Pradhan et al., 2012). Mayrose et al., (2011) reported differential response of wild and domesticated sunflower species towards water deficit. The domesticated species exposed to water deficit showed significant variation in its morphological features coupled with reduction in growth rate. While wild species growing under water deficit showed marginal impact on its growth rate. Under extreme water deficit, wild plants showed longest survival time as compared to the cultivated species. Such a difference in growth and survival between wild and cultivated species to drought was linked to variation in their gene expression profile (mRNA levels). Two protein coding genes, phosphatase 2C and Athb-8 gene showed difference in their expression, which was speculated to be the major reason for reduced growth and survival of the cultivated species. Wild potato species even showed higher tolerance to drought than the cultivated species which creates a wide genetic base to improve the stress tolerance of cultivated potato (Arvin and Donnelly, 2008). Wild relative of wheat (*Aegilops geniculata*) showed higher tolerance to drought (withholding of water

supply for 16 days) as compared to cultivated species. Higher sensitivity of cultivated wheat varieties to drought was portrayed by high levels of chlorophyll degradation and major reduction in yield. While *A. geniculata* under drought stress showed a marginal decline in yield; indicating its high tolerant to drought stress (Pradhan et al., 2012). Wild rice cultivars like *Oryza rufipogon, Oryza australiensis, Oryza glaberrima, Oryza officinalis* and *Oryza nivara* also have the potential for improving the performance of cultivated *Oryza sativa* towards water deficit (Xiao et al., 1998; Kamoshita et al., 2008). Comparative study on drought tolerance of wild watermelon (*Citrullus lanatus*), domesticated watermelon (*Citrullus lanatus* L. cv. Sanki), cucumber (*Cucumis* sp. cv. Shinsokusei) and maize (*Zea mays* L. cv, Honeybantam) was carried out to analyze their response to water deficit (Kawasaki et al., 2000). Domesticated watermelon and cucumber were reported to be drought sensitive owing to their major decrease in leaf water content and photosynthetic activity when exposed to water stress. While, wild watermelon showed highest drought tolerance by lesser decrease in leaf water content. Leaf water content in wild species was maintained due to specific change in its metabolism, protein content and amino acids which could resist drought stress (Kawasaki et al., 2000). All these studies attribute superior drought tolerance of wild species combined by specific alterations in phenotypic traits and underlying physiological and molecular mechanisms. An understanding about phenotypic variations and the underlying physiological and molecular mechanisms towards drought tolerance of wild species and drought susceptibility of cultivated species is still rudimentary. Studies are needed to figure out the mechanisms for drought tolerance of wild species which will aid in improving tolerance of crop species. Such studies are needed as demands for better crop yields are mounting because of the ever increasing human population, decrease in arable land cover and impact of climate change (specifically of crop water availability).

1.2. Phenotypic variations

Organisms respond to change in environmental conditions by altering their phenotypic traits. Capacity of a genotype to express a range of phenotypic variations as a response to changing environments is termed as 'phenotypic plasticity'. Phenotypic variations are analyzed through comparative studies of morphological traits amongst populations. These studies observe the response of populations of

related species occupying varied environments. Related populations are perhaps the best because they have close genetic affinities, and any observable differences will be more likely to be related to surrounding environment (Bradshaw, 2006). Phenotypic variation between related species are largely analyzed through plant morphological traits (such as plant size, branch number and internode length), plant growth and allocation of assimilates to different plant tissues (Schlichting, 1986; Sultan, 1987; Bradshaw and Hardwick, 1989; Sultan, 2000). Recent studies relate plasticity directly to functional and reproductive success of plants. Plant functional traits are quantitative traits that are related to the fitness and success of individuals in a given environment. Plasticity in plant functional traits allows a given individual to grow and reproduce successfully in contrasting micro-sites thereby contributing to the ability of species to occupy diverse and variable habitats in nature (Sultan et al., 1998). Traits involved in resource acquisition often show functionally relevant patterns of plasticity such as greater leaf area relative to plant biomass under low light (Gedroc et al., 1996; Sultan, 2000). Plant functional traits of species can be measured by using few easily quantifiable variables like seed size, plant height, leaf life span, leaf mass per area, etc. (Cornelissen et al., 2003). Identification of key plant functional traits like leaf shape, size, thickness, stomatal density, specific leaf area (SLA), leaf mass per unit area (LMA), flower size and timing of flowering, height at maturity, leaf pigmentation and plant chemical defense can aid in analyzing species response to climate change. Nicotra et al., (2010) advocated that these plant functional traits should have priority for the investigation of phenotypic plasticity and identification of molecular and genetic mechanisms across species.

Phenotypic variations in response to water deficit have been reported in diverse plant organs like leaves, roots and flowers (Gianoli and Teuber, 2005, Xu et al., 2008, Bell and Sultan, 1999, Carroll et al., 2001). Plasticity in leaves is good predictor of plants performance in a specific environmental condition. However, leaf morphological plasticity, its causes and regulation in different environments are incompletely understood (Xu et al., 2009). *Convovulus chilensis* growing naturally at three different precipitation regions showed plasticity in leaf area, leaf shape, leaf area ratio (LAR) and foliar trichome density. It reported high plasticity in plants coming from the population with prominent variation in water availability (Gianoli and Teuber, 2005). *Quercus acutissima* seedlings growing in varied soil water conditions also showed

plasticity in leaf traits such as leaf size, leaf shape and venation pattern (Xu et al., 2009). Leaf size and area were observed to decrease with water deficiency. These changes in leaf traits were accompanied by narrowing of leaf blade and reduction in leaf length. Leaf plasticity as exhibited by *Q. acutissima* seedlings in response to water availability suggest that they have important underlying mechanism to adapt to broad range of soil water conditions (Xu et al., 2009). In addition to these leaf traits (like leaf size and area), specific leaf area (SLA) and leaf dry matter content (LDMC) also act as vital indicators of plant resource use strategies and reflect the effect of environmental conditions on plant growth (Li et al., 2005). SLA of a species is a good positive correlate. Lower values of SLA tend to correspond with relatively high investments in leaf 'defences' (particularly structural ones) and longer leaf life span. Species in resource-rich environments tend to have larger SLA than those in environments with resource stress (Cornelissen et al., 2003). LDMC is related to the average density of leaf tissues. It has been shown to correlate negatively with potential relative growth rate and positively with leaf life span (Cornelissen et al., 2003). LDMC reflect a fundamental trade off in plant functioning between a rapid production of biomass (high SLA, low LDMC) and an efficient conservation of nutrients (low SLA, high LDMC) (Garnier et al., 2001).

Corolla size is an important attribute in the reproductive biology of plants. Resource-cost hypotheses postulates that reduced corollas can be advantageous for plants that live under stressful conditions. Variation in floral traits reflects plastic response to local environment (Holtsford and Ellstrand, 1992). In *Polemonium viscosum*, large corollas incur physiological costs because of their greater water uptake (Galen, 1999; Galen, 2000). Plant water status and corolla size were also directly related in *Epilobium angustifolium*, where drought stress caused 33% reduction in flower size (Carroll et al., 2001). Herrera, (2004) also observed reduction in size of *Rosmarinus officinalis* flowers in response to drought. It lead him to propose that small-flowers would be advantageous in relatively dry coastal areas, whereas the relatively moist soils of the mountains would allow plants to produce larger flowers. However *Clarkia unguiculata* populations showed minor reduction in flower size under drought stress (Jonas and Geber, 1999). Bell and Sultan, (1999) carried out comparative study of root system's plasticity in response to drought and flooding in two annual *Polygonum* species that had different ecological distribution with respect to soil water

content. *Polygonum persicaria* occurred in extremely dry to flooding microsites, while *Polygonum cespitosum* is found growing in only moderate soil moisture but not flooding. These closely related species (with differential ability to withstand water stress) showed variation in their plasticity traits like root biomass allocation, root length and dynamic adjustment of root deployment over time in response to both constant and changing soil moisture content. Such phenotypic variations shown by plants in response to stress has the potential to reduce the effects of stress (Sultan, 2000). It reflects sign of strength and aid in maintaining fitness.

Ability of an organism to express plasticity in a given trait (leaf, flower and roots) must be governed by underlying mechanisms. Variation in phenotypic traits of a species in reaction to environmental stress (water deficit) can be a result of complex events influenced by multiple interacting genes and gene products (Miklos and Rubin, 1996; Trewavas and Malho 1997; Schlichting and Pigliucci, 1998). Epigenetic processes involving small RNAs, DNA methylation, histone modification and transposable element activation are known to alter gene expression leading to plastic response (Chinnusamy and Zhu, 2009; Nicotra et al., 2010). Epigenetic changes occur rapidly as compared to DNA sequence-based changes. Epigenetic processes can contribute to environmentally induced phenotypic variation by modifying the gene expression (Angers et al., 2010). Previous studies indicate that epigenetic variation in natural populations can be independent from genetic variation, and that in some cases environmentally induced epigenetic changes may be inherited by future generations (Bossdorf et al., 2008). More recently, the XIV Congress of the European Society for Evolutionary Biology (held in Lisbon on 19-24th August 2013) also stated that epigenetic mechanisms can influence a trait by suppressing or promoting a gene activity and such epigenetic variations can persist through several generations. Such trans-generational epigenetic mechanism in *Arabidopsis* was demonstrated by heritable changes in flowering time and other traits as a result of epigenetics alone, unaided by any sequence changes (Pennisi, 2013). Nicotra et al., (2010) reported that an improved understanding of the molecular basis of environmentally induced changes in plant traits will yield insight into possible ecological and evolutionary responses in wild species and will be useful for engineering plasticity in crop species. Therefore, analyzing the phenotypic variations involved in higher tolerance of wild species will aid in developing similar variations in related cultivated species; thereby

increasing its tolerance to water deficit. Further identifying the underlying gene regulatory mechanisms like small RNAs causing better phenotypic variations in wild species (towards water deficit) has the potential for developing the same in cultivated species.

1.3. Physiological and biochemical variations

Growing plants respond to abiotic factors like light, temperature, water and nutrient availability linked to the surrounding environment. Any change in these abiotic factors away from normal range, influences plant growth and development associated with a cascade of underlying physiological and biochemical variations. Soil drying decreases plant growth due to tissue dehydration (Fitter and Hay, 1993). Incidence of soil water deficit sends signals to the leaves which results in closing of stomata; limiting the entry of CO_2 (Davies and Zhang, 1991; Hartung et al., 2002; Jiang and Hartung, 2007). Such a limitation of CO_2 fixation under drought stress causes reduction of NADP+ regeneration through Calvin cycle, leading to over gain of electron (reduction) in the photosynthetic electron transport chain. Under drought stress there is a higher leakage of electrons to O_2 which occurs by Mehler reaction (Smirnoff, 1993). Such a leakage of photosynthetic electron to the Mehler reaction was observed to increase by ~50% in wheat growing under stress as compared to unstressed wheat (Biehler and Fock, 1996). This leads to the accumulation of Reactive oxygen species (ROS). ROS production under drought and other stresses increases considerably (240–720 μM $s^{-1}O_2^-$ and a steady-state level of 5–15 μMH_2O_2) which under normal conditions is kept very low (240 μM $s^{-1}O_2^-$ and a steady-state level of 0.5 μMH_2O_2 in chloroplasts) (Polle, 2001; Mittler, 2002). ROS production and accumulation largely occurs in chloroplast (to some extent even in mitochondria) which may cause disruption of chloroplast activity.

Chlorophyll is important for photosynthesis as it captures light energy from the Sun. Relative chlorophyll content has a positive relationship with photosynthetic rate. Reduction in photosynthesis under drought stress is largely attributed to pigment photo-oxidation and chlorophyll degradation (Mittler, 2002; Cruz de Carvalho, 2008; Anjum et al., 2011; Jaleel et al., 2009; Farooq et al., 2009). In sunflower, reduced content of chlorophyll was measured as a complex phenomenon leading to decrease in photosynthesis (Reddy et al., 2004). Depending on the duration and severity of

drought stress, decrease or unchanged chlorophyll levels have been previously reported in various species (Kpyoarissis et al., 1995; Zhang and Kirkham, 1996). Recent reports showed drought induced decline in chlorophyll a, chlorophyll b, and total chlorophyll in different sunflower varieties (Manivannan et al., 2007). Similar observation for reduction in chlorophyll a and b was also reported by Anjum et al., (2003) and Farooq et al., (2009). *Gossipium* species and *Catharanthus roseus* also showed reduction in chlorophyll content towards water deficit (Massacci et al., 2008; Jaleel et al., 2008). However, decrease in chlorophyll content was observed to vary between the species and also because of the differences in the intensity of water stress (Guerfel et al., 2009). Severe water deficit showed major reduction in chlorophyll content in *Helianthus annuus* and *Vaccinium myrtillus* than low levels of water deficit (Kiani et al., 2008; Tahkokorpi et al., 2007). Cotton cultivars varying in drought tolerance (GM 090304- drought tolerant and Ca/H 631-drought sensitive) showed difference in chlorophyll degradation, with high degree of degradation observed in the sensitive cultivar (Parida et al., 2007).

Another pigment, anthocyanin is also known for its sensitivity to water deficit (Balakumar et al., 1993; Yang et al., 2000). Their characteristic red, blue and purple coloration is seen in various tissues of plants either in reproductive parts (flowers and fruits) or exclusively in leaves (Chalker-Scott, 1999). They are water soluble pigments that are derived from flavonoids; a ubiquitous plant secondary product (Harborne, 1965; Winkel-Shirley, 2002). Biosynthesis of anthocyanins occurs in the cytoplasm and gets transported into the plant cell vacuole where they are stored in the form of spherical pigmented inclusions (Conn et al., 2003; Tucic et al., 2009). Apart from their vital role in flowers (for recruiting pollinators), they are also present in the leaf tissue where it may be developmentally transient, appearing only in juvenile or senescing tissues, or they may be permanent. Similarly, they may be environmentally transient, appearing and disappearing with changes in environment stresses (Chalker-Scott, 1999). The function of anthocyanin behind its transient accumulation in green vegetative tissue has been obscure which is largely attributed to the range of inducers and diversity in the pattern of pigmentation (Steyn et al., 2002). Balakumar et al., (1993) showed increase in levels of anthocyanin in cowpea exposed to combination of UV-B radiation and water deficit. Similar observations were also reported in maize cultivars exposed to drought stress (Efeoglu et al., 2009). Its accumulation is largely

related to stress susceptibility of plants. This is mainly because; rise in anthocyanin negatively affects plant growth by decreasing photosynthesis. Following are the major rationales that demonstrate negative effects of anthocyanin accumulation on plant growth. Firstly, its accumulation increases the heat load of tissue (Schroeder, 1965; Hetherington, 1997). Such an increase in leaf tissue temperature can cause photoinhibition in plants growing under water deficit (Ludlow & Björkman, 1984; Steyn et al., 2002). It may lead to the formation of ROS, which in turn can cause photodynamic bleaching and perturbation of cellular metabolism (Foyer et al., 1994; Steyn et al., 2002). Secondly, synthesis and vacuolar sequestration of anthocyanin molecules represent a considerable metabolic investment for plant cells. It involves cost associated with biosynthesis of cyanidin from its precursors (4-coumaroyl-CoA and malonyl-CoA) which involve atleast seven enzymes (Shirley, 1996). Moreover there are costs associated with the conjugation of each cyanidin molecule to a monosaccharide molecule. Finally, there are costs associated with the transport of cyanidin-3-O-glucoside into the cell vacuole via a tonoplast Mg-ATP-requiring glutathione carrier (Alfenito et al., 1998; Gould, 2004). Hence metabolic cost associated with anthocyanin production is very high.

Drought induced accumulation of ROS causes oxidative stress in plants (Moran et al., 1994; Wellburn et al., 1996; Loggini et al., 1999; Boo and Jung, 1999). Over production of ROS during drought can cause lipid peroxidation, protein degradation and DNA fragmentation (Apel and Hirt, 2004). Lipid peroxidation is a widely used stress indicator of plant membranes. Reactive oxygen intermediates including O_2^-, H_2O_2, and OH^- radicals directly attack membrane lipids and cause lipid peroxidation (Mittler, 2002). The process of lipid peroxidation consist three phases; initiation (formation of free radicals), propagation (free radical chain reaction) and termination (formation of nonradical products); resulting in oxidation of unsaturated fatty acids. Malondialdehyde (MDA) produced during the oxidation of polyunsaturated fatty acids is considered as a suitable marker for estimation of lipid peroxidation. A decrease in membrane stability reflects the extent of lipid peroxidation caused by ROS. Lipid peroxidation acts as an indicator of the prevalence of free radical reaction in tissues (Anjum et al., 2011). Previous study of *Pisum sativum* showed 2-4 fold rise in the levels of lipid peroxidation in response to water deficit (Moran et al., 1994). These levels of lipid peroxidation (measured in terms of MDA) in response to water

deficit varied between the closely related plant species, with higher levels of MDA usually associated with the drought sensitive species *(*Turkan et al., 2005; Gao et al., 2008). Although accumulation of ROS during stress poses threat to cell organelles, it is also thought that ROS can act as signals for the activation of stress-response and defense pathways (Desikan et al., 2001; Knight and Knight, 2001).

In response to deleterious effects of ROS, plants have developed an internal protective mechanism in the form of enzymatic and non-enzymatic antioxidants. Non enzymatic antioxidants include ascorbic acid, tocopherol, reduced glutathione and carotenoids. Enzymatic antioxidants include superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) (Asada and Takahashi, 1987; Bowler et al., 1992; Willekens et al., 1997). Amongst both, major ROS scavenging mechanism consist of enzymatic antioxidants mediated by SOD, APX and CAT (Desikan et al., 2001; Ma and Eaton 1992; Pandolfi et al., 1995). The foremost ROS scavenging pathways of plants include SOD, found in almost all cellular compartments (Mittler, 2002). Takahashi and Asada, (1983) showed that phospho-lipid membranes are impermeable to charged molecules suggesting that SODs are essential in the cell compartment where free radicals are formed. Based on the metal co-factor used by the enzymes, SODs are classified into three groups: manganese SOD (MnSOD), iron SOD (FeSOD), and copper-zinc SOD (CuZnSOD). These SODs are located in different compartments of the cell. MnSODs are located in the mitochondria and the peroxisome, FeSODs are located in the chloroplast and CuZnSODs are located in the chloroplast, cytosol and possibly the extracellular space. Comparison of deduced amino acid sequences from these three different types of SODs suggest that MnSODs and FeSODs are more ancient types of SODs, and these enzymes most probably have arisen from the same ancestral enzyme, whereas CuZnSODs have no sequence similarity to MnSODs and FeSODs and probably have evolved separately in eukaryotes (Kanematsu and Asada, 1990; Smith and Doolittle, 1992; Alscher et al., 2002). Although MnSOD and FeSOD have high similarity in their primary, secondary and tertiary structure however Fe cannot restore the activity of MnSOD and *vice versa* (Fridovich, 1986). There are two distinct groups of FeSOD. The first group is a homodimer formed from two identical 20 kDa subunit proteins while second FeSOD group, found in higher plants, is a tetramer of four equal subunits with a molecular weight of 80-90 kDa. MnSOD is either a homodimeric or a homotetrameric enzyme

with one Mn atom per subunit. CuZnSODs even found in two groups; homodimeric form present in cytoplasm and periplasm, while other homotetrameric form present in chloroplast and extracellular region (Bordo et al., 1994). Therefore MnSOD, FeSOD and CuZnSOD have distinct origin, cellular location and structure. It indicates that each SOD isoform has vital function in plants which cannot replace each other's activity.

SOD isoforms are largely analysed by activity staining of nondenaturing-polyacrylamide gel and immunoblot analysis using antisera for the specific SOD isoform. Based on these techniques, different SOD isoforms (MnSOD, FeSOD and CuZnSOD) have been identified in model plant (*A. thaliana*) and several crop plant species like *Glycyrrhiza uralensis, Oryza sativa, Pisum sativum, Triticum* species and *Hordeum vulgare* (Kliebenstein et al., 1998; Pan et al., 2006; Wang et al., 2005; Moran et al., 1994; Zhang and Kirkham, 1994; Acar et al., 2001). In *A. thaliana* rosette tissue, three CuZnSODs (CSD1, CSD2, and CSD3), three FeSODs (FSD1, FSD2, and FSD3) and one MnSOD (MSD1) were identified by Kliebenstein et al., (1998). While in *G. uralensis*, Pan et al., (2006) identified four CuZnSODs, one FeSOD and one MnSOD. It indicates that number of SOD isoforms differs from species to species; and some of the isoforms can be sensitive to the surrounding environmental conditions (Pan and Yau, 1992; Ormrod et al., 1995; Alscher, 2002; Fernandez-Ocana et al., 2011). CuZn-SOD has been found in animals, fungi, slime molds and land plants as the major SOD. By contrast, prokaryotes with the exception of a few bacteria, protozoa and eukaryotic algae lack CuZn-SOD, instead possessing Fe-SOD and Mn-SOD as the major enzymes (Tanaka et al., 1996; Fridovich, 1995). CuZnSODs are also observed to be abundantly expressed in *A. thaliana* and *G. uralensis* (Kliebenstein et al., 1998; Pan et al., 2006). In *A. thaliana* ecotype *Cvi*, higher tolerance to various abiotic stresses including drought was proposed to be associated with elevated levels of CuZnSOD (CSD2) (Abarca et al., 2001). Drought stress causes a rise in the activity of CuZnSODs in *Arabidopsis* and important crop plant species such as *Oryza sativa* and *Pisum sativum* (Ke et al., 2009; Moran et al., 1994; Kliebenstein et al., 1998); indicating indispensable role of CuZnSOD under drought. Rise in the levels of CuZnSODs were also reported under oxidative, arsenate, salinity and temperature stress (Abercrombie et al., 2008; Gapinska et al., 2008; Gupta et al., 1993). Recent studies on transgenic sweet potato (*Ipomoea*

batatus) overexpressing CuZnSOD and APX showed superior drought tolerance and recovery from drought (Lu et al., 2010). It indicates major role of CuZnSODs in plants for imparting tolerance to drought. Compared to CuZnSODs, MnSODs and FeSODs show lesser expression in *A. thaliana*. Water deficit induced oxidative stress did not show a rise in the activity of MnSODs in model plant *A.thaliana* (Kliebenstein et al., 1998). Even drought stressed *G. uralensis* showed no change in MnSOD and FeSOD activities (Pan et al., 2006). However, transgenic plants having over expression of either MnSOD or FeSOD portrayed higher tolerance to water deficit. Studies on transgenic alfalfa and rice (over expressing MnSODs) showed more drought tolerance than compared to non-transgenic plants (McKersie et al., 1996; Wang et al., 2005). Transgenic Maize (over producing FeSODs) was more oxidative stress tolerant than non-transgenic species (Van Breusegem et al., 1999). It indicates that MnSODs and FeSODs have vital role in providing tolerance to drought stress. However their lower expression compared to CuZnSODs may curtail their role in stress tolerance. Therefore analyzing variation in above described physiological traits like change in pigment concentration (chlorophyll and anthocyanin), levels of lipid peroxidation and antioxidant defense provided by SODs can act as an indicator of stress sensitivity/tolerance of a species. Further, understanding the molecular basis for such a response towards water deficit is necessary, as variations in physiological traits are largely governed by changes in gene expression.

1.4. Molecular variations

Change in gene expression in response to stress is very crucial for the defense and survival of plants. Alteration in gene expression has been observed in *Arabidopsis*, Rice, Wheat and various other species in response to water deficit (Seki et al., 2001; Seki et al., 2002; Rabbani et al., 2003; Aprile et al., 2009; Krugman et al., 2010). Drought tolerant and sensitive Wheat cultivar showed difference in the expression of stress inducible genes (Aprile et al., 2009; Krugman et al., 2008), indicating differential gene expression of related plants species to water deficit. Regulation of gene expression largely occurs at two major control points; transcription (production and processing of mRNA) and translation (mRNA directed protein synthesis and further processing of protein molecule). It determines the amount and type of proteins it manufactures. Hence regulation of gene expression plays an important role in fine-

tuning the proteins synthesized and their impact on phenotypic variations. Nuclear gene expression is regulated at both transcriptional and post transcriptional levels, termed as Transcriptional gene silencing (TGS) and post transcriptional gene silencing (PTGS) respectively (Seo et al., 2009; Sakuma et al., 2006; Sunkar et al., 2006; Sunkar et al., 2012). As thousands of transcripts are produced every second in a cell, transcription can be thought as primary control point for regulating gene expression. TGS controls the process of transcription and specifies the amount of RNA produced. Transcriptional regulation of genes in response to stress has been studied extensively over the past couple of years. TGS in response to drought involves various cis-acting elements and transcriptional factors in the stress responsive promoters for plant adaptation to stress (Yamaguchi-Shinozaki and Shinozaki, 2006). In *Arabidopsis,* transcription factor DRBE2A regulates expression of many drought inducible genes, while MYB transcription factor (MYB96) regulates drought stress response by integrating ABA and auxin signals (Seo et al., 2009; Sakuma et al., 2006). Post transcriptional gene silencing is emerging as an important regulator mainly because of its rapid response to stress, which is needed in order to modify plants response to stressful conditions.

PTGS in plants and RNA interference (RNAi) in animals belongs to broad family of phenomena collectively called RNA silencing (Kooter et al., 1999; Li and Ding, 2001; Matzke et al., 2001; Vaucheret et al., 2001; Waterhouse et al., 2001; Hannon, 2002; Plasterk, 2002). The unifying features of RNA silencing phenomena are the production of small RNAs that act as specific determinants for regulating gene expression (Hamilton and Baulcombe, 1999; Hammond et al., 2000; Parrish et al., 2000; Zamore et al., 2000; Parrish and Fire, 2001; Tijsterman et al., 2002). In plants, PTGS was first revealed during a search for transgenic *Petunia* flowers that were expected to be purpler due to upregulation of a gene, coding for an enzyme chalcone synthase (chsA), which is involved in the production of anthocyanin pigment. However some of the transgenic *Petunia* plants having chsA coding region lost both endogene and transgene chalcone synthase activity, owing to variegated color of flowers (Napoli et al., 1990). The loss of chsA transcript responsible for the pigmentation was not observed to be associated with reduced transcription (Blokland et al., 1994). Such a loss of mRNA expression was termed as ‘cosuppression’ (Napoli

et al., 1990). However this mechanism was later recognized as PTGS and was observed in various plant species.

Small RNAs are largely categorized into micro RNA (miRNA) and small interfering RNAs (siRNA). Both siRNAs and miRNAs usually vary from 21-26 nucleotide (nt) in length, however the distinguishing feature that separates miRNAs from siRNAs is that, miRNAs are derived from hairpin like structure formed from single stranded RNA. While, siRNAs are derived from long double-stranded RNAs which originate due to RNA-dependent RNA polymerase (RdRp) activity or pairing of messenger RNAs derived from natural antisense pairs of genes. Endogenous siRNAs are further cataloged into numerous subcategories like trans-acting siRNAs (ta-siRNAs), natural antisense siRNAs (nat-siRNAs), heterochromatic siRNAs, and long siRNAs (lsiRNAs) based on their length, origin and function (Vaucheret, 2006; Jin et al., 2008) . TAS loci codes for ta-siRNAs, whose biogenesis depends on miRNA pathway and thereby, regulate gene expression similar to that of miRNAs (Sunkar and Zhu, 2007; Jin et al., 2008). nat-siRNAs originate from natural antisense pair of genes leading to formation of double stranded mRNAs; which consequently form nat-siRNAs (Borsani et al., 2005; Katiyar-Agarwal and Jin, 2010). Heterochromatic siRNAs, are derived from repetitive sequences such as centromeric repeat sequences, retroelements, transposons and ribosomal DNA. They play vital role in DNA and histone methylation (Chan et al., 2004; Xie et al., 2004; Zilberman et al., 2004; Kasschau et al., 2007). lsiRNAs are typically 30-40 nt in length (longer than other classes of siRNAs) and have been recently reported to function in response to bacterial infection in *Arabidopsis* (Katiyar-Agarwal et al., 2007). Although plants have diverse range of above described endogenous siRNAs, their functional role in plant development or stress is still ambiguous. Recently only nat-siRNAs and lsi-RNAs in plants were associated with stress response (Valencia-Sanchez et al., 2006; Chapman and Carrington, 2007; Katiyar-Agarwal et al., 2007; Hilbricht et al., 2008). On the contrary, miRNAs are discovered to have critical role in regulating gene expression in response to abiotic stresses (including water deficit) (Sunkar et al., 2007; Khraiwesh et al., 2012). Moreover, miRNAs react spontaneously to stress causing changes in mRNA levels which may aid in stress tolerance.

1.4.1. microRNAs - biogenesis and importance

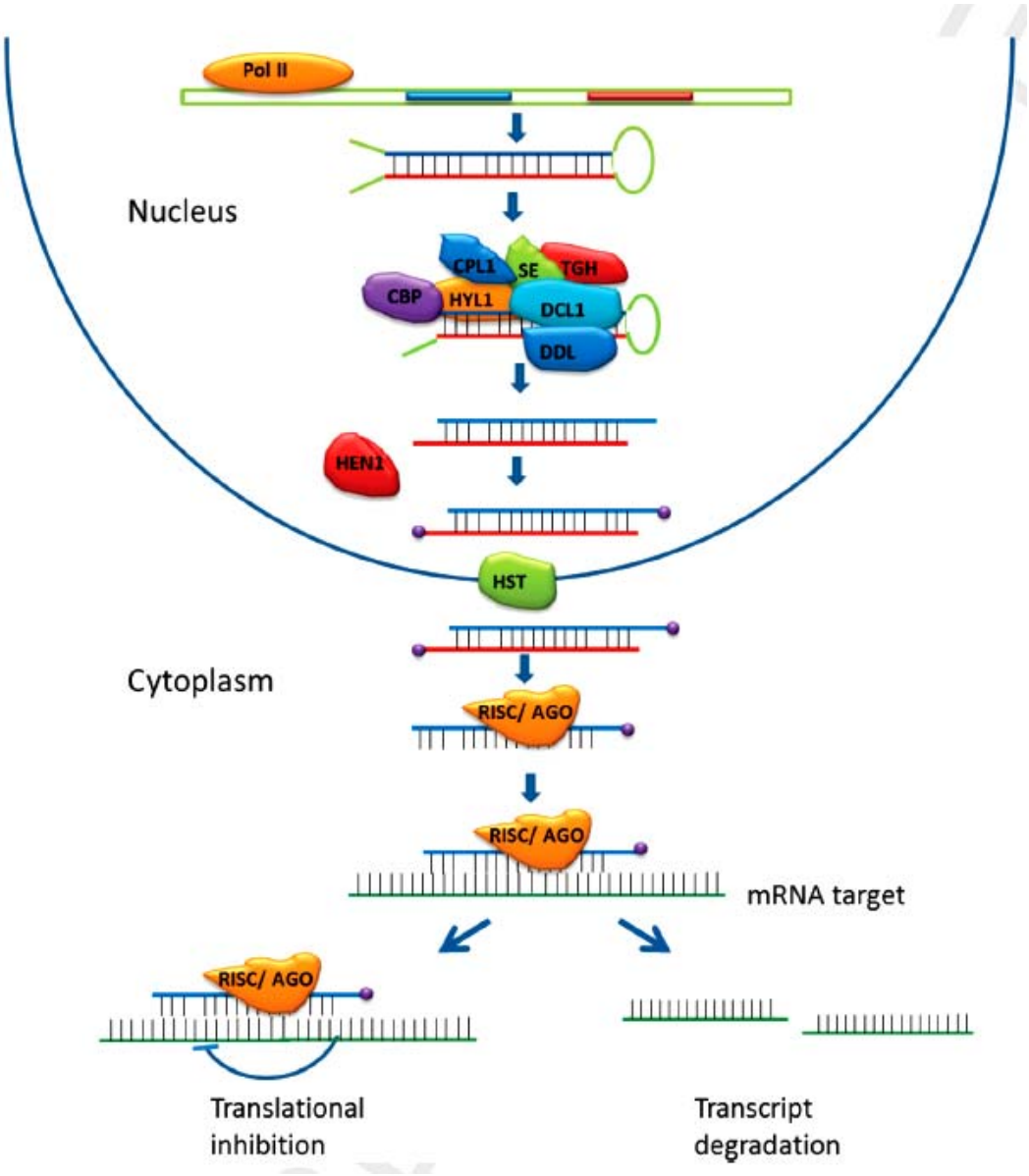
miRNAs are typically 21-24nt in length originating from non-coding RNA genes. miRNA genes may be similar to protein coding genes containing introns and exons. These are transcribed by RNA polymerase II. Similar to RNA pol II transcripts, miRNA transcripts are capped, spliced and polyadenylated to form the primary miRNA transcript (pri-miRNA) (Kim, 2005). This pri-miRNA transcript partially folds back due to complementary base pairing, forming an imperfect stem loop structure (hairpin like) with 5′ 7-methyl guanosine cap and a 3′ poly(A) tail (Zhan et al., 2012). Stem looped pri-miRNA is diced by RNase III enzyme DICER-LIKE1 (DCL1) to produce a precursor miRNA (pre-miRNA). Apart from major function of DCL1 in miRNA biogenesis, several other double-stranded RNA-binding proteins and DCL1 interacting proteins like HYL1 (hyponastic leaves1), SE (serrate, a C2H2zinc-finger protein), DDL (Dwadle-like), CBP80 (Cap-binding protein 80), CBP20 (Cap-binding protein 20), STA1 (Stabilized-1) and CPL1/FRY2 (C-Terminal Domain Phosphatase-like 1/FIERY 2) also aid in dicing of ~21nt long miRNA/miRNA* (where, miRNA* is the complementary strand of the mature miRNA) duplex from the stem loop structure (Sunkar and Zhu, 2007; Jones-Rhoades et al., 2006; Voinnet, 2009; Chen, 2010; Laubinger et al., 2008; Manavella et al., 2012; Ben Chaabane et al., 2013). The duplex is methylated at the 3’ termini by HUA ENHANCER1 (HEN1) methyltransferase enzyme (Yu et al., 2005). Reports on hen1 mutants showed heterogeneity in the size of miRNAs (than compared to abundance), indicating protective role played by methylation of miRNA/miRNA* against polymerase or terminal transferase which generally adds polyU to the 3’end of plant miRNAs (Park et al., 2002; Han et al., 2004; Li et al., 2005). Methylated miRNA/miRNA* duplexes are exported from the nucleus to cytoplasm with the help of HASTY (HST), a homolog of mammalian EXPORTIN 5 (Park et al., 2005). In the cytoplasm the duplex is separated and usually one strand is selected as the mature miRNA, whereas the other strand (miRNA*) is degraded rapidly (Kim, 2005). The mature miRNA gets associated with ARGONAUTE (AGO1) forming RNA-induced silencing complex (RISC) (Figure 2). RISC containing specific miRNA binds to the complementary mRNA leading to cleavage or translational repression of the target mRNAs, thereby causing PTGS in plants (Jones-Rhoades and Bartel, 2004; Bartel, 2004).

miRNA targeted gene regulation is important in plant development and stress response as they are observed to be preserved in diverse families of plants (Sunkar et al., 2007). Genome wide analyses of miRNA have revealed that several miRNA families are highly conserved among plant genomes (termed as conserved miRNAs); while others are highly specific to individual species (termed as novel miRNAs). Studies report that conserved miRNA families have been expanded by duplication followed by subsequent reduction of redundant homologs whereas novel miRNAs (which are often expressed only in restricted species) might initially evolve neutrally but develop more specialized roles (Chen and Rajewsky, 2007; Rajagopalan et al., 2006; Kim et al., 2012). Almost 10-15 conserved miRNA families were identified in ~11 diverse dicot plant species, using the available GSS, HTGS, and NR repositories and EST sequence data. Five families of conserved miRNAs (miR319, miR156/157, miR169, miR165/166 and miR394) were found in more than 40 species; six families (miR159, miR160, miR167, miR170/171, miR396 and miR399) were observed in 30-39 species; seven families (miR164, miR168, miR172, miR393, miR395, miR398 and miR408) in 20-29 species; and five (miR162, miR390, miR397, miR403 and miR437) in 10-19 species (Sunkar and Jagadeeswaran, 2008). Apart from dicots, several plant miRNA families were even observed to be conserved in monocot, gymnosperms, ferns and moss indicating its importance in plant evolution (Axtell and Bartel, 2005). For example, miR398 was observed to be conserved in 22 plant species (Sunkar and Jagadeeswaran, 2008). Targets of these conserved miRNAs are also observed to be conserved indicating important role of miRNA-target interaction amongst plants (Rhoades et al., 2002; Jones-Rhoades and Bartel, 2004; Sunkar and Zhu, 2004; Bonnet et al., 2004). Most of the evolutionarily conserved miRNAs are most abundantly expressed while novel miRNAs show low abundance due to their recent evolution in specific species. Kim et al., (2012) observed 44 miRNAs highly conserved in various tissues of *Brassica rapa*, suggesting that they are 'housekeeping' miRNAs important for regulating basic cellular functions in all tissues.

Thus far 8496 mature miRNAs are identified from 74 plant species which are compiled in miRBase (release-21) (http://www.mirbase.org/cgi-bin/browse.pl). These miRNAs are identified by either experimental approach (e.g. high throughput sequencing, microarray, northern blotting and RT-PCR) or computational approach. Recent advances in high throughput sequencing technologies have created a platform

for detection of millions of conserved as well as less abundant novel miRNAs in plants (Fahlgren et al., 2007; Sunkar et al., 2008; Chi et al., 2011; Szittya et al., 2008). Using high throughput sequencing several conserved and non-conserved miRNAs were previously identified from control and drought stressed libraries of plants whose genome is well characterized (Sunkar et al., 2008; Li et al., 2011; Wang et al., 2011). However for plants lacking genome sequence information the identification of the miRNAs becomes difficult. In such species miRNA identification is largely carried out by aligning the small RNA sequences with the known miRNAs present in the miRBase and using the publicly available ESTs (expressed sequence tags), GSSs (genome survey sequences), HTGSs (high-throughput genomics sequences) and NRs (nonredundant nucleotides) databases (Zhang et al., 2006; Sunkar and Jagadeeswaran, 2008). Several deep sequencing studies effectively identified miRNAs from plant lacking genome information. For example, Wang et al., (2012) identified 496 conserved and 2 novel RNAs from unique small RNA sequences of *Liriodendron chinense*; where in most of the reads were assigned as unknown small RNAs. In *Eugenia uniflora* (important Myrtaceae species lacking genome sequence information) 204 conserved miRNAs belonging to 45 miRNA families were identified (Guzman et al., 2012). In *Vigna mungo* (important legume crop lacking genome sequence information) 45 conserved miRNAs belonging to 19 families and 8 non-conserved miRNAs belonging to 7 families were identified (Paul et al., 2014). However most of these studies are carried out in plants growing under control/normal conditions only. Studies analyzing stress responsive miRNAs in such plants are largely missing. Present study address this gap by identifying miRNAs from control and drought stressed libraries of tolerant-wild and sensitive-cultivated Convolvulaceae species lacking genome sequence information. Identification and expression analysis of miRNAs from these species (having different sensitivity to water deficit) will help in recognizing their functional role under water deficit.

Figure 2 Primary microRNA transcripts are transcribed from miRNA genes by RNA polymerase II and these transcripts can adapt hairpin-like structures that are recognized and processed by the RNAse III enzyme DCL1 (DICER-LIKE1) with the assistance of several RNA binding proteins such as CBP20/80 (Cap Binding Protein CBP20/80), HYL1 (HYPONASTIC LEAVES 1), SE (SERRATE), DDL (DAWDLE), TGH (TOUGH) and CPL-1 (C-TERMINAL DOMAIN PHOSPHATASE-LIKE 1). DDL interacts with DCL1 and stabilizes primary miRNA transcript. TGH is a component of DCL1-HYL1-SE complex and facilitates primary miRNA recruitment to HYL1. CPL1dephosphorylates HYL1 for optimal activity. The miRNA/miRNA*duplex is stabilized by HEN1 (HUA ENHANCER1, a methyltransferase) which adds methyl group to the 2' OH group at the 3' end. The mature miRNA is transported to cytoplasm by HST (HASTY, exportin), where only miRNA strand gets loaded into RISC complex containing ARGONAUTE-1 protein. The RISC complex is guided by the miRNA to target mRNA causing target mRNA cleavage or translational repression.



1.4.2. miRNAs - Plant development

miRNAs play crucial role in the spectrum of plant growth and developmental processes which largely includes leaf morphology and polarity, lateral root formation, hormone signaling, transition from juvenile to adult phase, vegetative to flowering phase, flowering time, floral organ identity and reproduction (Mallory and Vaucheret, 2006; Sunkar et al., 2007). Many of the conserved miRNAs are responsible for regulating vital developmental processes. This includes various miRNAs few of which are described below. miR156 and miR172 modulate the vegetative-reproductive phase transitions based on their temporal expression (Aukerman and Sakai, 2003; Lauter et al., 2005; Schwab et al., 2005; Xie et al., 2006; Gandikota et al., 2007; Wang et al., 2009). Apart from these, miR156 also regulates crucial plant developmental events such as leaf trichome development (Yu et al., 2010), male fertilization (Xing et al., 2010), embryonic patterning (Nodine and Bartel, 2010), anthocyanin biosynthesis (Gou et al., 2011) and leaf growth (Xie et al., 2012). miR165/miR166 regulates adaxial-abaxial patterning of leaves and the radial patterning of stems in *Arabidopsis* (Emery et al., 2003; Juarez et al., 2004). miR319 and miR159 are responsible for characterizing leaf margin and size, by regulating expression of CYCLOIDEA genes (Palatnik et al., 2003; Ori et al., 2007).

1.4.3. miRNAs - Plant stress response

Role of miRNAs in plant stress response are evident from recent studies on model plants such as *Arabidopsis*, *Medicago*, and various other crop species (Sunkar and Zhu, 2004; Trindade et al., 2010; Xin et al., 2010; Jia et al., 2009). As several abiotic stresses regulate the expression of miRNAs, PTGS mediated by miRNAs can be considered to play vital role for adaptation to stressful conditions (Sunkar and Jagadeeswaran, 2008). Stress responsive miRNAs subsequently impinge upon their targets and fine tune the expression. One of the key benefits with the miRNA mediated gene regulation is that miRNAs can respond spontaneously to stress and regulate the existing pool of mRNA targets without any *de novo* synthesis (Leung and Sharp, 2007). Several miRNAs responsive to abiotic stresses caused by climate change (heat, drought, UV-B radiation and ozone) have been reported and reviewed previously (Yu et al., 2011; Sunkar and Zhu, 2004; Liu et al., 2008; Zhou et al., 2010; Zhou et al., 2007; Jia et al., 2009; Iyer et al., 2012; Ghorecha et al., 2013). Drought

mediated miRNA response have been extensively studied in the past few years. Different plant species such as *Arabidopsis thaliana*, *Oryza sativa*, *Triticum dicoccoide*, *Medicago truncatula*, *Phaseolous vulagris* and *Populus trichocarpa* reported list of drought responsive miRNAs; which varied between the species (Sunkar and Zhu, 2004; Liu et al., 2008; Zhao et al., 2007; Zhou et al., 2010; Kantar et al., 2011; Trindade et al., 2010; Arenas-Huertero et al., 2009; Lu et al., 2008). On the bases of all these reports it can be deduced that miRNA responsiveness varies between the plant species, their varieties and the intensity of drought stress. *Arabidopsis* exposed to water deficit showed major upregulation of miRNAs (miR393, miR397, miR402, miR167, miR168, miR171, and miR396) while downregulation of only miR398. These miRNAs negatively regulate the expression of their targets. Evidence for such a response of miRNAs towards water deficit in *Arabidopsis* came from both, miRNA sequencing and microarray (Sunkar and Zhu, 2004; Liu et al., 2008). Studies in similar lines were even carried out in various other crop and model plants. Two of the major crop plants, rice (*O. sativa)* and wheat (*T. dicoccoides)* showed alterations in expression of several conserved and species specific miRNAs (Zhao et al., 2007; Zhou et al., 2010; Kantar et al., 2011). In *O. sativa* seedlings exposed to water deficit showed upregulation of only one miRNA (miR169f and g) which was identified from root tissue (Zhao et al., 2007). On the contrary, 11 down-regulated miRNAs (miR170, miR172, miR397, miR408, miR529, miR896, miR1030, miR1035, miR1050, miR1088, and miR1126) and eight up-regulated miRNAs (miR395, miR474,miR845, miR851, miR854, miR901, miR903, and miR1125) were identified from mature *O. sativa* plants exposed to drought stress (by withholding water in pot) (Zhou et al., 2010). These indicate differential response of *O. sativa* with respect to plant age and the method for imparting drought stress. Another major crop plant species *T. dicoccoides* (wild ancestor of domesticated *Triticum durum)* showed 13 drought responsive miRNAs (miR1867, miR896, miR398, miR528, miR474, miR1450, miR396, miR1881, miR894, miR156, miR1432, miR166 and miR171) from leaf and root tissues as analyzed through microarray platform (Kantar et al., 2011). Apart from crop plants, model leguminous plant, *M. truncatula* exposed to drought stress showed significant upregulation of miR398a,b and miR408 while transient downregulation of miR169 and miR166 (Trindade et al., 2010). *P. vulagris* and *G. max*; also showed variation in miRNA

response to drought. *P. vulagris* showed significant upregulation of 5 miRNAs (miR2118, miR159.2, miRS1, miR1514a and miR2119) and a moderate upregulation of 6 miRNAs (miR168, miR395, miR397, miR399, miR403 and miR408) (Arenas-Huertero et al., 2009). Two soybean (*G. max*) cultivars, one drought tolerant and another sensitive showed difference in miRNA expression wherein, miR166-5p, miR169f-3p, miR1513c, miR397ab and miR-Seq13 were upregulated in sensitive genotype while downregulated in tolerant genotype (Kulcheski et al., 2011). Woody plant, *P. tichocarpa* also showed differential expression of miR171l-n, miR1445, miR1446a-e and miR1447 under water deficit (Lu et al., 2008). Hence miRNA responsiveness is known to vary even between related species having differential sensitivity to water deficit. Analyzing such differential miRNA expression in related tolerant-wild and sensitive-cultivated plant species can aid in identifying potential role of miRNAs in stress tolerance of wilds.

Role of some conserved miRNAs in plant development and in response to drought are listed below:

miR168

miR168 regulates post transcriptional expression of *Arabidopsis* ARGONAUTE1 (AGO1) protein (RNA slicer enzyme), which binds with all mature miRNAs in the cytoplasm forming RISC (Vaucheret et al., 2004). Therefore alterations in levels of miR168 can affect the regulation of other miRNAs which relies on the availability of AGO1. It indicates that, AGO1 homeostasis is regulated by transcriptional co-regulation of miR168 and AGO1 genes and posttranscriptional stabilization of miR168 by AGO1 (Vaucheret et al., 2006). In *Arabidopsis*, AGO1 transcription activity was reported to increase under drought treatments, suggesting that transcriptional elevation of miR168a is required for maintaining a stable AGO1 transcript level during the stress response (Li et al., 2012).

miR169

miR169 regulates the expression of its target, NFYA5- transcription factor (member of the *Arabidopsis* NF-YA family), an important TF for expression of number of drought responsive genes. In drought stressed *Arabidopsis*, downregulation of miR169 strongly induces its target NFYA5 leading to drought stress tolerance (Li et al., 2008). Sequence analysis in rice revealed two proximate DREs (Dehydration-

Responsive Element) in the upstream region of the miR-169g promoter, suggesting that miR-169g could be regulated directly by the transcriptional factors CBF/DREBs, in response to drought stress in plants (Zhao et al., 2009). It indicates promising role of miR169 towards drought tolerance in *Arabidopsis* and rice.

miR171

miR171 targets SCARECROW-LIKE (SCL subclass of highly conserved GRAS family) transcription factors in *Arabidopsis*. miR171 in *Arabidopsis* represses differentiation of axillary meristem by repressing the expression of its target (Schulze et al., 2010; Reinhart et al., 2002). However in addition to its known function in *Arabidopsis*, miR171in barley is recently observed to alter the vegetative to reproductive phase transition by activating the miR156 pathway and repressing the expression of the TRD (THIRD OUTER GLUME) and HvPLA1 (Plastochron1) genes (Curaba et al., 2013). Under drought stress expression of miR171 is induced in *Arabidopsis* (Liu et al., 2008).

miR172

miR172 targets APETALA2 (AP2) transcription factor, which plays a key role in establishment of meristem and organ identity during floral development; and flowering time (Park et al., 2002; Chen, 2004). Over expression of miR172 in *Arabidopsis* causes early flowering (Jung et al., 2007). Downregulation of miR172 in *Arabidopsis* and *O. sativa* under drought (Liu et al., 2008; Zhou et al., 2010) can be thought to delay flowering. Interestingly, expression level of miR172 in *Arabidopsis* is discovered to correlate inversely with miR156, which together regulated the transition from vegetative to reproductive phase and *vice versa* (Wu et al., 2009). Abundance of miR172 and conversely lower levels of miR156 may cause the plant to enter the reproductive phase.

miR319

miR319 regulates the expression of TCP (for TEOSINTE BRANCHED/CYCLOIDEA/PROLIFERATING CELL FACTORS [PCF]) genes encoding plant-specific transcription factors (Axtell and Bowman, 2008; Palatnik et al., 2003; Ori et al., 2007; Nag et al., 2009). miR319 expression is regulated by environmental stimuli like drought and salt stress, suggesting its involvement in plant stress response. Accumulation of miR319 is observed to display morphological

changes and exhibited enhanced drought and salt tolerance associated with increased leaf wax content and water retention but reduced sodium uptake (Zhou et al., 2013). Therefore miRNAs has the potential to be used for the development of novel molecular strategies to genetically engineer crop species for enhanced resistance to environmental stress.

miR393

miR393 regulates mRNAs for the F-box auxin receptors TIR1 (Transport Inhibitor Response Protein 1) and AFB (Auxin Signaling F-box Protein 1). Overexpression of a miR393-resistant form of *TIR1* (*mTIR1*) enhances auxin sensitivity and led to pleiotropic effects on plant development including inhibition of primary root growth, overproduction of lateral roots, altered leave phenotype and delayed flowering. Furthermore, the interaction between miR393 and its target indicates a fine adjustment to the roles of the miR393-*TIR1* module, which is required for auxin responses in plant development (Chen et al., 2011). Drought is known to induce the expression of miR393 in *Arabidopsis* (Liu et al., 2008).

miR398

miR398 is known to regulate the expression of targets CSD1 (Cytoplasmic CuZnSOD), CSD2 (chloroplast CuZnSOD), Copper chaperone for SODs (CCS) and COX 5b (cytochrome c oxidase) in *Arabidopsis* (Sunkar and Zhu 2004; Jones-Rhoades and Bartel 2004; Sunkar et al., 2006). CSD1 and CSD2 genes code for CuZnSODs which are known as foremost enzymes in providing protection against oxidative stress (Sunkar et al., 2006) that generally occur during water deficit. Recently, Juszczak and Baier, (2012) linked expression of miR398 to Plastocyanin, CuZnSOD 2 (CSD2) and copper chaperon of CSD2 (CCS1) forming miR398-CSD2-CCS1 regulon which was observed to be subjected to natural variation in *Arabidopsis*. They further said that tolerance of *Arabidopsis* accession (*Cvi-0*) to harsh habitats (including drought) was associated with high levels of CuZnSOD regulated by specific levels of miR398 and its capacity to overwrite miR398 regulated copper regulon.

miR408

miR408 regulates the expression of mRNAs for copper containing proteins like plantacyanin and laccases (Abdel-Ghany and Pilon, 2008). No reports in *Arabidopsis*

are currently available for miR408 regulation under drought; however miR408 is reported to be induced in plants grown in soils containing low concentration of copper (Yamasaki et al. 2007; Abdel-Ghany and Pilon 2008). In *M. truncatula*, drought induces the expression of miR408 which negatively regulates its target plastocyanin (Trindade et al., 2010). In one of the studies on transgenic tobacco, over expressing miR408 was linked to downregulation of antioxidant enzymes such as SOD, POD and CAT (Feng et al., 2010). Therefore rise in levels of miR408 can potentially provide tolerance towards drought stress.

As evident from above discussion most of the miRNAs involved in developmental response are also involved in stress reactions, implicating vital role of miRNAs in plant stress adaptation. miRNA mediated regulation of vital transcription factors such as *NF-YA* subunits/CBFs (CAAT box binding factors), *SCLs*(Scare-Crow like transcription factors), *AP2*-like factors (apetala 2-like transcription factors), and *GRFs* (Growth regulating transcription factors) *SPL*s (Squamosa promoter binding protein-like transcription factors), *MYB*s(MYB-domain containing transcription factors) and *TCP*s (Teosinte branched 1, Cycloidea, PCF (TCP)-domain protein family), CuZnSODs (CSDs) and Copper chaperone for SOD (CCS1) are essential for plant response, adaptation and survival under water deficit.

1.5. Perspective of the current study

Climate change has become a global issue due to devastating human activities which is likely to continue for many centuries. Although there are uncertainties associated with future rates of change, it is clear that these changes will be increasingly manifested in important and tangible ways, such as changes in extremes of temperature and precipitation, decreases in seasonal and perennial snow and ice extent, and sea level rise. One of the major effects of climate change is decrease in precipitation causing deprivation of soil water content leading to drought stress in plants. Plant communities are adversely affected by drought; however the intensity of the effects varies between wild and cultivated species. Wild species are highly tolerant to drought stress owing to their regular exposure to similar conditions. While cultivated species are susceptible to water deficit as they are grown in benign environment where resource availability (such as water) are optimized. Angiospermic plants show huge difference in their life history traits. Annual plants live for less than

a year, wherein unfavorable environmental cues trigger flowering leading to seed production and senescence. Perennials live for many years by undergoing repetitive cycles of vegetative and flowering phase which is mediated by environmental conditions. Hence perennials can be thought to have unique underlying mechanisms (molecular and physiological) that aids in their survival under stress by bringing in suitable phenotypic variations. In the present study, two perennial plant species *Ipomoea campanulata* (wild) and *Jacquemontia pentantha* (cultivated) belonging to family Convolvulaceae are analyzed. *I. campanulata* is tolerant to water deficit while *J. pentantha* is sensitive. Both the species belong to the same family (Convolvulaceae) making it possible to evaluate their differential response to water stress. In response to water deficit, both the species show variation in their phenotypic traits and adaptability. Phenotypic responses to drought are associated with underlying physiological, biochemical and molecular alterations (specifically miRNA expression). Hence, evaluating the basis for the differential response of wild and cultivated species towards water deficit helps in understanding the adaptability of tolerant once. Analyzing phenotypic, physiological, biochemical and miRNA alterations in wild but drought tolerant and cultivated but drought sensitive species will craft a holistic view for identifying basis for superior tolerance of wilds. Keeping these in mind, the work has been carried out with the following objectives,

1) Selection of related wild and cultivated species responding differently to water stresses both *in situ* and *ex situ.*
2) Analyzing phenotypic variations in wild and cultivated Convolvulaceae species towards water stress.
3) Analyzing physiological and biochemical alterations in these species exposed to water deficit.
4) Analyzing miRNA expression in these species in response to water deficit.