With the name of ALLAH who is Merciful and Beneficent

Master of the Day of Judgment

Only You (ALLAH) we worship and You (ALLAH) we seek help and forgiveness

Guide us the right path

Path of those upon whom ALLAH bestowed favor

And

Will also be rewarded on

ROZ-E-JAZA
Biometric and biochemical markers for drought tolerance in diversified germplasm of maize (Zea mays L.) crop

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF

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DEDICATED TO

Last Prophet
HAZRAT MUHAMMAD

And

Four rightly guided successors of Islam

Hazrat Abu Bakr Siddique (Yaar e Ghar) (Radi Allah Anhu)

Hazrat Umar Farooq (Radi Allah Anhu)

Hazrat Usman e Ghanee (Radi Allah Anhu)

Hazrat Ali ul Murtada (Radi Allah Anhu)

And

Syedina Bilal Habshee (Radi Allah Anhu)

Syedina Uwais Karnee (Radi Allah Anhu)

And

My Respected Teachers & My Parents
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SUMMARY

Drought stress limits the crop growth and productivity. In order to feed ever-expanding world population, it is necessary to improve crop productivity, particularly under water stress environment. Screening and selection of crop cultivars with greater yield potential under water stress conditions is a suggested way. However, selection based on agronomic traits is not usually useful in developing drought tolerant cultivars. To overcome this, selection should be based on potential physiological indicators for drought tolerance. However, exact places of cellular damages caused by drought stress are not well understood. Moreover, it is also not known how these physiological changes are being regulated. For this purposed, we need to understand detailed mechanism of water stress tolerance in plants.

In the present study, a series of experiments were conducted to determine the genetic variability in maize (a major cereal crop to fulfill the requirement of food all over the world) for drought tolerance at germination, vegetative and yield stages. In germination experiment, seeds of six maize (Zea mays L.) cultivars (DTC, EV-77, EV-78, EV-79, Faisalabad maize and 6621) were subjected to varying levels of PEG-induced water stress (0, 3, 6, and 9%) to screen out the drought tolerant maize cultivar at seedling stage. It is obvious from the results that increasing water deficit induced by PEG\textsubscript{6000} reduced germination percentage, germination rate and energy of emergence in all maize cultivars (EV-77, EV-78, EV-79, Faisalabad maize, DTC and 6621). Among cultivars DTC appeared to possess tolerance potential to drought for germination attributes while 6621 seems to lack such ability. Moreover cv. DTC was higher in seedling vigor, root length and dry biomass tolerance indices. In screening experiment, cv. DTC showed tolerance in germination attributes under water stress, while the cv.6621 was drought sensitive at the germination stage. In addition to this, cv. EV-78 was intermediate in drought tolerance at germination stage.

In adult experiment, it was assessed whether maize cultivars which exhibited drought tolerance at the germination stage maintained their degree of drought tolerance at the adult vegetative growth stage. In addition, what kind of physiological and biochemical mechanism of stress tolerance is being operated in drought tolerant and drought sensitive maize cultivars. For this
purpose, a series of pot experiments were conducted to get reproducible results at the Botanic Gardens of Bahauddin Zakariya University, Multan, Pakistan. Twenty seeds of each of three maize cultivars selected from the germination experiment were sown in plastic pots and thinned four plants per pot after one week. Drought stress was imposed after three weeks of sowing as cyclic drought (0, 1, 2, 4 drought cycles) by withholding water till wilting of leaves. Imposition of water stress as cyclic drought caused a drastic reduction in fresh and dry biomass of all maize cultivars. Among the cultivars, DTC was the highest in fresh and dry biomass accumulation than the other cultivars at all levels of drought cycles. Moreover, cv 6621 was the lowest and cv. EV-78 was intermediated in this morphometric attribute at all levels of drought stress.

Leaf water potential \( (Ψ_L) \) reduced in maize cultivars (DTC, EV-78 and 6621) due to 4 cycles of water stress. Drought tolerant cultivar, DTC had greater \( Ψ_L \) whereas cv. 6621 had minimal leaf water potential \( (Ψ_L) \) after four drought cycles. Total free amino acids and total soluble proteins decreased in in drought stressed plants of all maize cultivars examined in the present study. Maize cultivars also differed in both these biochemical attribute and cv. DTC was higher in both total soluble protein and free amino acids than the other two maize cultivars under water stress conditions.

Net CO\(_2\) assimilation rate (A), internal or sub-stomatal CO\(_2\) (\(C_i\)) concentration and transpiration rate (E) decreased markedly due to drought stress in the three maize cultivars. However, this reducing effect of water stress on A and \(C_i\) was minimal on cv. DTC. Maximum reduction in these gas exchange attributes was observed in cv. 6621 after 4 drought cycles. In addition, drought tolerant cv. DTC had lower transpiration but higher A and \(C_i\) values than the other maize cultivars. Due to this reason, drought tolerant cv. DTC had greater water use efficiency (WUE=\(A/E\)) under severe water stress conditions as compared to other maize cultivars. Overall cv. DTC had greater photosynthetic rate which is associated with greater \(C_i\), lower transpiration rate and higher WUE than EV-78 and 6621 maize cultivars.

Chlorophyll fluorescence variables (Fm/Fo, Fv/Fo, Fv/Fm), which depict PSII functional status, showed that Fm/Fo and Fv/Fo were lower in drought sensitive cv. 6621 than in cvs. DTC and EV-78. The rate of accumulation of closed reaction centers (Mo) increased in drought sensitive cv. 6621 whereas it was lower in water stressed plants of cvs. EV-78 and DTC. All energy fluxes such as absorption fluxes (ABS/RC, ABS/CSo, ABS/CSm), trapping fluxes (TRo/RC, TRo/CSo,
TR/CSm), electron transport fluxes (ETo/RC, ETo/CSo/ET/CSm) were increased under the induction of four drought cycles in drought sensitive cv. 6621, whereas all energy fluxes were decreased in moderately drought tolerant and drought tolerant cultivars cvs. EV-78 and DTC with respect to their controls. Likewise greater increase in dissipated energy fluxes (DIo/RC) was found in drought sensitive cv. 6621. In addition, moderately drought tolerant cv. EV-78 and tolerant maize cv. DTC were lower in dissipated energy fluxes. Comparison among DTC, EV-78 and 6621 maize (Zea mays L.) cultivars revealed that performance indices (PI) was maximal in drought tolerant cv. DTC than in sensitive cv. 6621. Moderately drought tolerant cv. EV-78 was intermediate in this bio-physical attribute.

In order to identify the yield indicators related to the drought tolerance, a field experiment was conducted in the fields of Botanic Gardens of Bahauddin Zakariya University Multan, Pakistan. To assess contribution of different yield components in degree of water stress sensitivity, different yield components were also assessed. Drought stress at the reproductive stage especially during tassel formation in maize (Zea mays L.) hindered the fertilization process because pollens remain immature and thus yield reduction occurs. In the present results, yield (kg/ha) and yield components of maize cultivars (DTC, EV-78 and 6621) such as 1000- kernel weight (g), number of kernel number/cob, kernel weight/cob (g), were decreased under water stress. Maize cultivars (DTC, EV-78 and 6621) showed the genetic variations regarding these attributes. Kernel yield was maximally reduced in water stress sensitive cv. 6621 and relative yield reduction (RYR) in this cv. 6621 was 75%. In contrast, kernel yield was maximal in water stress tolerant cv. DTC in which relative yield reduction was 48%. In addition, cv. EV-78 remained intermediate in kernel yield and showed 64% relative yield reduction due to water stress.

Yield is a polygenic complex trait; selection for yield under water limited environment is a difficult phenomenon due to variations in phenotypes versus genotypes and larger interaction between the plant species and environment. In addition, drought stress from the beginning of anthesis to maturity decreases the maize kernel yield through the reduction in the rate and duration of grain filling.
Among the abiotic stresses, drought is the most devastating environmental component accelerates the significant cut back in growth, yield and downgrade crop production on global scale (Liu et al., 2011; Li et al., 2013; Campos et al., 2014). The main reason for the occurrence of drought is the climatic change due to increase in temperature variation and increase in CO$_2$ concentration (280 to 385 ppm today and may reach up to 550 ppm by 2050), changes amount and pattern of rain fall (Houghton, 2009; Washington, 2013). Crop productivity is characterized to water intensive process as land cultivation is responsible for the consumption of more than 70% of the entire available water (Eckardt et al., 2009). In Asia, wheat, rice and maize production fall due to increasing water deficit and climatic changes (Challinor et al., 2010; Dolferus et al., 2011). Abiotic factors such as drought and salinity accounts 70% yield reduction, that are being exacerbate by the climatic change (Peleg et al., 2011). In recent years, the damage from the droughts to agriculture in some countries was extensive, and the figure of losses due to drought rank first in the list of all natural hazards (Gosal et al., 2009). Pakistan being an agrarian country, deficiency of water at acute level will have the destructive effects on economy, as the agriculture sector directly contribute almost one fourth of its GDP. In addition, faster increase in human population and dwindling land and water resources further aggravate this problem (Godfray et al., 2010).

Globally, growth of population is likely to increase above nine billion in less than 40 years. Drought phenomenon has raised widespread concern on food supply for increasing population worldwide, which is estimated to reach 9.1 billion in 2050 (Gregory & George, 2011). According to FAO Declaration of the World Summit on the Security of Food 2009, 70% more food require till 2050 and to achieve this 44 million metric ton cereal production is required annually for growing population. Penalties of drought spells caused hunger and impoverishment with the serious malfunctioning in social and economic standards of Thailand, India etc. (Singh & Ballabh, 2008).
Maize (*Zea mays* L.) is the third highest yielding cereal and oilseed crop worldwide. In general maize kernel contains 2-6% oil and from this, 85% associated to the seed’s scutellum (Serna-Saldívar, 2010). A bushel of corn (weighing 56 lbs) yields 1.55 pounds of corn oil (almost 3% by weight). In maize (*Zea mays* L.), the high oil content of some varieties is due to an enlarged embryo structure. Corn oil extracted from the maize germ contains high contents polyunsaturated acids and show the oxidative stability. In refined corn oil, triglycerides are 99%, almost 65% proportion of polyunsaturated fatty acids, monounsaturated fatty acids are 33%, and 15% of saturated fatty acids. In Thailand, yield losses due to drought are as high as 45% (Jongdee et al., 2006), while in Cambodia, it ranges from 12-46% (Ouk et al., 2006). (Jones & Thornton, 2003) predicted that in Latin America and Africa, future maize losses estimated $2 billion per year due to climate change.

Water scarcity greatly influence various stages of crop growth and development (Maes et al., 2009) but juvenile stages; seed germination and seedling growth are particularly of great concerns because germination results in optimal number of seedlings that governs overall success of a crop in terms of yield (Lobell et al., 2008). For example, drop in soil moisture contents from 50-20 % field capacity caused the decreased emergence of about 94.3-82.7 % in winter and spring rapeseed genotypes. Ouk et al. (2006) observed the 12 to 46% loss in yield production of lowland rice due to drought stress. Hence, germination and seedling growth attributes such as germination percentage and rate with physiological indices are frequently used as predictors to appraise drought tolerance in crop plants (Zhou et al., 2011).

How water shortage affects the plant growth and in what ways physiological traits play a role in drought tolerance that results in genetic variability has been discussed by number of authors (Erice et al., 2010; Ahmed et al., 2013; Aimar et al., 2014). Their main focus has been on photosynthetic capacity, antioxidant potential and maintenance of plant water status in relation yield under water deficit environment (Chaves et al., 2003; Neumann, 2008; Brestic & Zivcak, 2013; Guha et al., 2013; Sengupta et al., 2013; Araus & Cairns, 2014; Chen et al., 2014; Gajanayake et al., 2014).
Water status in plants is highly sensitive to water deficit and therefor play a significant role in determining the plant tolerance against the water shortage. Reduction in plant growth and productivity mainly due to drought stress is mainly due to changes in plant water status such as relative water content (RWC), water potential that result in reduction in cell division and cell enlargement. Water relation characteristics of leaves may be helpful to study the maintenance capacity of functional status of species under water deficit environment (Rodríguez et al., 2012). The optimal water content (80-90%) under non stressed conditions did not bring alteration in normal physiology of plants (Lawlor & Cornic, 2002b). Conversely, when the RWC drop below 70% (mild water stress, the most frequently occurring stress condition in nature), negative cascade is initiated in the physiological processes of the plant. These deviations from the normal conditions caused the turgor loss as well as the fall in the status of leaf water potential. As a result of decreased in leaf relative content and water potential, there is also reduction in cell areas of mesophyll and bundle sheath cells and increase in cell wall thickness. For example, dehydration in leaves correlate with a decrease in their water potential resulted reduction in the cell size, leaf plate area (Suarez, 2011), and an increase in the cuticle width (Zhuang et al., 2011). However, plant tolerance to drought confers by maintaining the optimal cell turgor to continue the metabolism even under acute water shortage. Similarly, capacity to maintain the optimal level of relative water content (RWC) under induction of water stress was noticed in drought tolerant cultivars of bean plants (Zlatev et al., 2006). Additionally, accumulation of greater amount of proline and other osmotic compounds in bean cultivars take part in the drop of water potential as well as in osmotic adjustment (Zlatev et al., 2006). Number of studies reported that the processes linked to plant metabolism are less tolerant to cell turgidity and volume as compared to water potential (Jones & Corlett, 1992; Lawlor & Cornic, 2002a; Martínez et al., 2007; Martorell et al., 2015). Physiological mechanisms associated to maintain the turgor pressure of leaf while the decline in osmotic potential might be due to drop in fraction of osmotic water or because of imbalance in mechanism of osmotic adjustment (solute accumulation in the symplast) was noticed (Jones et al., 1980). Drought tolerance of Ml-173 (Tunisian Medicago laciniata population originating from the arid region) was found to be linked with marked reduction in osmotic potential (OP) and less reduction in RWC due to buildup of solutes such as potassium ions (K⁺), proline and soluble sugars (Yousfi et al., 2010). Moreover, variations in tissue elasticity under water stress that modify the association between cell volume and turgor
pressure turgor, might contribute in tolerance against water stress, as experimental in sunflower (Maury et al., 2000), black spruce (Blake et al., 1991), and common bean (Martínez et al., 2007).

Nutrient uptake of plant is usually reduced under conditions of drought stress due to reduced uptake rate of nutrient by the root and its transportation to the shoot because of limited rate of transpiration, disturbing the active transport and membrane permeability (Ge et al., 2012). The uptake of nutrients from the soil related to status of water in soil and the plant roots. Because decrease in soil moisture contents related to the fall in diffusion rate of nutrients from the soil solution to the roots absorbing surface (Bramley et al., 2010). The reduction in uptake and transpiration of water are generally related with decrease in shoot water contents and stomatal aperture size, showing that leaves were in the condition of water stress (Barrett-Lennard et al., 1988). Losses of nutrients in plants throughout the dry season are the results of senescence and decay of leaves (Buwalda & Greenway, 1989).

Optimal quantity of nitrogen is crucial for the biochemistry of non-enzymatic components like the secondary metabolites, coenzymes, polyamines, and the photosynthetic pigments (Senadheera & Maathuis, 2009) in plants. As a result of soil solution potential on nitrogen uptake and its allocation in plants may also play a role in contributing to the effect of drought on N contents in grasses likewise to the water stress effect on the transfer of mineral N in the soil solution to the root surface. However, significance of this physiological effect varies among different species (Gonzalez-Dugo et al., 2012). Many scientists reported that uptake of nitrogen (N) was reduced in soyabean and rice (Tanguilig et al., 1987), wheat (Debaeke et al., 2006) and also in bean (Zayed & Zeid, 1997) under water stress environment. In addition decreased uptake of N also perturbs the transpiration rate and transport from roots to shoots in plants.

Phosphorus is one of six essential macro nutrient for proper functioning of plant growth (Ribot et al., 2008; Han et al., 2014). Nawaz et al. (2012) reported that drought stress at early stage decreased the nitrogen uptake by 38% whereas it was 46% during late stages of drought stress. In addition to this, the phosphorus (P) and potassium uptake (K) were declined to 49 and 37%, respectively during the beginning of water stress environment whereas this uptake
reduction was drop to 51% during later stages of water deficit. Drought stress (at 60% field capacity) significantly decrease the P contents in roots and shoots, and root K⁺ of canola cultivars (Shafiq et al., 2014). (Ashraf et al., 2014) reported that water stress lowered potassium (K⁺) and calcium contents (Ca²⁺) while iron and phosphorus contents were increased in maize. In the context of nutritional status of plant, potassium ion (K⁺) contribute a central role in many physiological functions necessary for growth (Zörb et al., 2014), yield, quality, maintain a high osmotic potential in the sieve tubes (Marschner, 2012) and stress resistance of all crops. Tsonev et al. (2011) showed positive effects of K nutrition on the rate of photosynthesis only in crops subjected to some drought treatment. The assimilation of CO₂ required the sufficient supply of K by a crop is greater under limited water than that of well-watered conditions (Römheld & Kirkby, 2010). The differences in absorption of K among different plant species are attributed to variations in root structure, such as root density, rooting depth, and root hair length (Ahmad & Maathuis, 2014; Nieves-Cordones et al., 2014; Wigoda et al., 2014). The uptake capacity of K by the roots was hampered under water stress. Thus, foliar spray of K had been suggested under stress environment (Römheld & Kirkby, 2010).

Therefore, transport of nutrient in plants mainly base on synthesis, translocation and utilizations of photosynthates because of translation of these processes is regulated by the balanced nutrient metabolism. So, the perturbations in uptake of nutrients by different plant organs distress all physiological mechanisms that are linked to nutrient uptake and their utilization efficiency due to induction of drought stress. Consequently, this directed to fluctuation in biomass allocation pattern under water stress that reduced the growth and yield.

Osmotic adjustment (OA) has been suggested to be necessary and play a substantial contribution in plant adaptations to drought tolerance (Zhou & Yu, 2010). It is widely known that sodium ion (Na⁺), potassium ion (K⁺) and soluble sugars are the main stabilizer elements in OA, whereas calcium ion (Ca²⁺) and proline act as trivial contributor in OA in the seedling of sugar beet cultivars under water deficit (Wu et al., 2014). In addition, (Martínez et al., 2007) argue that these compounds assist the stressed cells in two ways; firstly, as osmolyte of cytoplasm, thus facilitate the uptake of water and its retain capacity. Secondly, these compounds protect and stabilize the macromolecules and structures (proteins, membranes, chloroplast, and
liposomes) from injury induced under stress conditions. The degree of osmotic adjustments in plants under water deficit is determined by many factors such as the rate and span of duration at which water stress developed, intensity of water deficit, type of cultivar, tissue age and developmental stage of plant (Nio et al., 2011a). High yielding wheat genotype Kohdasht had the highest water retention property after the induction of drought stress mostly due to effective OA and stomatal closure (Soleimani et al., 2014). Moreover, pattern of solutes accumulation was varying at different growth stages in wheat leaves during leaf osmotic adjustment under drought stress (Nio et al., 2011b). It was also observed that various solutes accumulated in wheat cultivar Hortog at different times to maintain the mechanism of osmotic adjustment during development of water stress. The major (54%) contributor of OA was the K⁺ upto the 30 days of drying, glycine betain accounted the 19%, proline and glucose give 21% of OA later at the day 37.

Water stress is considered to induce the oxidative stress that alters the metabolism of crop plants (Foyer & Shigeoka, 2011). Drought stress induced the generation of reactive oxygen species (ROS) act as highly reactive oxidized biological molecules such as singlet oxygen (O₂⁻), hydroxyl radical (OH⁻) and hydrogen per oxide (H₂O₂) can be damaging to carbohydrates, proteins, lipids, nucleic acids and other cell tissues (Marok et al., 2013). To scavenging this toxic effect of ROS, plants respond to develop complex antioxidant machinery that comprises the enzymatic components (peroxidases (POX), catalases (CAT), superoxide dismutases (SOD), and ascorbate peroxidase (APX) and non-enzymatic components (α-tocopherols, β-carotene, ascorbates and glutathione (Uzilday et al., 2014). This antioxidant machinery serves as the physiological indicators of stress tolerance (dos Santos et al., 2014). For example, greater activities of SOD, POD and the minimum level of malondialdehyde saw in drought tolerant orchard grass (Baoxing) as compared to drought sensitive orchard grass (01998) under the imposition of drought stress (Ji et al., 2014). Another study also found that moisture stress caused increased activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) in Panicum sumatrensein (Ajithkumar & Panneerselvam, 2014). As drought stress intensified, more increase in malondialdehyde and electrolyte leakage showing an increase in oxidative stress. Furthermore, increase in accumulation of proline and total soluble sugars, enhanced activities of SOD and POX, accumulation of free proline and total soluble sugar contributed to defensive mechanism of plant against the damaging effect of oxidative
stress (Ge et al., 2014). In adding to lethal toxicity of ROS, it was recently stated that ROS molecules play an important role in growth and development of the plant under stressed environment. Hence, this feature make it to produce the optimum ROS in cell to stabilize its regular operative (Mittler et al., 2011).

Photosynthesis is one of the most and complex and sensitive process to water deficit environment in terms of plant production (Chaves et al., 2009; Guerfel et al., 2009). Shutting down of stomata is the first action response to drought stress and it’s an effective way to conserve water under water stress condition. The maximum assimilation rate of CO$_2$ (A) under ambient CO$_2$ (Ca), intercellular CO$_2$ (Ci) and the chloroplastic CO$_2$ (Cc) concentrations and day light of fully hydrated leaves is translate as photosynthetic potential ($A_{pot}$). But the inhibitory effect of drought stress on the process of photosynthesis may be related to low CO$_2$ availability because of decreased stomatal conductance as well as mesophyll conductance (Flexas et al., 2014) due to impairment of carbon assimilation metabolism (Peeva & Cornic, 2009). Massive publicized research work reported that stomatal limitations limit CO$_2$ assimilation capacity due to impairment of water potential which may distress the photosynthesis under drought stress (Chaves et al., 2009; Hu et al., 2010). Moreover, water limited environment decreases both photosynthetic rate (A) and internal CO$_2$ concentration (Ci) and this concurrent to the fall in relative water content of leaves (RWC) and in water potential (Cornic & Massacci, 1996; Flexas et al., 1998). Water stress mainly damaged the transfer of electrons from the plastoquinone pool to terminal acceptors of photosystem I; along with this it limit to stomatal components and non-stomatal components of photosynthesis directly limited the carbon assimilation.

Genotypic variations also exist in gas exchange characteristic due to change in drought intensity and duration. Guha et al. (2010) stated the minimal change in leaf gas exchange attributes associated to sustain the transpiration and stomatal conductance in drought tolerant mulberry genotypes for longer period of time even under the induction of drought stress. Such behavior is analogous to the anisohydric physiology of plants that exert low stomatal control on leaf gas exchange in spite of high evaporative demand. Morales et al. (2013) documented that four-week water stress induced reduction in photosynthetic rate is lesser in drought tolerant raspberry (Rubus idaeus L.) cultivar than drought sensitive.
The responsive mechanism of photosynthetic parameters to drought stress was helpful to indicate the drought tolerance mechanism in crop plants. A/Ci curve showed the relationship between net photosynthetic rate and intercellular CO$_2$ concentration. The photosynthetic parameters $V_{\text{cmx}}$ (the maximum rate of carboxylation), $J_{\text{max}}$ (maximum potential rate of carboxylation and the triose phosphate utilization were derived from the A/Ci curve. In a study on Leymus chinensis, the Maximum values of $V_{\text{cmx}}$, $J_{\text{max}}$ and triose phosphate utilization were observed under the soil moisture content of 15.56, 15.89 and 16.23% in greenhouse experiment while these were the 16.89, 17 and 16.79% in a field experiment, respectively (Lin et al., 2008). The photosynthetic parameters of Leymus chinensis under the soil moistures of 18-19% and 15-16% could recover from the water stress rapidly; even some of them would increase (Lin et al., 2008). It inferred that slight water stress could improve functioning of photosynthetic parameters. This study also elucidates those photosynthetic parameters of Leymus chinensis would not recover to the normal level when the soil moisture is less than 10-12%. Moreover, the response of A to the Ci (A-Ci) describe that A was limited under water limited environment (Carmo-Silva et al., 2012). Thus, the limitation rate by mesophyll conductance (gm) from An-Cc was higher in the leaves of non-irrigated shrub than daily irrigated. Maximum carboxylation efficiency derived from An-Cc contrary to those that are calculated from An-Ci, were higher in non-irrigated shrub than shrub that is daily irrigated (Rho et al., 2012).

In general, water use efficiency (WUE) considered as the ratio between transpiration and photosynthesis in physiological context. However, this ratio is not in optimal functioning under drought stress. It has been shown earlier that WUE is the key physiological indicator to describe capacity of genotype to conserve water under limited water supply and this efficiency of water use combine to the drought resistance and maximum potential of yield (Fang et al., 2010; Richards et al., 2010; Zhang et al., 2014). Significant variations in WUE levels were observed by number of scientist among cultivars of winter wheat (Hu et al., 2006; Dong et al., 2011; Miranzadeh et al., 2011). For example, in studying of 19 cultivars of winter wheat, a 31.3% difference in WUE was found (Dong et al., 2011). Similarly, in another study, differences were found in WUE among pea varieties for green seeds in response to dry environment (Nemeskéri et al., 2015). High yielding cultivars generally showed relatively high WUE level under drought
stress and therefore the selection of cultivars that show the higher degree of water use efficiency could increase the yield (Zhang et al., 2005; Zhang et al., 2010).

The biochemical limitations to photosynthesis under water stress conditions are generally associated with low carboxylation efficiency and low electron transport rate (Zong et al., 2014). So, lower photosynthetic CO$_2$ fixation causes decrease in ATP and NADPH consumption in Calvin cycle and finally results the over-reduction of electron transport carriers owing to deficient electron acceptor (NADP$^+$) (Zivcak et al., 2013). However, the consequence of water deficit on performance activity of Rubisco vary with the plant species and severity of water deficit fluctuating from intensive decrease to slight or no blockage of enzyme activity (Galmés et al., 2013). In C4 plants, RuBisCO strictly localizes and CO$_2$ fixes in bundle sheath cells and the availability of CO$_2$ around the RuBisCO is maximized by CO$_2$ concentrating mechanism and the photorespiration rate is decreased (Gowik & Westhoff, 2011). These unlike characteristics between C3 and C4 plants can also a source of variations in the adjustment of other metabolic processes. Consequently, mesophyll limitations down-regulated the photosynthesis in the leaves of ‘Bluecrop’ blueberry than stomatal and biochemical limitation under moderate drought stress (Rho et al., 2012).

Stomatal closure, the early response of water deficit, which limit the CO$_2$ absorption may boost an imbalance between photochemical activity of PSII and the electron demand of the Calvin-Benson cycle, governing to surplus absorbed excitation and consequently photoinhibitory damage to the reaction centers of PSII occur (Baker & Rosenqvist, 2004; Foyer et al., 2012). As a result, there is an unbalance between NADPH/NADP ratio in the stroma because of the lesser use of NADPH in the Calvin cycle functioning, responsible of decreasing the NADP$^+$ content that is the main electron acceptor of PSI. Numerous reports have publicized that drought stress negatively alter the functionality of both photosystems (PSI and PSII) mainly PSII. This accelerated to lower electron transport through these photosystem (Liu et al., 2009b). When plant face the highly luminous environment due the water stress, the problem is worsened due to surplus electrons present in the thylakoid and photosystem transport system, consequently result in the over-excitation of PSII and PSI reaction centers (RCs) and increasing the production of reactive oxygen species (Sharma et al., 2012). Many studies in documented that water deficit
may be the cause of injury to the OEC of PSII and hold it down the PSII reaction centers (De Ronde et al., 2004) correlated to breaking of D1 protein (Meyer & de Kouchkovsky, 1993; Liu et al., 2014; Ramalho et al., 2014). Furthermore, when photosynthetic electron chain is over-reduced and inactivation of oxygen evolving manganese complexes occurred, that may affect PSII to damaged triggered by long lasting P680+/water stress on the concomitant new synthesis of D1 protein may outcome ROS generation, consequently this directed to the photoinhibition and oxidative stress (Asada, 1999; Ramalho et al., 2014). Proteins of PSII such as D1, D2 and LHCII likewise mRNA coincides to psbA, psbD and cab, dropped greatly because of water stress, this was attributed to decrease in transcription and translation as well as fast degradation of protein and mRNAs (Duan et al. 2006, Liu et al. 2009). It was stated that stability against over-reduction of acceptor side was intensify in wheat leaves under water stress (Zivcak et al., 2014). This was complemented by rapid development of transthylakoid pH gradient even at low light intensities, pertinent to unusual increase in NPQ and down regulation of electron transport chain. In addition to this, it was escort by rapid increase in redox poise at the acceptor side of PSII and at the PSI donor side. The acidification of thylakoid lumen in drought stressed tissue could be related to the increased fraction of PSI not participated in linear electron flow, that may directed to cyclic flow of electrons in spite of low light, further drought-induced decrease in amplitude of IP step of fast chlorophyll a fluorescence.

Under water stress, stomatal closure reduces the CO₂ fixation that resulted in over reduction of photosynthetic electron transport chain components. This may lead to switch rubisco from carboxylase activity to oxygenase activity – photorespiration. Photorespiration is a general phenomenon of C3 type of mechanism considered energetically negative because it competes carbon assimilation by consuming O₂ at the cost of CO₂ (Foyer et al., 2009). Photorespiration has been shown to function in photoprotection under drought stress (Wingler et al., 1999), and maximum in drought tolerant transgenic lines (Blumwald et al., 2009). Greater increase in photorespiration rate under water stress environment can be seen as acclamatory response to avoid over-excitation of PSII units under severe drought stress (Massacci et al., 2008). So, linear electron flow of energy can be in part transmitted to photorespiration (Kozaki & Takeba, 1996), that give to transthylakoid ΔpH, this was keep the higher level of electron flow; centrally important in drought stress environment (Singh & Raja Reddy, 2011).
Nevertheless, the proton flow in photosynthesis can also be modulated, due to cyclic electron flow around the PSI, could be upregulated under stressed environment (Bukhov & Carpentier, 2004; Johnson, 2011). This process shown to be crucial because it pays to the contribution of photoprotective mechanism and equilibrate production ratio of ATP/NADPH (Munekage et al., 2004).

Monitoring factors (phosphorylation and dephosphorylation of PSII) act as to restore the function of PSII under drought stress. Liu et al. (2009a) reported that drought caused the rapid dephosphorylation of proteins linked to PSII, connected to the phosphorylation of LHCII b4 and CP29 in (Hordium vulgare). Intrinsic and extrinsic phosphatases membrane proteins are involved to accelerate the process of dephosphorylation. Though, it was also reported that decline in dephosphorylation exacerbated the damages induced by stress and inhibited to repairing of photosystems, when the stress is retrieved by rehydration. Additionally, the structure of thylakoid persisted to integral state under water deficit with the exception of CP29 transferred slightly from granal to stromal thylakoid, although the rest of PSII proteins remained intact and undamaged. However, chloroplast proteins activated under drought stress triggered to liberate the TLP40, act as the membrane phosphatase inhibitor. It was hypothesized that phosphorylation of CP29 may disconnecting the LHCII from the PSII complex and broken down the trimer of LHCII results its degradation. Contrary to this, dephosphorylation of PSII proteins may play a role in the repair of PSII proteins and signal transduction during the stress induction (Liu et al., 2009b).

Non-photochemical quenching is an important photoprotective mechanism in plants that dissipate the excess light energy in PSII under induction of water stress (Lambrev et al., 2012; Ruban & Murchie, 2012). Different pathways of energy dissipation served as the photoprotective mechanism. First, light energy can be refixed in the photorespired CO₂. Secondly, Mehler type of reactions contributed in balancing of electron flow and thirdly the heat dissipation via the xanthophyll cycle and the down regulation of photochemical efficiency are also engaged in the excess light energy dissipation. The mechanism of non-radiative energy dissipation was obvious upon exposure to water stress due to increased NPQ and decreased efficacy of excitation apprehension by open RCs (Oliveira & Salgado, 2014). In addition to this, it was suggested that
Drought causes coordinate changes in down regulation of photosynthesis by dissipation of excess energy at PSII by non-photochemical mechanism connected to increase in photorespiration, limiting the photodamages (Silva et al., 2012). Coefficient of non-photochemical quenching (qN) was increased in rice genotypes during the post-anthesis water deficit conditions (Gauthami et al., 2014).

Decrease in photosynthetic rate is at least partially attributed to the decrease of RuBPC activity under drought stress and that drought inhibits RuBPC activity by decreasing the ratio of PAs/fPut and increasing the release of ethylene in pakchoi (Huang et al., 2014). Phosphoenolpyruvate carboxylase (PEPC), NADP-malic enzyme (NADP-ME), and pyruvate phosphate dikinase (PPDK) take part to concentrating the CO₂ in C4 type of photosynthesis. Greater activities of PEPC, NADP-ME, and PPDK were observed in various crop plants under different kind of abiotic stresses, like drought, salinity, heavy metal toxicity and the lack of phosphate and iron in soil. The functions of PEPC, NADP-ME and PPDK seem to be essential for the growth of plants under stress environment as compared to normal conditions (Doubnerová & Ryšlavá, 2011).

Chlorophyll a fluorescence technique is extensively used to check the drought tolerance and resistance via the screening and selection method against environmental stresses (Baker, 2008; Fang et al., 2010; Murchie & Lawson, 2013). Chlorophyll a fluorescence can be employ to determine the photosynthetic efficiency and gives the knowledge of connected relation between structure and function of PSII reaction centers and core complexes (Gomes et al., 2012). Functionality status of photosynthetic apparatus is very important physiological indicator to determine the susceptibility of various crops under harsh set of environmental condition (Maxwell & Johnson, 2000; Baker, 2008; Fang et al., 2010; Murchie & Lawson, 2013).

Prompt and delayed fluorescence technique is very useful to investigate the effect of drought on photosynthesis. The shape of the delayed fluorescence induction was profound to be the most sensitive indicator for various changes in electron transport of plants under water stress. A study depicted that variation in second delayed fluorescence induction could be inferred as reduction in the rate of intersystem electron flow in tolerant plants of Plantago (Krasteva et al.,
Furthermore, this study suggest that decrease in reduction rate of P700+ was observed when the light reflection at 820 nm. This study also proposed that suppression of intersystem of electron transport might be related to the adjustment of electron transport chain for further switching of cyclic electron transport in PSI to secure the photosynthetic electron transport protection against drought induced stress.

Of various chlorophyll fluorescence techniques (Murchie and Lawson, 2013), use of JIP test gaining a significant importance as this is a rapid tool which is also workable in field. The JIP-test is study of behavior of photosynthetic apparatus under stress conditions and is sensitive to the change in environment caused by abiotic stresses (Živčák et al., 2008). OJIP transients can be divided into O, J, I, and P phases, describe the three reduction processes of electron transport chain. It has been argued that rise in OJ step characterize reduction of PSII acceptor side (Stirbet & Govindjee, 2011). The next JI phase has been shown the kinetic properties likely for the oxidation and reduction of plastoquinone pool. The IP is the slowest step of fluorescence rise that taken the time of almost 30-300 ms, respectively, was equivalent to the re-reduction of plastocyanine (Singh et al., 2007) and P700+ in PSI (Stirbet & Govindjee, 2011). JIP test decode the primary fluorescence dimensions of OJIP kinetics into many biophysical and phenomenological parameter that enumerate the PS II status (Ceppi et al., 2012). Moreover, recently performance index (PI_{ABS}) derived from JIP-test calculation was recognized as more sensitive indication of drought stress as compared to maximum quantum yield of primary photocemistry (Oukarroum et al., 2007; Strasser et al., 2004). PI_{ABS} showed as an integrative calculation of three independent attributes. First character is the density of active RCs, second parameter including the efficiency of electron transfer by the trapped exciton into the electron transport chain and the third attribute described as the probability that an absorbed photon could be trapped by the reaction center. Water stresses decreased the IPABS (Oukarroum et al., 2007; Živčák et al., 2008) and drop the stomatal conductance and rate of CO₂ assimilation in crop plants. Redillas et al. (2011) reported that transgenic rice overexpressing OsNAC10 with improved drought tolerance had higher efficiency of energy utilization evaluated through analysis of chlorophyll transients with corresponding normalization. Variations in PSII units and constancy of oxygen evolving complex (OEC) are also observed in barley (Hordeum vulgare L.) cultivars showed differential drought tolerance (Oukarroum et al., 2007).
Drought avoidance is the capacity of plants to sustain the metabolic activities by maintaining the cellular hydration at lower water status. Drought avoidance mechanism contains the effective water acquisition through massive, deeper root system, and greater root penetration ability (Miyazawa et al., 2011). Arid legumes such as Cyamopsis tetragonoloba (L.), Vigna aconitifolia (Jacq.), Vigna unguiculata (L.) are characterized by their tap root and slow growth classified as drought avoiders (Parida & Das, 2005). Abiotic stresses enrich the deposition of cuticular waxes (increasing impermeability of cuticle) to prevent the water loss consequently enhanced the drought avoidance (Bolger et al., 2005; Lopes et al., 2011). Thus, it can be suggested that both natural and man-made selection given the preference to drought avoidance over drought tolerance except in the case of resurrection plants. Plants that escape the drought period having high potential of developmental plasticity, being able to complete their growth and reproductive cycle before the onset of drought period. Optimum gas exchange rate in selfers was favored for selection to complete their life cycle fastly and thus escape the seasonal drought (Mazer et al., 2010). Higher drought tolerance ability in chickpea was observed due to early flowering and considered it to have the mechanism of drought escape (Bueckert & Clarke, 2013). This strategy of plants is important for the arid regions where native annual species may have short life period with high rate of growth due to efficient gas exchange characteristics, uses the maximum assimilates from soil during water reservoir. For example, ability of cowpea cultivars to escape the terminal drought should be exploited in areas with very small duration of rainfall season (Hall, 2012). This is linked with the plants ability to reserve the photo-synthase in roots and stem and mobilize them to synthesize the fruiting bodies as in cereals and some leguminous plants. Similarly, warm dry conditions can selectively favor the correlated evolution of traits that contribute in resource acquisitive mechanism and escape mechanism of earlier reproduction in spite of lower fertility rate (Brouillette et al., 2014). This mobilizing ability of photo-products is increased in plants that are subjected to water stress environment. Shortening in the duration of crop growth due to onset of early flowering constitutes an important characteristic of drought escape. Similarly, Brassica rapa escape the drought period through early flowering due to optimal maintenance of water use efficiency (Franks, 2011). These findings also suggested a trade-off between the drought escape and avoidance, further it was considered that drought act to shorten the growing season. Thus, earlier flowering based categorization of drought escape is fundamentally important than phenotypic plasticity. But, the short period of
growth usually outcome the lower economic yield, particularly in indeterminate crops like cotton. California poppy complete its life cycle in limited weeks before the onset of drought but Coffee and Cacao produced flowers and fruits when rain trail drought period (Alvim & Peirão, 1985).

Grain yield of different crops is the primary measuring character for the extent of resistance against drought stress. Secondary traits may be playing a particular role to improving the selection criterion under stress conditions. Ribaut et al. (2009) reported that classifications of secondary traits based on their performance contribution are the determinants of primary traits (concentration of transpired water, water use efficiency (WUE) and harvest index (HI). Selection of screening methods for the secondary traits should be established: primarily, advancement in precision where the variations in yield heritability is reduced due to stressed environment; secondly, avoid the confounding effects of timing of drought stress on yield during flowering dates; finally, focus for the selection of specific type of stress, and (Susumu Hiraga 1, 2001) measurements should be economical, easier approach and faster than the grain yield of primary traits (Araus et al., 2008). A desirable secondary trait must meet the several rationale (Royo et al., 2007; Ribaut et al., 2009): genetically associated to the final grain yield, less affected by the impact of stressed environment due to higher heritability, genetic diversity within species, do not show the yield reduction under normal environment, fast, uniformity, reliable, cost effective measurements and ensured assessment at individual plant level as well as for small plot areas.

Aims and objectives

1. Assessment of variation for drought tolerance in germplasm of six maize (Zea mays L.) cultivars (DTC, EV-77, EV-78, EV-79, Faisalabad mays and 6621) at the germination and seedling growth stages.
2. Assessment of degree of water stress tolerance in selected maize cultivars at the vegetative and reproductive growth stages.
3. Evaluation of various morpho-physiological and biochemical characteristics selected maize cultivars that could be used as reliable selection criterion at vegetative growth stages.
Crop production is severely affected due to reduction in growth by the number of abiotic stresses such as salinity, water stress, temperature extremes, flooding, cold stress, light stress, toxic concentration of metals, and deficiency of nutrients (Cavatte et al., 2012; Holzkämper et al., 2013; Jha et al., 2014). It has been enumerated that 50% of the yield losses was due the abiotic stresses (Morgounov et al., 2014). The estimated losses of yield are 20% owing to salinity stress, while the 17% yield destruction was due to drought, high and low temperature destroy the yield 40 and 15% respectively, and other factors reduce the yield to almost 8% (Ashraf et al., 2008).

**Major threats to global food security**

FAO (2013) reported that food security encompasses food availability (crop productivity including food wastes), food access (shows income linked with purchasing ability as well as market factors) with its stability and availability (prevalence of extreme events due to climate change) and food utilization (express nutritive quality as well as its security).

In the 21st century, environmental stresses are the major threat indicators to food security worldwide (Battisti & Naylor, 2009). Abiotic stressors induced yield losses will become more predominant in next decades due to worldwide variation in weathers. Drought differs from other natural disaster that its effects often accumulate slowly over a longer period of time and may linger for years even after the termination of the event. Climate change and drought will increase percentage (10 to 20%) of people at the menace of hunger. According to recent estimate, more than 8 hundred million people are underfed (Fao, 2014) and food production needs to increase by 70% in 2050 to feed the population worldwide. Population growth coupled with environmental extremes due to climatic change will exacerbate the existing risks to food security (Khan et al., 2014). A study reported that the correlation exits between hunger, poverty and water shortage (Falkenmark, 1990). Additionally, predictable economic evaluation standards, irrigated land area can yield decrease economic values of water at margin than to usage of water by competing divisions. Consequently, the future use of natural resources especially to sustain agriculture yield
under limited water supply has been of critical interest for researchers (Hoekstra & Mekonnen, 2012; Vanham et al., 2013).

**Water scarcity**

Water shortage is largely categorized into physical and economical types. Physical scarcity of water entails in those areas where surface and ground water resources are insufficient to supply the water demand of those regions. While the economical water shortages is when water resources are scarce because of mishandling the optimal available water resources. In number of countries, economic water supply is more widespread than physical which is due to mishandling and overexploitation of water assets in agriculture, industrial, urban and other sectors as in Pakistan (Economic survey of Pakistan, 2008).

Pakistan being an agrarian country has maintain one of the well-developed canal system in the world that fulfill the demanding need of water to nearly about 86% of the irrigated areas (18 million ha) give production about 90% of the foodstuff/fibre necessities of the country. The remaining 13% of the total cultivated area (~23.38 million ha) is entirely rainfed (Jaleel et al., 2008). Drought spell of the years 1998-2002 is the worst disaster during 50 years history of Pakistan. This extensively longest drought spell loss the 25 PKR billion to the national exchequer and economic growth rate depressed to 2.6% (GoB, 2007). This dry spell critically affected 1.91 million people lives, 9.31 million livestock, 80% fruit orchards and 1.76 million livestock had perished. Moreover, 22 districts out of 29 of this province were deadly affected. World bank notified that drought would predictably hit the economic growth of Pakistan. During years from 1999-2000, more than 60 million people of Central Asia and South west Asia were suffered due to persistent drought of many years. Thus, it is one of the major global perspectives (IRI, 2001) with Afghanistan, Iran, Western Pakistan, Turkmenistan, Tajikistan, and Uzbekistan experiencing the most severe impacts.
Fig. 2.1. Irrigated and rain-fed areas of Pakistan

**Worldwide impacts of drought on maize yield**

Number of studies documented the impacts of droughts on corn production in various part of the world (Endfield and Tejedo, 2006; Halvinka et al., 2009; Trigo et al., 2010; Ozdogan, 2011; Velde et al., 2012; Evangelista et al., 2013; Ju et al., 2013; USDA, 2013). These studies reported that drought causes 12-27% yield losses in various countries such as Botswana, China, Czech Republic, France, Mexico, Turkey and USA. Variation in yield losses in maize due to drought has been due to additional climatic factors such as high temperature.
Increasing crop yield is key challenge of growing world population

Literature concerning to the economic problems is probably to cause the drop in food production over the next century (Jaggard et al., 2010). With the growing world population, increase in crop yield is the vital challenge especially for the plant scientist as well as for the agriculture industry. Progress over the last 50 years in agriculture sector has been made. For example, from one acre of land, a US farmer can produce sufficient food to fulfill the demand of 151 persons that is additionally the double production of the year 1960.

Can plant scientist able to maintain or uplift that development to nourish 9 billion people for their life perspective of long time future (Gregory & George, 2011)? In addition to these, demographic and economic models that develop the increasing demand of food thankful to the growth of population (Foyer & Shigeoka, 2011), expansion of cities (Satterthwaite et al., 2010), and a shift to more meat consumption (Wang et al., 2010) play crucial role to accept the challenge. Therefore, this specifies that worldwide food safety is critical unless the food productivity increases to 70% (Godfray et al., 2010).

Yield and yield components under drought stress

Maize kernel yield depends on plant density, cobs number per plant, number of kernel rows per cob, and number of kernels per row of cob and these yield components are function of numerous developmental stages of the plant and can differ with intensity of stress (Khalily et al., 2010; Harrison et al., 2014; Oyekunle & Badu-Apraku, 2014). Of these yield components, kernel number is determined by plant vigor 10-15 days before anthesis and this growth stages is most vulnerable to drought stress (Spitkó et al., 2014). For example, kernel number/cob was decreased to 60% in field grown maize cv. Loft under long drought conditions (Asch et al., 2001). Several studies have reported that drought stress substantially influence grain filling and grain size (Badu-Apraku et al., 2011; Badu-Apraku & Oyekunle, 2012; Khodarahmpour & Hamidi, 2012; Aparna et al., 2014). For example, 100- grain weight was the most important component of yield to determine the yield variation in maize when the different concentrations of water stress were induced at the low sensitive stages of growth (Mansouri-Far et al., 2010). Similarly, in other cereal crops such as barley, wheat and rice total number of grains depends on inflorescence initiation, spikelets development on spikes and intensity of drought stress at these developmental
stages (Fig 3,4,5) (Ji et al., 2010). In addition, number of grains can also be decreased far along when the abiotic stress factors coincides to anther dehiscence. Failure of discharge the pollens from the locules of anther halts dispersion in self-fertilizing species like in wheat and rice. Extended stress events that correspond with the young microspore and at the stage of dehiscence are probable to express the collective effects of both stages. The duration and time scheduling of the stress determine abort number of florets. Contrary to this, maize ovary was also most sensitive at the reproductive stage of development during drought spell (Zinselmeier et al., 1995). In the same context, translocation of soluble carbohydrates of stem to grain is considered drought adaptive character associated to increase partitioning. Demand of increasing yield may have been achieved by preventing floret abortion and increasing translocation of carbohydrates during grain filling stage needs to be accomplished through complex physiological attribute-based breeding.

**Biomass growth in relation to drought stress**

Water deficit is an important abiotic component that limits the growth due to drop in the developmental functioning of different plant organs (Trachsel et al., 2010). Deep rooting is important character in crop plants to access the water from deeper layer of soil profile (Vadez, 2014). A study reported that water conservation strategy of drought tolerant wheat cultivar (RAC875) was associated to root hydraulic properties and root anatomical features (Schoppach et al., 2014). However, benefit of deep roots in terms of adaptations to water limited environment depends on the duration of stress, soil water holding capacity and the quantity of water exist in the soil. Another developmental attribute related to growth is plant height, a study depicted that it was deceased to 40 and 25%, respectively at imposition of two stress levels of drought in field grown maize (Zea mays L.) cultivar (Loft) (Asch et al., 2001). Another central morphometric trait (leaf area) affected due to water stress in many crop plants. Leaf mass ratio (Manzoni et al., 2013), specific leaf area showed varying plasticity in association to drought water index in red beet under drought stress (Stagnari et al., 2014). Because leaf area is affected by the leaf phenology, morphology of stem, rate of emergence, and its size. Thus, even a slight change in these factors due to water stress can also modify the leaf area. In addition to this, the cessation of new tiller formation in wheat is due to the reduction in leaf area under drought stress. Reduction in leaf area directly linked to decrease in leaf gas exchange under water deficit.
environment and this decline not only reduced the water loss but also the cause of decrease carbon assimilation of whole plant, and thus limited the growth (Álvarez et al., 2009).

Maximum reduction in growth of leaves as compared to growth of roots during the development of water stress causing the increased ratio of root/shoot owing to maximum supply of photosynthates to the roots (Chang et al., 2014), thus leading to greater proportion of roots. This maximum root biomass would contribute to improve the capability of root system to get more water per unit shoot area by searching maximum soil volume that otherwise may not be promising one (Postma et al., 2014).

**Water relation characteristics under drought stress**

Generally photosynthesis affected due to alterations in leaf water potential and relative water content (RWC) under water stress. These are the good indicators of plant water status in relation to drought tolerance. RWC measures the volume present in leaves relative to water concentration at full turgor pressure (Blum, 2011). When relative water content (RWC) reached to 70% due to water stress, the process of photosynthesis is reduced. A study depicted that RWC decreased to 39% in Pusa 362 (drought tolerant chickpea cultivar) while these were the 35% in SBD 377 (drought sensitive chickpea cultivar) under drought stress (Khanna et al., 2014). Decline in RWC during the water deficit has been observed by number of authors in different plant species (De A. Silva et al., 2014; Goodarzian Ghahfarokhi et al., 2014; Soleimani et al., 2014). Collectively, it was hypothesized that leaf water potential, osmotic potential and turgor potential maintain the plant water potential gradient under stress conditions (Raza et al., 2014).

Moreover, water potential of leaves at the stage of turgor loss ($\pi_{tlp}$) defines as the soil water potential below which the plant cannot able to take enough amount of water to recover from the wilting stage. In addition to this, many kinds of plant species close their stomata when the leaf water potential was less negative as compared to the stage of turgor loss, enabled the plant to survive on stored amount of water that gradually transpired opened the lowest epidermal conductance (Bartlett et al., 2012). Finally, at the end of drought cycles, stressed plants showed decrease in osmotic potential even under the full turgid condition and this turgor loss linked to the decrease in leaf tissue elasticity (Sai Kachout et al., 2011). Leaf water potential of tolerant maize (*Zea mays* L.) genotype (C-3014) was reduced to 84% however it was declined to 126% in susceptible genotype (C-3015). So, the higher level of water potential in C-3014 suggested that it
was better able to retain water content than C-3015 maize genotype (Castro-Nava et al., 2014). Similarly, soil and leaf water potential falls to more negative values with respect to control in Capsicum annum L. cv. Canon when water stress is induced (Campos et al., 2014). Generally negativity of water potential related to the hydraulic conductance in leaves that directly impact the stomatal opening or closure (Sack & Holbrook, 2006). Trifolium angustifolium L. (annual legume) retained the lowest value of leaf water potential under limited water supply and seemed the drought adaptive mechanism similar to perennials plants and Onobrychis caput-galli (L) Lam. (annual legume) showed the isohydric behavior by not changing the water potential under limited supply of water (Kostopoulou et al., 2010). Moreover, xerophytic evergreen shrub (Larrea tridentate) maintained the turgor pressure mainly by improving the cell wall elasticity and retaining the maximum proportion of bounded water under moisture stress (Zhang et al., 2014).

Nutrient uptake and drought stress

Water deficit induced the reduction in growth by depressing the nutrient uptake, acquisition and redistribution (Rouphael et al., 2012). It is evident that nitrogen (N), phosphorus (P), potassium (K⁺) and calcium (Ca⁺²) are the essential nutrients for plant growth and metabolism like the activities of enzymes, protein synthesis and for the integrity maintenance of plant cell boundary membranes. Decrease in nutrient concentration associated to reduced uptake of nutrient due to fall in transpiration rate under water deficit environment. Reduction in shoot and root nutrient accumulation (N, P, K⁺, Ca⁺²) positively correlated to decrease in growth. This reduced uptake of nutrients directly associated to lesser solubility and was the cause of alteration in physiological processes. So, nutrient deficiency during dehydrated conditions was the cause of senescence and decay of leaves (Warren et al., 2011). Moreover, uptake capacity of nutrient during the later stages of vegetative growth can be linked to changes in dry biomass allocation (Novák & Vidovič, 2003). Potassium (K⁺) as positive ion plays a key role in osmotic adjustment by decreasing the osmotic potential of cell. Potassium element was considered as the cationic solute, accountable for the movement of stomata under shortage of water (Ruiz-Lozano et al., 1995). Water stress collapse the uptake concentration of potassium (K) and phosphorus (P) in different organ of maize at various growth stages especially affected the root uptake capability of nutrients (Ge et al., 2014). Water deficit also decrease the status of nitrogen (N) nutrition as well as its uptake, attributed to alter the transpiration based N transport in soil solution (Gonzalez-
Dugo *et al.*, 2012). Another study stated that when drought stress induced at the heading and dough stages on barley plants, phosphorus concentration decreased drastically while this effect was more pronounced during latter stages of development (Youssef *et al.*, 2012).

**Photosynthesis is the key physiological process affected due to drought stress**

**Photosynthetic apparatus:** The process of photosynthesis in higher plant and algae takes place in chloroplast and contains highly organized membranous system called thylakoid membranes, harbor light capturing apparatus of photosynthesis and engaged to mechanize the structural properties for optimal utilization of light energy. The photosynthetic complexes are composed of series of thylakoid subunits of protein. Both photosystems (PSI and PSII) are made up of central reaction center enfolded by the light harvesting system (LHCl and LHClII). Under desiccation, LHClII was removed from PSI and some of PSII complexes disassembled with few losses of PSII core and LHClII (Sárvári *et al.*, 2014). In addition, the LHC family was the most abundantly found protein complex present in thylakoid membrane, covered almost half of the total chlorophyll of chloroplast (Peter & Thornber, 1991). Chlorophyll ‘a’ is the element of reaction center as well as the LHC while the chlorophyll b is limited to LHC. Water shortage lowered the concentration of Chl b than normal irrigation (Lalarukh *et al.*, 2014). Primarily, the main function of the LHC was gathered the light energy and make it possible to drive the electron transfer to the reaction center (Kang *et al.*, 2012). Chlorophyll molecules are responsible to convert the light energy into chemical form of energy such as ATP and NADPH (Taiz & Zeiger, 2006). The degree of chlorophyll quantity was a central physiological trait to check phenomenon of delayed senescence/ stay green effect in relation to drought stress (Ramírez *et al.*, 2014). Water stress degrades the chlorophyll pigment. A study reported that chlorophyll and carotenoid pigment were significantly reduced in hexaploid and diploid wheat under the induction of 10 days of drought stress (Chandrasekar *et al.*, 2000). Liu *et al.* (2006) observed the marked increase in electrolyte leakage and decreases in chlorophyll ‘a’ and ‘b’ pigment in wheat cultivars at the imposition of water deficit.

**Photosynthesis**

Plant growth is reduced under water limited environment due to reduction in carbon balance, which is largely based on photosynthetic performance (Flexas *et al.*, 2009). However,
the first drought-induced symptom is the shutting down of stomata to conserve water, indeed stomatal regulation play a vital role in regulating the hydric status of the plan (Huang et al., 2013). This partial or complete closure of stomata is the primary cause of decrease in net CO₂ assimilation due to reduced uptake of CO₂ to the carboxylation sites in the leaves (Flexas et al., 2004; Chaves et al., 2009; Wall et al., 2011). In addition to this, decrease in stomatal conductance is combined with irradiance persistence, but when leaves are continuously exposed to light energy relative to intercellular CO₂ then rate of production of reducing power exceeds its rate of consumption during the Calvin cycle (Pinheiro & Chaves, 2011). Under these conditions, down-regulation and photo inhibition of photosynthesis can act as a defensive mechanism in C3 type of photosynthetic mechanics. This photo-protection can be supplemented by thermo regulated dissipation that occurs in light harvesting complexes and encompasses in xanthophyll and lutein cycles (Demmig-Adams & Adams, 2006; Camarero et al., 2012). So, it is considered that there is competition between photoprotection mechanics with photochemistry for the absorbed energy, resulting in the down-regulation of photosynthetic processes due to decreased efficiency of photosystem II (PSII). A study reported that 40-60% photosynthesis takes place under severe drought stress one day after irrigation and this recovery lingers during the subsequent days (Flexas et al., 2009). Stomatal conductance (gs) declined to 114 and 13 mmol m⁻² s⁻¹ under moderate and severe water stress, respectively in bell pepper (*Capsicum annuum* L.) (Campos et al., 2014). Similarly, the CO₂ assimilation (A) and transpiration rates decreased during water stress but recovered after re-watering. Moreover, CO₂ assimilation (A) was in maximum range (~6 µmol m⁻² s⁻¹) during morning and then falls to minimum value in afternoon (2.5 µmol m⁻² s⁻¹), gs was less than (~0.03 µmol m⁻² s⁻¹) for most of daily courses in mature olive trees (*Olea europaea* L., cv. Leccino) (Marino et al., 2014). Moreover, study on *Jatropha curcas* L. accessions (Suriname, Tanzania, Brazil) showed differential performance in photosynthesis and the Suriname accession displayed more reductions in net photosynthesis, as compared to Tanzania and, mainly, the Brazil accession, during drought stress (Fini et al., 2013).

**Water use**

Physiologically and developmentally, plants are designed to reduce the water use by evolution in water deficit environment. The main target of the plant’s breeder is that how we reduce the water use under stressed environment to achieve the maximum production because yield production describe the function of water use. Similarly, reduction in leaf area including its
index related to the shorter plant size which are the major phenotypic translations to regulate water use and defensive against the water shortage injury (Blum, 2005a). It was recently reported that drought tolerance associated to effective use of water instead of water use efficiency (WUE) and it thought to be desire achieving target for the plant breeders (Blum, 2009). Another effective manipulation of water use in crop plants is the senescence of particular older leaf tissues whereas the younger photosynthetic tissue holds its turgidity, assimilation capacity, stomatal conductance as well as maintains the osmotic adjustment. In a study of corn genotypic difference to drought tolerance mechanism, the highest water use was observed in I$_{100}$ (full irrigation) as 738.1 mm for Safak maize genotype, while the lowest was found in I$_0$ (no irrigation) treatment as 260.0 mm for Ant-I’90 maize genotype (Aydinsakir et al., 2013).

**Water use efficiency**

Water use efficiency (WUE) is the physiological attribute of the plant, stated as the concentration of transpired water over the amount of CO$_2$ consumed during photosynthesis (Taiz and Zeiger, 2006). It also showing the plants ability to store the water resources of soil, hence combine the water stress tolerance and high yield potential. In addition to this, WUE is expressed as plant production alternative to the gas exchange attribute (Blum, 2005b). In short, apparent genotypic variations in terms of WUE are generally interpreted because of varying capacity of water use of different genotypes. Moreover, it was inferred that harvested yield and water available to the plant correlated to the best of WUE. Most probably reduced water use associated to maximum WUE, extent of WUE sensitivity and tolerance is generally predicted to the shorter (node appearance) and longer vegetative period (heading and grain filling) (Varga et al., 2015). However, under most dry land conditions where crops depend on erratic seasonal rainfall, the maximization of soil moisture use is a essential component of drought resistance (avoidance), which is generally expressed in lower WUE (Blum, 2005b).

Genotypic variations in WUE of maize genotypes depicted that the highest water use efficiency was found in I$_{50}$ (mild water stress) as 15.7 kg ha$^{-1}$mm$^{-1}$ for Safak maize genotype, while the lowest one was found in I$_0$ (no irrigation) as 5.5 kg ha$^{-1}$mm$^{-1}$ for Ant-I’90. It was concluded that Safak was more tolerant genotype to water stress than that of Ant-I’90 (Aydinsakir et al., 2013).
C4 photosynthesis under water stress

There have been less studied reports on C4 type of photosynthesis under drought stress as compared to C3 photosynthesis. Likewise the C3 plants, decrease in stomatal conductance associated to the fall in leaf water status and it steadily correlate to the reduction in rate of photosynthesis (Ghannoum et al., 2003; Carmo-Silva et al., 2008). This concurrent decrease of $A$ and $g$ particularly at 70% RWC has been taken as important liking in C3 and C4 plants. But different studies shown that $C_i$ decreases at the initial stages of water deficit like in maize (Leakey et al., 2006), sugarcane (Du et al., 1996) and sorghum (Williams et al., 2001). Whereas at the later stages of drought stress, it shows the increasing response while $A$ successively falls (Du et al., 1996; Kalapos et al., 1996). Moreover, certain studies reported no change in $C_i$ of C4 photosynthesis at induction of water stress (Lal & Edwards, 1996).

C4 plants are often considered to have become skilled under water shortages conditions because they retain the photosynthetic rate in spite with the closed stomata. In the perspective of an increasingly shortage of water all around the world, the exploitation of C4 photosynthesis has greater advantage to increase the yield production to secure the food. For example, maize (Zea mays L.) is the C4 third main cereal crop comes after wheat and rice in terms of annual yield production (Lobell & Gourdji, 2012) and containing the NADP-malic enzyme. In addition to C3 photosynthesis, C4 plants have CO$_2$ concentrating mechanism pathways. While the NADP-malic type species, atmospheric CO$_2$ is mainly fixed to oxaloacetate (El-Samad Hamdia et al.) by the carboxylation of phosphoenolpyruvate (PEP) with the help of PEP carboxylase enzymes present in mesophyll cells, then oxaloacetate (El-Samad Hamdia et al.) is transported to the chloroplast of mesophyll cells. Furthermost OAA is reduced to malate dehydrogenase (NADPMDH). Afterward, malate (4-carbon compound organic acid) is transferred to the bundle sheath cells of chloroplast, then it decarboxylated with the help of NADP-malic to accumulate CO$_2$ as well as reducing power. This CO$_2$ is refixed via Ribulase-1,5-bisphosphate carboxylase in the Calvin Benson cycle and the resulting pyruvate is given back to the mesophyll cell of chloroplast, then by the phosphate by the enzyme pyruvate orthophosphate dikinase to generate the PEP. Elevation in CO$_2$ concentration in the locale of Rubisco in the bundle sheath cells destroy the activity of oxygenasw, therefore decline the process of photorespiration and results in rise of photosynthesis when compared to C3 photosynthesis (Kanai & Edwards, 1999). Moreover, C4 photosynthesis is more competitive under stress conditions such as high light intensities linked
with increasing temperature and water limited conditions that promote carbon impairment through photorespiration (Lopes et al., 2011) Though, limited studies reported that CO₂ pumps of C₄ species are more defensive against water limited environment than enzymes of C₃ photosynthesis (Ghannoum, 2009). The uptake of CO₂ is reduced by the cause of closing of stomata accelerate to over-excite the PSII reaction centers (Ahmad et al., 2009) a of r (Ghobadi et al., 2013) and as a result increase in the development of reactive oxygen species (ROS).

**Development of reactive oxygen species (ROS)**

Abiotic stresses like drought stress overproduce the reactive oxygen species (ROS) as a result of highly electron leakage towards oxygen during respiratory and photosynthetic processes (Du et al., 2014). These ROS causes the damaging effect to protein, carbohydrates, lipids molecules and DNA, ultimately leading to the oxidative stress in crop plants (Filippou et al., 2011). So, the over production of ROS is harmful to lipid, proteins and nucleic acids molecules, whose oxidation may lead to harmful effects such as enzyme inhibition, chlorophyll degradation, disruption of membrane integrity, injury to organelle function and reduction in metabolic efficiency and carbon fixation (Wan et al., 2014). These species contain the free radicals such as O₂•⁻, H₂O₂, ¹O₂, HO•, ROOH, ROO•, RO• and showed the high affinity towards reactivity. So, the accumulation of ROS in response to various abiotic stresses is a major cause of loss in crop productivity. Photosystems I and II in the chloroplast are the main positions for the production of ¹O₂ and O₂•⁻, while in the mitochondria, complex I and complex III of the electron transport chain are the sites of O₂•⁻ production (Gill & Tuteja, 2010).

**Antioxidant response against ROS production**

Plant responds the defensive enzymatic and non-enzymatic antioxidant system against production of damaging effect of reactive oxygen (ROS) in response to drought stress (Sairam et al., 2011; Ji et al., 2014). Higher activities of superoxide dismutase (SOD), guaiacol peroxidase (POX) and catalase (CAT) were observed in ‘Baoxing’ orchardgrass than ‘01998’ orchardgrass was reported under drought stress and rehydration. It was suggested that higher activities of SOD, POD and CAT and lower malondialdehyde (Royo et al., 2007) concentration in ‘Baoxing’ grass plants may be involved in scavenging the reactive oxygen species (oxidative stress) and removing the H₂O₂ because of changes in the gene expression level (Ji et al., 2014). It was also hypothesized that POD also play a key role of recovery from drought induced damages. In addition to this, sugarcane resistant genotype (IACSP 94-2094) showed additional active
superoxide dismutase (Cu/Zn-SOD VI) isoenzyme in comparison to commercial brazil genotype (IACSP 95-5000) when grown under 70 and 30% soil water dehydration, may be contributing in the better performance of IACSP 94-2094 against induced stress (Boaretto et al., 2014). In addition, the total glutathione reductase (GR) activity increased considerably in less tolerant sugarcane genotype (IACSP 94-2094). However catalase (CAT) activity seems to have more direct role in H$_2$O$_2$ detoxification under water stress and the shift in isoenzymes in the tolerant cultivar might have subsidized to this response, which may be dependent upon the place where the excessive H$_2$O$_2$ is being produced under stress. The improved performance of IACSP 94-2094 sugarcane genotype under water deficit was related to more efficient antioxidant system response (Boaretto et al., 2014). Drought caused a more pronounced increase in CAT activity in duram wheat cultivars at the milk ripeness and in bread wheat cultivars at the completion of flowering (Huseynova, 2012).

**Chlorophyll a fluorescence kinetics is the excellent monitor to check the crop productivity under drought stress**

Chlorophyll a fluorescence kinetics has been an excellent monitor to check the specific effect of environmental stresses in crop development by assessing the structural and functional integrity of photosynthetic apparatus, photochemical yield of PSII photochemistry, and the electron transport activity.

**Chlorophyll a fluorescence**

The analysis of chlorophyll a fluorescence has been a widespread non-invasive technique used extensively to study the performance efficiency of photosynthetic apparatus under stress conditions. It gives the information on large number of events of photosynthesis based on quantity as well as quality as described by many authors.

When photosynthetic plants kept in darkness for a period of 10 minutes then upon illumination, chlorophyll a fluorescence shows the characteristic variations known as the fluorescence transients, fluorescence induction and Kautsky effects. Chlorophyll a fluorescence induction transients measured under continuous light conditions has two phases. First is the fast increasing phase (occur within a second) is called the OJIP phase. Second is the slow decreasing
phase (within a few minutes) characterized as the PSMT. In this phase, ‘S’ is the semi-steady state, ‘M’ shows the maximum and ‘T’ designates the terminal steady state.

In fast OJIP fluorescence transients, ‘O’ is the origin of first measured minimal level, ‘J’ and ‘I’ are the intermediate stages, and P is the peak level of fluorescence transients (Strasser, 1992; Stirbet & Govindjee, 2011). Another level called ‘K’ appears between ‘O’ and ‘J’ at about 300 µs under the abiotic stress environment (Huang et al., 2013). The O-J phase (termed as ST) of fluorescence transients described the photochemical reduction of Qₐ in reaction centers of PSII while the JI phase characterized the kinetic properties of reduction and oxidation of the plastoquinone pool. Several studies interpreted that increase in F₀ also rise the number of inactive reaction centers may slow down the primary reduction of Qₐ (Haldimann & Strasser, 1999; Kalaji et al., 2011), disconnection of PSII light harvesting antennae from the PSII core complex (Guha & Reddy, 2014), accumulation of reduced Qₐ (Qₐ⁻¹) narrated by the Redillas et al. (2011) with the loss of structural and functional integrity of PSII (Čajánek et al., 1999). Therefore, increase in F₀ might not be due to the inactivation of PSII reaction centers. But also owing to the decrease in electron transfer beyond Qₐ⁻¹ resulting from accumulation of Qₐ⁻¹ (as evident from increased Vj) and also could be due to the fractional decrease of Qₐ to Qₐ⁻¹ per total Qₐ as supported by the increase of Mo. This type of response occur in those plants whose photosystem II is tolerant to drought stress and the effect of water stress is usually manifested as lowered efficiency of electron transport to the photosystem I considered as photoprotection strategy under drought stress conditions (Redillas et al., 2011). The O-I phase (Vₒ₁ < 1) of chlorophyll a fluorescence transients indicates the sequence of events from the exciton capture by PSII to the reduction of the plastoquinol (PQ) pool (Adamski et al., 2011). The positive response of Vₒ₁ phase in drought stressed plants indicate that cultivar was able to maintain the reduction rate of PQ process was not affected during the diurnal time in both controlled and stressed plants. The I-P phase, a region of the MTP (J-I-P) represents the electron transfer through the PSI and quantify the measure of reduced and acceptor (plastoquinol (PQH₂) side at the PSI and designates the rate-limiting stage of photosynthetic electron transport chain (Schansker et al., 2005; Redillas et al., 2011). This I-P fluorescence transient stage was evaluated following the two distinct approaches based on the time intensities. First, is the normalization of Vₒ₁ fluorescence transients between the time range of 30-300 ms expressed as (Vₒ₁ ≥ 1). This time span imitates the electron transfer from PQH₂ to plastocyanin and PSI.
Secondly, the transients normalization to the time range of 30-200 ms (linear time scale) expressed as behavior of electron flux that reached to the acceptors of PSI (Adamski et al., 2011) and reflects the velocity of ferredoxin reduction beyond the PSI (Schansker et al., 2003). The maximum wavelength amplitude of $V_{OI} \geq 1$ designates the pool size of the electron acceptor end of the PSII. But early minimum decrease in $V_{OI} \geq 1$ both in drought stressed plants as well as in the control plants and subsequently again rise under the diurnal course time might be an acclimation process of the *Morus indica*, cv.VI, elucidating its attempt to maintain the pool size of PQ (Guha & Reddy, 2014). This $V_{IP}$ transient stage exhibited the hyperbolic manner as described by the Michaelis-Menten equation in which the inverse of time to attain to 0.5 $V_{IP}$ is an approximation time of the rate of reduction of electron acceptors of PSI (Adamski et al., 2011). Usually, a sluggish reduction rate showed the traffic jam of electrons initiated due to transient block at the acceptor side of (inactive ferredoxin-NADP+ reductase) (Schansker et al., 2003; Schansker et al., 2005). Guha & Reddy (2014) reported that both controlled and drought stressed plants primarily showed a slightly lower reduction rate of final electron acceptor of the PSI but after that rise in the reduction rate of final electron acceptor clearly indicating no significant PSI association under the drought stress. Moreover, I-P phase revealed the re-reduction of plastocyanin and P700$^+$ in photosystem I (Schansker et al., 2005; Tóth et al., 2007; Adamski et al., 2011). According to results of Gomes et al. (2012) on a study of chlorophyll a fluorescence in two passion cultivars, Yellow master (FB200) and Maguary (FB300) at exposure to 11 days of water withholding stress and after six days of rewatering. Results depicted that under water stress, reduction of $V_{OP}$ was observed in J-I phase in FB300 cultivar but all the phases of OJIP were declined in FB200 passion cultivar. Whereas the J-I phase in FB-200 showed the sign of recovery within six days of rehydration but this period was not sufficient for O-J and I-P phases. In addition to these results, $V_{OP}$ recovery in FB300 passion cultivar followed by an increment of efficiency of electron transfer to photosystem I (Karpouzas et al., 2014) acceptor side supported with an appearance of positive increase of I-P phase under water stress.

Kinetic differences of $V_{OK}$ in chlorophyll a fluorescence were compared by the double normalization of time interval of 50 and 300µs to make the so called L-band which has a peak at around 150µs (Oukarroum et al., 2007). Guha et al. (2013) recorded the marginal changes in the L-band of mulberry stressed plants were capable to prevent the dissociation of LHCII from PSII complex by virtue of which maintenance of energetic connectivity. In addition, any damage to
donor side of PSII linked with the appearance of K-band in the OJIP transients at 300 µs time intensity under drought stress (Guissé et al., 1995; De Ronde et al., 2004; Guha & Reddy, 2014) and the presence of distinct K-band might be due to an imbalance between the donor and acceptor sides of PSII (Ouzounidou et al., 1997). Guha et al., (2014) reported the occurrence of K-band present only in drought stressed plants but the rise was very minimal regardless of the time during the diurnal courses, showing the stability of OEC. Glycine betain and proline have been shown to protect the oxygen evolving complex (OEC) under water stress conditions (De Ronde et al., 2004). In earlier study, (Guha et al., 2010) reported mulberry leaves showed higher level of glycine betain and proline contents that could also be linked with the stability of OEC.

**Maximum quantum yield of PSII (F\textsubscript{V}/F\textsubscript{M})**

It is the most frequently used parameter as the indicator of photoinhibition and other types of abiotic stress injury in photosystem II apparatus (Roháček et al., 2008). It enumerates the maximum photochemical efficiency, of open PSII reaction centers (RC). Its value is almost constant (0.832) for many different plant species when measured under non-stressed conditions (Björkman & Demmig, 1987). For stressed or injured plants (F\textsubscript{V}/F\textsubscript{M}) is significantly reduced might be due to the emission of fluorescence from PSI contributing in F\textsubscript{O} phase (Vredenberg et al., 2007; Henriques, 2009). The insensitivity of F\textsubscript{V}/F\textsubscript{M} to water stress is a well-known phenomenon as it considers the inverse changes in the figure of original (F\textsubscript{o}) and maximum fluorescence (F\textsubscript{M}) and if one rises with respect to fall in the other or vice versa, the ratio F\textsubscript{V}/F\textsubscript{M} might not change significantly (Elsheery & Cao, 2008). In addition, no change in F\textsubscript{V}/F\textsubscript{M} was observed when temperature increased from 25 °C to 42 °C in fruits of apple peel (Chen et al., 2009) also found that F\textsubscript{V}/F\textsubscript{M} was insensitive to the temperature rise (25 °C to 35 °C) in leaves of tomato.

**Biophysical parameters of OJIP**

Strasser et al. (2004) classify the group of biophysical parameters characterize the photosynthetic plants under stress conditions which can be used in screening the physiological traits in terms of plant vigor.

1) Energy fluxes per absorbed photon fluxes includes TR\textsubscript{O}/ABS (\(\Phi P_O\)), DI\textsubscript{O}/ABS (\(\Phi D_O\)), ET\textsubscript{O}/ABS (\(\Phi E2O\)), RE\textsubscript{O}/ABS (\(\Phi RE1O\))
2) Energy fluxes per reaction center fluxes consists of ABS/RC, TR\(_O\)/RC, ET\(_O\)/RC, DI\(_O\)/RC

3) Energy fluxes per excited cross section at fully open reaction comprises ABS/CS\(_O\), TR\(_O\)/CS\(_O\), ET\(_O\)/CS\(_O\), DI\(_O\)/CS\(_O\) and at closed reaction centers ABS/CS\(_m\), TR\(_O\)/CS\(_m\), ET\(_O\)/CS\(_m\), DI\(_O\)/CS\(_m\)

4) Density of reaction centers contains RC/ABS, RC/CS\(_O\), RC/CS\(_m\)

5) Probability of electron transport \(\Psi_{ET2}\), \(\Psi_{RE1}\), \(\delta_{RE1}\)

6) Performance indices and driving forces covers PI\(_{ABS}\), PI\(_{TOT}\) and df\(_{TOT}\)

The energy flux of ABS/RC is measure of the average total absorbance per active PSII reaction center showing the antenna size. Studies depicted that increased ratio of ABS/RC under induced water stress refers only to the active PSII reaction centers which might be due to the inactivation of some PSII reaction centers (Oukarroum \textit{et al.}, 2009) and the regrouping of antenna from inactive to the active PSII reaction centers (Van Heerden \textit{et al.}, 2003). Greater ABS/RC ratio in drought stressed plants might be due to regrouping of antennae from inactive PSII reaction centers to active reaction centers (Van Heerden \textit{et al.}, 2003). TR\(_O\)/RC is trapping flux leading to Q\(_A\) reduction/reaction center. But the very high rate of effective dissipation (DI\(_O\)/RC) was observed in \textit{Morus indica}, cv.VI under drought stress due to decreased electron transport per PSII reaction centers (TR\(_O\)/RC) (Guha & Reddy, 2014). Such down-regulation of DI\(_O\)/RC might have prevented over reduction of electron transport chain as well as facilitated dissipation of excessive energy in order to minimize the photo oxidative damage in the thylakoid membrane (Van Heerden & Laurie, 2008; Deng \textit{et al.}, 2010).

Electron transfer from photosystem II to Q\(_A\) then PQ to photosystem I electron acceptor can be characterized according to their sensitivity to drought stress and ranked as \(\varphi_{RO} > \varphi_{ED} > \varphi_{PO}\) (Campos \textit{et al.}, 2014). Decrease in RC/CS\(_m\) (energy fluxes per excited cross section area at fully open reaction center) change the functionality of PSII RC can reduce the free energy gap between S\(_2\)Q\(_A^-\) and S\(_2\)Q\(_B^-\) contributing to the photoprotection of PSII by decreasing the active reaction center density. The behavior of other phenomenological fluxes particularly ET\(_O\)/CS\(_m\) was reduced due to water stress which might have significantly affected the performance indexes PI\(_{CSM}\) and PI\(_{Priibil\ et\ al.}\). The specific and phenomenological flux derivatives as a whole phenomenological fluxes specified the down-regulation in overall processing of light energy per leaf sample cross-section under drought stress.
**Drive energy cascade:** energy fluxes, bifurcations & energy conservation competences

Demonstration of events linked with corresponding energy fluxes

Quantum yields with their efficiencies

Correlation with fluorescence signals

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**Fig. 2.6.** A diagrammatic presentation of JIP analysis (revised after Tsimilli-Michael and Strasser, 2008)
Mechanism of drought tolerance

Different crop species show the varying vulnerability/resistance to water stress at different stages of growth from germination to maturity stage. So, crop plants have various complex adaptable mechanisms to cope the water deficit environment, and the adaptations are attributable to a plant’s ability to exercise one of these mechanisms. The components of adaptive mechanisms have been categorized as drought avoidance, escape and tolerance.

Drought resistance and drought tolerance

Plants adapted to simultaneously rainy and dry weather, complete their flowering and maturity during the wet season can be classified as drought resistant. Drought resistant strategies include mechanisms of “drought tolerance”. It’s the ability to maintain physiological metabolism even at reduced leaf water potentials (Levitt, 1980; Kozlowski & Pallardy, 2002; Poorter & Markesteijn, 2008). In other words, plants that can withstand the period of water deficit without any damage or loss to metabolism are grouped as drought tolerant. In a study, drought resistance indices, mean productivity (MP), geometric mean productivity (GMP), stress tolerance indices (STI), stress susceptibility index (SSI), and drought response index (DRI), MCC544, MCC696 and MCC693, considered the chickpea genotypes had drought resistant mechanism (Ganjeali et al., 2011).

Drought escape

Crop plants that complete their life cycle in a short period of time allowing them to reproduce during the wet season or before the onset of drought conditions are classified as drought escape. For example, Brassica rapa plants escape the drought stress by the early flowering because of increasing the water use efficiency rather than adapting the mechanism of drought avoidance (Franks, 2011). Moreover the selections of cultivars that have drought escape mechanism are more important than phenotypic plasticity. This strategy of breeding for drought escape had successfully brought the yield stability in chickpea (Kashiwagi et al., 2013) However, these early maturing chickpea cultivars had to pay a yield penalty due to the cut in their total photosynthetic period.

Drought avoidance

It’s the ability of plants to avoid or postpone the decline of leaf water potential during drought stress (Levitt, 1980; Poorter & Markesteijn, 2008). Plants can avoid drought period either by water saving strategies like stomatal closure and shedding of leaves (Rouhi et al., 2007)
or traits that confer the ability to extract water by deep rooting (Huang, 2008; Leitner et al., 2014) and maintain the higher conductivity of water from soil rapidly enough to compensate transpirational water losses (Pirnajmedin et al., 2014). These two avoidance mechanisms are commonly stated as water saving and water spending strategies, respectively (Kozlowski & Pallardy, 2002; Mediavilla & Escudero, 2004). Root system involved in less reduction of water uptake efficiency were found to delay the transpirational decline (Kashiwagi et al., 2014). Leaf shedding is a typical avoidance mechanism (Touchette et al., 2009) which decreases water demand of plant and is important for maintaining water potentials in meristems and roots at an “above dangerous” point (Ibrahim, 2013). Leaf conductance and duration of stomatal opening in drought stressed plants decreased to conserve the water loss through transpiration contributing to maintain turgor status of the leaves. Thus, develop a mechanism of drought avoidance. In addition to this, resistance against the soil drought was due to trade between changes in xylem vulnerability to embolism, reliance on water reserves of sapwood and reduced leaf area leading to tradeoff of avoidance under drought tolerance (Pineda-García et al., 2013).

**Osmotic adjustment**

Osmoregulation is main drought tolerant mechanism in which plants assimilates the solutes in cell sap that regulates intercellular osmotic potential and turgor pressure during drought stress (Soleimani et al., 2014). These solutes consist of organic molecules (amino acids, betaeines and sugars) and inorganic solutes. So, the osmolyte accumulation can be estimated by the osmotic adjustment. This is defined as a decrease of osmotic potential within cells due to solute accumulation during a period of declining leaf water potential (Babita et al., 2010; Montesinos-Pereira et al., 2014). Higher turf performance was related to the osmotic adjustment, stimulated the water retention in leaves and protect the stability of cell membrane from the damaging effect of drought (Burgess & Huang, 2014). Proline, play as the role of compatible solute in osmotic adjustment under water stress, act as free radicle scavenger, a metal chelator, balancing the cell redox, buffer the cytosolic pH, stabilize the subcellular structures and membranes including photosystem II and as well act as the signaling molecules (Anwar Hossain et al., 2014). Under normal growth conditions, the endogenous level of proline ranges 0.2-0.7 mg g\(^{-1}\) dry biomass. However, under stressful growth conditions, endogenous level of free proline increases up to 50 folds. In addition to proline, glycinebetaine was found to be most abundant and important compatible solute for osmotic adjustment under drought. GB stabilized the
proteins, enzymes, membranes, protect the photosynthetic apparatus by photo-damaging and maintain the electron flow in thylakoid membrane under various stress environments (Gupta et al., 2014). Similarly accumulation of soluble sugars also contributes osmotic adjustment and osmoprotectant particularly in young growing leaves and this is the most important adaptive response in plants under drought condition (Cattivelli et al., 2008a; Xu & Zhou, 2008). For example, in sorghum, drought stress increased the accumulation of total soluble sugars which was positively associated with osmotic adjustment and their osmoprotective effects on cytosolic and chloroplastic proteins by replacing water molecules (Bohnert et al., 1995).

**Non-photochemical quenching**

Down regulation of PSII quantum efficiency by sustaining the regulation of excess heat energy dissipation (non-photochemical quenching) considered to be a photo protective mechanism. Thus, NPQ is one of the most adaptive mechanism by which plants protect themselves from excess light. It act as to prevent the generation of surplus reductive energy that may be injurious to the plants. Significant processes that contribute to NPQ were pH dependent, enzymatic de-epoxidation of xanthophyll and conformational alterations of PSII antenna proteins (Müller et al., 2001). It has been shown in several studies that NPQ increased under water limited conditions (Lambrev et al., 2012; Luo et al., 2014; Ramalho et al., 2014). For example, it was reported that regulation of thermal energy dissipation give the maximum percentage of instantaneous as well as daily absorbed energy by more than to 80 and 72%, respectively in *Jatropha curcas* plants under water deficit condition (Tominaga et al., 2014).

Defensive mechanism that may subsidize in non-photochemical quenching (NPQ), is known as pH based energy dissipation (qE). In this, cause of induction of NPQ is the low hydrogen ion concentration (pH) in the lumen of thylakoid and high difference in ionic concentration ($\Delta pH$) that are generated because of disturbance in electron transport under high light irradiance. So, this low proton concentration in the thylakoid lumen activate the qE by donating the proton to protein (PsbS) (Niyogi & Truong, 2013). Then these uplift/stimulate the violaxanthin deepoxidase that changes the violaxanthin to antheraxanthin and zeaxanthin in xanthophyll cycle (Demmig et al., 1987). Moreover, energy produced due to electron gradient from the linear electron flow may be in part, retransferred to the photorespiration (Kozaki & Takeba, 1996; Suorsa et al., 2012) that also takes part to transthylakoid pH difference because it maintain the maximum electron transport level particularly in the case of water deficit (Singh &
A study depicted that regulation in proton gradient that is generated by electron transfer and proton motive force play an important role in the protection of PSI against the photodamages under fluctuating light flashes (Suorsa et al., 2012). Nevertheless, proton circulate in photosynthesis might be also modulated due to cyclic electron movement revolve around PSI, could be up-regulated under stress environment (Bukhov & Carpentier, 2004; Huang et al., 2012). This process is vital in both ways for the plants. First, it contribute in photoprotection and secondly, balancing the output energy currency (ATP/NADPH) in plants (Rumeau et al., 2007).

This high energy level comes back to relax state within minutes after plant exposed to dark conditions. High energy state quenching (qE) terminated into transition relaxed state (qT) and take in the phosphorylation of light harvesting protein reversibly. This reversible phosphorylation takes part in balancing the distribution of light energy between PSI and PSII at low light flashes. Moreover, relaxation kinetics cannot discriminate the qE and qT forms of quenching, but the latter makes little output in overall quenching processes.

Photoinhibition (qI) (processes that relaxes in time span of hours), the term applied to both defensive progressions as well as for damage of PSII reaction centers (Posudin et al., 2010). In a report it was revealed that iron sulphur protein spots (ISPs) slow down the linear electron transport under water stress/high light flashes to safe the photosystem I from photoinhibitory damage (Sanda et al., 2011). In addition to this, photoprotection takes place in the photosystem II light harvesting antenna while the injury to RCs occurs within RCs of PSII. Thus, it was studied that photoinhibition because of water stress directly correlate with greater light intensities signifies as important factor in measuring the productivity of photosynthetic activity (Sofo et al., 2010)

Approaches screening, selection and breeding

Conventional breeding technique

Genetic variation at intraspecific and inter-specific for desired trait (stress resistant and tolerant) can be achieved through the plant breeding and conventional breeding techniques getting the high quality yield. The green revolution since from 1940s (Dr. Norman Borlaug was the founder of green revolution) mainly based on the traditional breeding, the purpose is to increase the wheat and rice yield on global scale especially in South Asia (Raja ram, 2005). Traditional breeding have been used as general practice to study the genetic variability in
different crop lines. Genetic variability has been identified by measuring the desirable traits under abiotic stress conditions have been induced in different economically important crops. Moreover, cultivars of different crops like wheat, rice, maize and soyabean have been introduced through different measures of conventional breeding (Valkoun, 2001; Mishra et al., 1996; Banziger et al., 2004; Van Toai et al., 1994). Thus, the conventional breeding improves the high quality yield crops at cellular, molecular, biochemical and physiological level against abiotic stresses. Another important selection factor may be the availability of germplasm with the low genetic variations (Ashraf, 2010). This breeding methodology was initiated to develop the drought tolerant cultivar at International Maize and Wheat improvement Center (CIMMYT), Mexico in 1970s. Most of the drought related cereal breeding programs of different institutes like International Maize and Wheat improvement Center (CIMMYT), International Center for Tropical Agriculture (CIAT) and International Rice Research Institute (IRRI) based on the tolerant and resistant strategies of cultivars for selection that give the maximum yield under drought environment. Banziger et al., (2004) reported that these institutes produced maize yield increases from 59 to 233 kg ha\(^{-1}\) cycle\(^{-1}\) as reviewed by (Ashraf, 2010). Similarly, selection for yield productions under drought conditions was also high as 146 kg ha\(^{-1}\) annually (Campos et al., 2004). Superior maize hybrids developed by scientist of CIMMYT than other private enterprises regarding high yield under water limited environment (Banziger et al., 2004). Another drought tolerant maize cultivar ‘Obatanpa GH’ in 2006 was developed by the plant breeders of Crop Research Institute (CRI), Kumasi, Ghana in collaboration with International Institute of Tropical Agriculture (IITA), Ibadan, CIMMYT, Mexico and Sasakawa, Global 2000.

Thus, breeding is difficult due to the complexity of the plant’s adaptation under stress. So, this proposes that a number of interested traits may combine and contribute to adapt the plants for tolerance against a range of abiotic stresses. Thus, there is a need to seek more efficient approaches for genetically tailoring crops for enhanced drought tolerance.

**Marker assisted breeding**

Different strategies proposed to produce more grain involved in improved agronomic management and breeding of novel genotypes that adapted to stress environments. So, for this instance, novel marker assisted breeding (Haefele et al., 2013) technique of biotechnology have been introduced to acclimatize different varieties of major cereal crops to heat (Pinto et al.,
2010), drought (Araus et al., 2008; Fleury et al., 2010), salinity (Ren et al., 2005) In this technique, desirable genes can be tagged easily and selected in a breeding population for the choice of trait. Thus, examining breeding value of each of the genomic regions, the breeders can shuffle genes which are diverse in origins.

**Role of quantitative trait loci (QTL) for the drought tolerance**

Plant breeders have made progress to develop the drought resistant lines through conventional breeding but in fact, this technique is highly time consuming, costly, laborious and slow in attaining the progress. Otherwise, quantitative trait loci (QTL) mapping followed by marker assisted breeding (Haefele et al.) could be an efficient approach for recognizing the genomic sections related to the reproductive stage traits and crop performance under stressed environment and pyramiding the desirable alleles to improve the drought resistance in crops (Ashraf, 2010). Likely, the drought tolerance related to switching the number of minor genes called polygenes has additive properties in their expression. Thus the loci of chromosomes containing polygenes referred as the quantitative trait loci (QTL) and loci mapping is helpful in location, number and mass of phenotypic traits with its pattern of gene expression (Vinh and Paterson, 2005).

Number of QTLs resistance traits against drought has been identified using massive population in rice (Kamoshita et al., 2008; Courtois et al., 2009). Another study reported that grain yield and its physiological related components were concomitant to the QTLs (Peleg et al., 2009; Serraj et al., 2011) In addition, QTLs for the drought resistance of primary traits (thickness, penetration index and pulling force of roots), secondary traits (phenology, rolling and drying of leaves) and integrative traits (grain yield) for drought resistance have also been reported by the number of scientist (Lafitte et al., 2007; Venuprasad et al., 2007; Kumar et al., 2009; Zhou et al., 2010; Gowda et al., 2011). Use of DNA markers and QTL mapping has made it convenient to assess the role of polygenes to cut up the complex trait. For assessing the legacy of stress tolerance, Ashraf et al. (2008) analyzed the number of DNA markers (AFLPs, CAPS, RFLPs, RAPDs, PCRindels, SSRs) with DNA sequencing. Maize segregating population developed from the cross between N87-1×9526 (drought resistant versus drought sensitive), which have genotyped at 103 SSR loci (Feng-Ling et al., 2008). The resulting F_{2:4} populations were tested under two water treatments. Two QTL for plant height, five QTL for anthesis-silking
interval, four QTL for root mass and one QTL for grain yield out of 12 identified QTL. But most of them exhibited the over-dominant gene action

**Introggression breeding**

Drought is a major abiotic threat of environment limits the yield production of crops. So, there is need to develop adaptable varieties that can withstand the drought stress and still produce the maximum yield under non-stressed conditions. Introggression breeding technique identify the abiotic stress tolerant cultivars that could survive drought and salinity and submergence were commenced as the International Rice Molecular Breeding Program by the Zhikang Li at the International Rice Research Institute (IRRI) in 1998 (Li *et al.*, 2005; Ali *et al.*, 2006; Varshney *et al.*, 2013). Marcaida III., et al (2014) reported physiological efficiency and performance of recently developed Green Super Rice (Songsri *et al.*) cultivars exhibited the favorable response against drought. These GSR cultivars bred through an advanced introgression breeding approach that entails less irrigation water and chemical inputs without compromising the quality and yield of grains. GSR cultivars, IRI-5-S10-D1-D1 and IR 83142-B-19-B perform well under conditions of severe drought stress, with grain yield production almost parallel to the drought check (1.79 tons ha$^{-1}$). Whereas during the moderate stress, there was a relative yield advantage of 25% and 40% for the two GSR cultivars over the drought check, respectively. This study describe that physiological traits (dry biomass accumulation, leaf area index and panicle yield), and their responses enabled the Green Super Rice Cultivars to endure the drought stress. Thus, these are the significant meters for the selection of varieties in drought-prone environments, predominantly at the reproductive stage under severe water shortage conditions. In spite of the reduced performance of some cultivars to cope with severe drought stress, three GSR cultivars produced the grain yield (2.0-2.9 tons ha$^{-1}$) that was the same or higher than the drought check under moderate drought stress. Therefore, introgression breeding technique introduced in newly developed drought tolerant cultivar through the GSR breeding strategy was found to be effective against drought stress.

**Transgenic techniques**

Plant scientists move their directions to the gene revolution for security of food due to advancement in biotechnology. These revolutions comprise alterations in quantitative and
qualitative trait loci of an organism by shifting the preferred genes from one species to another. Transgenic techniques can rapidly develop gene pool for engineering the stress tolerance (Lusser et al., 2012). In addition, high output phenotyping together with the genetic engineering not only give the precise strategy for desirable trait selection but also shortens the breeding cycle (Tardieu

Use of omics technologies

Use of omics technologies for drought tolerance is another tool for crop development. Omics is a blended term of high throughput genomics, proteomics and metabolomics. The cohort of expressed sequence tags (ESTs) from cDNA libraries and the genomic sequence of *Arabidopsis* and *Oryza sativa* L. gives the significant information for the discovery of gene of interest (Sreenivasulu et al., 2007). Digital expression analysis of EST with gene marking in wheat, resulted in the identification of several pathways associated with abiotic stress tolerance (Houde et al., 2006).

Use of crop wild relatives (CWR)

A taxon of wild plant is a derivative of closely related genetic make-up and function to a crop. The use of crop wild relatives (CWR) gives the breeding efficiency, tolerance against abiotic stresses and quality traits to the crop (Kell et al., 2008). In addition, CWR are the useful resource for the long term availability of food crops due to genetic diversity as well as contribute in beneficial traits against stress resistance leading to improve the yield production (Vredenberg et al., 2007). Moreover, the characterization of phenotype associated to the genotypic expression and evaluation of interested traits in breeding and selection using CWR is an urgent requirement to secure the worldwide food demand (Khoury et al., 2015).

Role of proteomics in drought tolerance

Advancement in molecular biology techniques played a vital role in dissecting the mechanism of tolerance. For example, Ali et al. (2014) suggested that for assessing exact mechanism of drought tolerance proteomic studies complemented GC-MS or MALDI-TOFF will help in determining the proteins that have a role in sensing stress and communication a message for altered plant metabolism (Ali et al., 2014). In another study with wheat cultivars differing in degree of drought tolerance (Bazargani et al., 2011) found that separation of proteins by one
dimensional and two-dimensional gel electrophoresis (2-DE) with subsequent identification proteins by MALDI-TOF-MS proved to be very effective and have identified 152 differentially expressed protein spots. Of all differentially expressed proteins, around 23% proteins are responsible for defense and detoxification while 26% proteins are related with cellular metabolism. In addition, 17% differentially expressed proteins are related with storage function in plants. It was also observed that expression of drought tolerant wheat varieties had expressed different proteins as compared to drought sensitive wheat cultivars (Jiang et al., 2012). Water stress responsive proteins were identified after proteomic analysis in rice cultivars (IR64 and Azucena). In this study 15 proteins express the differential response to water stress out of 700 proteins (Yang et al., 2011)

To evaluate the effect of drought on the protein composition may also give significant information between environmental stress and plant development (Barnabás et al., 2008). So, the proteomic analysis is useful to study the changes in gene expression under drought stress (Hu et al., 2010).

**Morpho-physiological indicators of drought tolerance in plants**

Understanding physiology of plant responses against drought stress induction and interpret degree of drought tolerance by measuring key traits may be helpful in breeding program. Therefore, it is important to identify the most susceptible aspects of growth and yield performance under drought stress.

1) Morphological characteristics like leaf area, root morphology, root shoot ratio, dry biomass production (De Jong Van Lier et al., 2009; Futakuchi et al., 2012) directly associated with physiological performance and helpful in screening and selection of tolerant varieties to attain the maximum output of yield (de Souza et al., 2013).

2) Gas exchange attributes (A, E and \(g_s\)) describe the extent of variation in stomatal limitations in drought tolerance (Huang et al., 2013).

3) Relationship between leaf water potential or relative water content and drought tolerance can be drawn (Jongdee et al., 2006; Cattivelli et al., 2008b; Moradi et al., 2014).

4) Water use efficiency (WUE) and the effective use of water (WU) can be used as selection criteria for the cultivars/lines that have tolerance potential against drought stress (Blum, 2005b).
5) Plants ability to accumulate the osmolytes can be estimated by the osmotic adjustment (OA). So, the OA considered as the major contributor in drought resistance of the crop plants (Barcia et al., 2014).

6) Higher activities of SOD, POD, CAT, POX in drought tolerant cultivars showed the defensive mechanism against generation of ROS due to drought stress (Ji et al., 2014). Drought tolerance has been associated with changes of antioxidant metabolism in tolerant cultivars (Soleimani et al., 2014).

7) Integrated response of PSII photochemistry, maximum quantum yield (Fv/Fm), performance index on the basis of absorption (PI_{ABS}) also revealed photosynthetic performance in drought tolerant cultivars (Lepeduš et al., 2012; Jamil et al., 2014).

8) Anthesis silking interval (ASI) is another criterion in identification of drought tolerant varieties.
Plant tolerance to water stress is linked with its various physiological processes. To identify physiological attributes that contributes in water stress tolerance in maize at different developmental growth stages, a series of experiments were operated at the Botanical Garden of Bahauddin Zakariya University, Multan, Pakistan. Initially, at the start, three maize cultivars were selected as tolerant, moderately tolerant and sensitive cultivar out of six maize cultivars in screening experiment at the germination stage as well as during the growth stages of seedling. In addition, to determine the water stress tolerance of selected maize cultivars, various physiological and biochemical attributes were analyzed at the vegetative growth stage. In the last experiment, the selected maize cultivars were evaluated twice in field experiments based on yield and yield components.

Meteorological data for all experiments (1st, 2nd and 3rd year experiments) were taken from Weather Station, Central Cotton Research Institute (CCRI), Old Shujabad Road, Multan. The data were presented in Box and Whisker Plots (Fig. 3.1 & Fig. 3.2).

Six cultivars (DTC, EV-77, EV-78, EV-79, Faisalabad mays and 6621) of maize (Zea mays L.) were used for the present study. Seeds of maize (Zea mays L.) cultivars (DTC, EV-77, EV-78, EV-79, Faisalabad mays) were obtained from Ayub Agriculture Research Institute, Faisalabad Pakistan while the seeds of cultivar 6621 got from the Syngenta Pakistan Limited, Multan.
**Fig. 3.1.** Meteorological data showing the maximum and minimum temperatures (°C) for the growth of maize (*Zea mays* L.) cultivars during 2010-2012.
Fig. 3.2. Meteorological data showing the rainfall and the relative humidity (RH) for the growth of maize (*Zea mays* L.) cultivars (DTC, EV-78 and 6621) during 2010-2013.
Experiment No. 1

Screening for PEG\textsubscript{6000}-induced water stress tolerance in maize cultivars at juvenile stage

The primary objective of conducting this experimental trial was to screen out the degree of water stress tolerance in six maize (\textit{Zea mays} L.) cultivars (DTC, EV-77, EV-78, EV-79, Faisalabad mays and 6621) at the stages of germination of seedling using polyethylene glycol (PEG\textsubscript{6000}) induced water stress. Experiment was conducted under laboratory conditions at the Institute of Pure and Applied Biology, Bahauddin Zakariya University Multan, Pakistan during October-November 2011 at 32±3°C and relative humidity range from 36-75%. Germplasm of all six cultivars of maize (DTC, EV-77, EV-78, EV-79, Faisalabad mays and 6621) were surface sterilized in the 5% solution of sodium hypochlorite (NaClO) for the time period of 5 minutes for further experimentation. Twenty surface sterilized seeds of all maize cultivars were placed on to the appropriately labeled plastic trays (30 × 25 × 10 cm) on 6 layers of filter paper (Whatman No. 1). The plastic trays were not covered. Simulated water stress was applied using polyethylene glycol (PEG\textsubscript{6000}, SIGMA) during the start of experiment. Varying concentrations of PEG (0, 3, 6 and 9\% PEG\textsubscript{6000} equivalent to 0, 0.0466, -0.0759, and -0.0876 MPa) were applied to germinating seeds of maize cultivars. Osmotic potential of PEG solutions were measured using osmometer and compared with curve described by (Michel & Kaufmann, 1973). Experiment was arranged in completely randomized design (CRD) with three replications. Seeds of all six maize cultivars were allowed to germinate for 12 days. Daily germination was recorded after passing every 24 hours and data were recorded for 10 days. Seeds were considered germinate when the emerging radicals and plumules were \( \geq 1 \) mm in length. The experiments continued for 12 days then fresh weights and lengths of shoots and roots were taken. Seedling mass was oven dried at 70°C for 24 hours then measured the dry weights of seedling roots and shoots. The following attributes and indices were calculated as follows:

\[
\text{Germination percentage} = \frac{\text{Total number of germinated seeds in each treated sample} \times 100}{\text{Total number of seeds in each treatment}}
\]

\[
\text{Energy of emergence (EG)} = \text{Percentage of germinated seeds after 4 days of sowing relative to the total number of seeds (Ruan et al., 2002)}.
\]

\[
\text{Germination rate (GR)} = \frac{(100/n) (N3/3+N5/5)}{}
\]
Where n= total number of seeds, N3 = Number of germinated seeds on 3rd day, N5= Number of germinated seeds on 5th day (Uniyal & Nautiyal, 1998; Singh et al., 2007).

**Promptness index (PI)** = nd2 (1.00) +nd4 (0.75) +nd6 (0.5) +nd8 (0.25); where ‘n’ designates the number of germinated seeds at day (d) (Ashraf et al., 2006).

**Germination tolerance index (GTI%)** = Promptness index (PI) of stressed seeds / Promptness index (PI) of controlled seeds × 100 (Ashraf et al., 2006).

**Seedling Vigor Index** = Length of seedling (cm) × germination percentage (modified formula of (Abdul-Baki & Anderson, 1973).

**Plant height tolerance index (PHTI)** = plant height of stressed plants / plant height of control plants × 100

**Root length tolerance index (RLTI)** = root length of stressed plants / root length of control plants × 100

**Dry biomass tolerance index (DBTI)** = dry biomass of stressed plant / dry biomass of control plants x 100 (Ashraf et al., 2006)

**Statistical analysis:** Mean, standard deviation and standard error (+S.E) were calculated by using MS Excel 2010. Data of various morphological and physiological attributes were placed to Two Way Analysis of Variance (2WCR ANOVA) via using a computer package of COSTAT (Cohort Software, Berkeley, California). The comparison of mean values were analyzed with the Least Significant differences test (LSD) following Snedecor & Cochran (1980).
Experiment No. 2

Assessment of degree of drought tolerance in maize (*Zea mays* L.) at the vegetative growth stage.

Drought is a main abiotic stressor component of the environment that inhibits the growth of crops by limiting the photosynthetic performance. Significant objective of conducting this experiment was to observe the drought induced stress in terms of different drought cycles (D0, D1, D2, and D4) counteracting the inhibitory effects of water shortages on photosynthetic physiology related to the growth stage of maize cultivars differing in drought tolerance. In order to assess the differential tolerance potential/status of maize cultivars in response to drought stress, an experiment was to direct at the Botanical Garden of Bahauddin Zakariya University Multan, Pakistan during 3 December, 2011 to 10 April, 2012.

In this experiment, 3 maize cultivars (DTC, EV-78 and 6621) were evaluated for drought resistance potential in 4 treatments (D0, D1, D2 and D4 drought cycles), arranged as randomized complete block design with four replications.

Seventy two seeds of maize cultivar were superficially sterilized with 5% NaClO3 soln. for 5 minutes. Six surface sterilized seeds of tested maize cultivar were sown in each plastic pots (30 cm height and 24 cm in diameter) filled with 12 kg garden soil. Physical and chemical composition of soil given in the Table 3.1. Pots had a drainage hole at the bottom covered with muslin cloth. The physico-chemical characteristics of garden soil are given in Table 1. After seed sowing, the plastic pots were watered with tap water to field capacity to induce the germination of seedling. After one week of emergence of maize seedling, the plants were thinned to four in number per pot which were almost of uniform size and equidistantly placed.
Table 3.1. Physical and chemical analysis of soil

<table>
<thead>
<tr>
<th>Soil physical and chemical properties</th>
<th>values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand</td>
<td>46 %</td>
</tr>
<tr>
<td>Silt</td>
<td>24 %</td>
</tr>
<tr>
<td>Clay</td>
<td>30 %</td>
</tr>
<tr>
<td>Textural class</td>
<td>Loam</td>
</tr>
<tr>
<td>Electrical conductivity</td>
<td>0.5 mS/cm</td>
</tr>
<tr>
<td>pH</td>
<td>8.2</td>
</tr>
<tr>
<td>Organic matter</td>
<td>0.63 %</td>
</tr>
<tr>
<td>Available P</td>
<td>5.0 ppm</td>
</tr>
<tr>
<td>Available K</td>
<td>140 ppm</td>
</tr>
<tr>
<td>Zinc</td>
<td>0.74 ppm</td>
</tr>
<tr>
<td>Copper</td>
<td>0.18 ppm</td>
</tr>
<tr>
<td>Iron</td>
<td>4.40 ppm</td>
</tr>
<tr>
<td>Manganese</td>
<td>0.84 ppm</td>
</tr>
</tbody>
</table>

After the establishment of the plants (3 weeks old plants), water stress was imposed in terms of different drought cycles (D0, D1, D2 and D4).

**D0**= Normal watering,

**D1**= one drought cycle; one time withholding water upto wilting stage and then re-watered with 3L full strength Hoagland nutrient solution.

**D2**= Two drought cycles; two times withholding water up to wilting stage and then re-watered with 3L full strength Hoagland nutrient solution

**D4**= Four drought cycles; four time withholding water up to wilting stage and then re-watered with 3L full strength Hoagland nutrient solution.

After the completion of drought cycles, two sample plants from each replicate were harvested and the other two samples were left for assessing the yield attributes. After harvest the plants were dried in oven at 80 °C for time period of 72 hours and the accumulation of dry weights was recorded.
Table 3.2. Composition of full strength Hoagland nutrient solution

<table>
<thead>
<tr>
<th>Chemical compound</th>
<th>Stock soln. concentration g/L</th>
<th>Volume of stock soln. per liter of final soln.</th>
</tr>
</thead>
<tbody>
<tr>
<td>KNO₃</td>
<td>101.10g/L</td>
<td>6ml</td>
</tr>
<tr>
<td>Ca(NO₃)₂•4H₂O</td>
<td>236.16g/L</td>
<td>4ml</td>
</tr>
<tr>
<td>(NH₄)₂PO₄</td>
<td>115.08g/L</td>
<td>2ml</td>
</tr>
<tr>
<td>MgSO₄•7H₂O</td>
<td>246.49g/L</td>
<td>1ml</td>
</tr>
<tr>
<td>KCl</td>
<td>3.728mM</td>
<td></td>
</tr>
<tr>
<td>H₃BO₃</td>
<td>1.516mM</td>
<td></td>
</tr>
<tr>
<td>MnSO₄•H₂O</td>
<td>0.338mM</td>
<td>Dissolve in</td>
</tr>
<tr>
<td>ZnSO₄•7H₂O</td>
<td>0.575mM</td>
<td>1 liter</td>
</tr>
<tr>
<td>CuSO₄•5HO</td>
<td>0.125mM</td>
<td></td>
</tr>
<tr>
<td>H₂MoO</td>
<td>0.081mM</td>
<td></td>
</tr>
<tr>
<td>Fe-EDTA</td>
<td>6.922mM</td>
<td></td>
</tr>
</tbody>
</table>

Before harvest following physiological and biochemical attributes were analyzed:

**Total chlorophyll:** Youngest fully expanded 3rd leaf from the top of the plant was selected to record the total chlorophyll contents by using the handy chlorophyll meter (Minolta SPAD-502, Japan). Three readings were taken from the different points of leaves three times to calculate the average value.

**Leaf water potential:** Fully developed youngest leaf was selected and excised from each replicate of all treatments to measure leaf water potential. The readings were made at 8 am with the help of Scholander type apparatus (Chas W. Cook and Sons, Birmingham, U.K).

**Gas exchange attributes:** Gas exchange characteristics were recorded by an open system LCA-4 ADC infrared gas analyzer (Analytical Development Company, Hoddeson, England). Measurements were recorded from 9:00 to 11:00 am with adjusting the following standardization: surface area of leaf (11.50 cm²), ambient CO₂ concentration (371µmol/mol), surrounding temperature (45±3°C), leaf chamber temperature (Tch) mottled from 25-28 °C, leaf chamber volume gas flow rate (v) (296 mL/min), molar gas flow rate of leaf chamber (U) 400 µmol/s, ambient pressure (P) (97.95 kPa), PAR (Qleaf) at the leaf surface (upto 770 µmol m⁻² s⁻¹). Data for sub-stomatal CO₂ concentration (Cᵢ), net CO₂ assimilation rate (A), rate of transpiration (E), water use efficiency (A/E) was recorded on the youngest fully expanded 3rd leaf from the top of plant of each sample.
**Determination of total soluble protein:** Total soluble protein (mg/g fresh wt.) was measured by following the method of (Bradford, 1976). Fresh leaf mass (0.2 g) was taken and homogenized in 3 ml phosphate buffer with the help of mortar and pestle. Then transferred this homogenized extract to the centrifuge tube and washed the pestle mortar with 1 ml phosphate buffer. It was centrifuged at 6000 x g for the time period of 5 minutes and supernatant was obtained and stored at 4 °C. Finally it was used for the determination for total soluble protein and total amino acids. The optical density was recorded at 595 nm using a spectrophotometer (Hitachi U-2000, Tokyio Japan).

\[
\text{Total soluble protein} = \frac{\text{Reading of sample} \times \text{Vol.of sample} \times \text{Dil. factor}}{\text{Fresh wt. of leaf mass} \times 1000}
\]

**Total free amino acids:** Total free amino acids was estimated as described by Hamilton & Van Slyke (1943). Leaf extract (5 ml) of each sample was taken in test tubes. 1 ml of 10% pyridine and 1 ml of 2% ninhydrin were mixed to the extract of leaf samples. The reagents in the test tubes were allowed to react for half an hour at 100°C. After this, the mixture was left for cooling and diluted up to 50 ml. The optical densities of all solution samples were noted at 570 nm using a spectrophotometer (Hitachi U-2000).

\[
\text{Total free amino acids} = \frac{\text{Sample reading} \times \text{volume of sample} \times \text{Dilution factor}}{\text{Fresh wt. of leaf} \times 1000}
\]

**Statistical analysis:** Mean, standard deviation and standard error (+S.E) were calculated by using MS Excel 2010. Data on the total chlorophyll, leaf water potential, photosynthetic gas exchange characteristics, total soluble protein and total free amino acids were subjected to Two Way Analysis of Variance (2WCR ANOVA) using a COSTAT software (Cohort Software, Berkeley, California). The comparison of mean values were calculated by Least Significant Difference test following Snedecor & Cochran (1980).
Chlorophyll $a$ fluorescence measurement and study of OJIP transients

Transients of chlorophyll $a$ fluorescence were noted on third leaf with portable fluorescence meter, FluorPen FP 100. Leaves were acclimatized to darkness for $\frac{1}{2}$ an hour by covering the leaves with aluminium foil to make sure that all reaction centers (RCs) of PSII were adapted to dark state. Data of OJIP parameters were calculated as described by the (Tsimilli-Michael & Strasser, 2001). Chlorophyll $a$ fluorescence kinetics was recorded from 10 micro seconds ($\mu$s) to 1second (s) as follow: $F_0$, the initial fluorescence was measured at 50$\mu$s, $L$ (150 $\mu$s), $K$ (300 $\mu$s), $J$ (2000 $\mu$s) and $I$ (30,000 $\mu$s) are the intermediates ($F_L$, $F_K$, $F_J$, and $F_I$, respectively) and $P$ (500,000 $\mu$s) as the maximum fluorescence ($F_M$) (Table 3.3).The entire digitized fluorescence kinetics with their differences was normalized as described by Strasser et al. (2004); Oukarroum
et al., 2007 Redillas et al., 2011. (a) The fluorescence transients were normalized twice between the origin (O) and peak (P) end of fluorescence phases and the change in between these two extremes (F₀ and Fₘ) of fluorescence kinetics expressed as V_OK. Further, these transients (Sreenivasulu et al., 2012) were double normalized within time range of 30 (F₀) to 300 µs (Fₖ) calculated as the V_OK = [(Fᵣ - F₀)/(Fₖ - F₀)] to check the fluorescence increase at about 300 µs. In addition, change in differences (ΔV_OK) of transients regarding to reference was also calculated and revealed as the L band. (b) The chlorophyll a fluorescence transients were double normalized between F₀ and Fₖ phases expressed by way of the V_OJ i.e. V_OJ = [(Fᵣ - F₀)/(Fₖ - F₀)] and change in fluorescence transients were described as (ΔV_OJ) to visualize the response of maize cultivars and assess the K-band. To determine the OI step, the chlorophyll a fluorescence data was recoded between the time span of 50 to 10⁶ µs formulated as V_OI (<1) [(V_OI = (Fᵣ - F₀)/(F₁ - F₀))] and the kinetic change was calculated as follows: V_OI [(ΔV_OI = (Fᵣ - F₀)/(Fₖ - F₀)]]. The IP was assessed on two lines: (1) V_OI transients normalization between time period of 3x10⁴-10⁵ µs stated as as V_OI (≥1) [(V_OI = (Fᵣ - F₀)/(F₁ - F₀))] and the transient normalization to the time span of 30000 to 200000 µs equate as V_IP [V_IP = ((Fᵣ - F₁)/(Fₕ - F₁)]. For the quantification and performance of PSII response of maize cultivar under drought stress, biophysical and phenomenological parameters derived from OJIP analysis were assessed.

Other parameters during the fast chlorophyll a fluorescence induction were also calculated. They were e.g. specific energy fluxes and their ratios i.e ABS/RC, TR₀/RC, ET₀/RC and DI₀/RC showed the absorption, trapping, electron transport and dissipation per reaction centers (RC). Flux ratios also associated to photosynthetic yields. Fᵥ/Fₘ showed the maximum quantum yield of primary photochemistry, ET₀/TR characterize the efficacy of trapped exciton can move an electron to an electron transport chain, ET₀/ABS shows electron transport quantum yield. Moreover, ABS/CS, TR₀/CS, ET₀/CS and DI₀/CS (Table 3.3) determined as the phenomenological energy fluxes per cross section (CS) (Boureima et al., 2012). In addition, RC/CS calculated as the ratio of active reaction centers of PSII based on per excited cross section.

The associated parameters of fast chlorophyll fluorescence like the Fᵥ/F₀ showed the maximum yield of primary photochemistry of PSII, TFm designates the time to attain the
maximum fluorescence, Area, RC/ABS indicated the ratio of active reaction centers based on the absorbance and the performance index were also recorded (Stirbet & Govindjee, 2011)

Table 3.3. Measured parameters of OJIP test, their formula and description:

<table>
<thead>
<tr>
<th>OJIP parameters</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_O$</td>
<td>Initial fluorescence showed that RCs of PSII are open at 50 µs.</td>
</tr>
<tr>
<td>$F_J$</td>
<td>$F_{2ms}$ shows the intensities of fluorescence at 2 ms (J step)</td>
</tr>
<tr>
<td>$F_I$</td>
<td>$F_{30ms}$ describe the fluorescence value at 30 ms</td>
</tr>
<tr>
<td>$F_M$</td>
<td>Maximum fluorescence at the peak of OJIP transients revealed that all reaction centers of PSII had been closed and adapted to dark state</td>
</tr>
<tr>
<td>$F_V$</td>
<td>$F_M$-$F_O$ designates the maximum variable chlorophyll fluorescence</td>
</tr>
<tr>
<td>$V_J$</td>
<td>$V_J$ = $(F_J$-$F_O$) / $(F_M$-$F_O$) describe the relative variable fluorescence at J level</td>
</tr>
<tr>
<td>$V_I$</td>
<td>$V_I$ = $(F_I$-$F_O$) / $(F_M$-$F_O$) describe the relative variable fluorescence at the I step</td>
</tr>
<tr>
<td>$F_M$/$F_O$</td>
<td>Ratio of maximum quantum yield of photochemistry and competitive non-photochemical in PSII.</td>
</tr>
<tr>
<td>$F_V$/$F_O$</td>
<td>Ratio between variable and minimal chlorophyll fluorescence. It translates the primary yield of photochemistry and estimation of PSII</td>
</tr>
<tr>
<td>$F_V$/$F_M$</td>
<td>$(F_M$-$F_O$)/$F_M$ describes the maximum productivity of photochemistry of PSII.</td>
</tr>
<tr>
<td>$M_O$</td>
<td>$4(F_{300µs}$-$F_O$)/(F_M$-F_O$) formulate the primary slop of fluorescence kinetics.</td>
</tr>
<tr>
<td><strong>Area</strong></td>
<td>Zone between the curve of OJIP and the line $F_M$</td>
</tr>
<tr>
<td><strong>Fix area</strong></td>
<td>Area below the fluorescence curve between $F_{40µs}$ and $F_{1s}$ (subtraction of background)</td>
</tr>
<tr>
<td>$S_M$</td>
<td>Area/$F_V$ denotes the normalized area above the OJIP curve (multiple turn-over or multiple events of QA reduction)</td>
</tr>
<tr>
<td><strong>$S_s$</strong></td>
<td>Total normalized area relate to only OJ step (single volume of reduction of QA events or single turn-over).</td>
</tr>
<tr>
<td><strong>N</strong></td>
<td>$S_M$.$M_O$. (1/$V_I$) denotes number of QA redox turn-overs to $F_M$ (total no. of electrons moved to electron transport chain during 0 to t.</td>
</tr>
<tr>
<td>Symbol</td>
<td>Definition</td>
</tr>
<tr>
<td>--------</td>
<td>------------</td>
</tr>
<tr>
<td>$\Phi_{Po}$</td>
<td>Photosystem II photochemistry primarily associated to the maximum quantum yield.</td>
</tr>
<tr>
<td>$\Psi_o$</td>
<td>The chance that a trapped exciton transfer an electron to an electron transport chain beyond plastoquinone $Q_{A}^\cdot$</td>
</tr>
<tr>
<td>$\Phi_{Eo}$</td>
<td>Quantum yield of electron transport from $Q_{A}^\cdot$ to the electron acceptors.</td>
</tr>
<tr>
<td>$\Phi_{Do}$</td>
<td>The probability of dissipation of the absorbed photon.</td>
</tr>
<tr>
<td>PI</td>
<td>Performance index on absorption basis</td>
</tr>
<tr>
<td>ABS/RC</td>
<td>Absorption flow of reaction centers showing the size of antenna.</td>
</tr>
<tr>
<td>TR$\sigma$/RC</td>
<td>Rate at which exciton is took by the reaction center causing in the reduction of plastoquinone ($Q_{A}^\cdot$). leading to reduction /reaction center</td>
</tr>
<tr>
<td>ETo/RC</td>
<td>Reoxidation of $Q_{A}^\cdot$ through transport of electron in active reaction center</td>
</tr>
<tr>
<td>DIo/RC</td>
<td>The flux of energy dissipated per reaction center</td>
</tr>
<tr>
<td>$T_{FM}$</td>
<td>Time to reach the maximal fluorescence ($F_{M}$)intensity</td>
</tr>
<tr>
<td>Phenomenological Fluxes</td>
<td></td>
</tr>
<tr>
<td>ABS/CSo</td>
<td>Ratio between absorption flux and excited cross section at fully open reaction centers (RC)</td>
</tr>
<tr>
<td>TRo/CSo</td>
<td>Trapped electron flux related to related to the excited cross section at fully open reaction centers (RC)</td>
</tr>
<tr>
<td>ETo/CSo</td>
<td>Trapped flux per excited cross section at fully open reaction centers (RC)</td>
</tr>
<tr>
<td>DIo/CSo</td>
<td>Dissipated energy flux per excited cross section at fully open reaction centers (RC)</td>
</tr>
<tr>
<td>ABS/CSm</td>
<td>Absorption flux/excited cross-section at closed RC</td>
</tr>
<tr>
<td>TRo/CSm</td>
<td>Trapped flux/excited cross-section at closed RC</td>
</tr>
<tr>
<td>ETo/CSm</td>
<td>The flux of electron transport per excited cross section at closed RC</td>
</tr>
<tr>
<td>DIo/CSm</td>
<td>Ratio between dissipated energy and excited cross section at closed RC</td>
</tr>
<tr>
<td>Reaction center densities</td>
<td></td>
</tr>
<tr>
<td>RC/CSo</td>
<td>Density of RCs per excited cross section (RC open)</td>
</tr>
<tr>
<td>RC/CSm</td>
<td>Reaction center density per excited cross section (RC closed)</td>
</tr>
</tbody>
</table>
Statistical analysis: Mean, standard deviation and standard error (+S.E) of the OJIP parameters and radar plot were calculated by using MS Excel 2010. Two Way Analysis of Variance (2WCR ANOVA) was applied to determine the significant variations between control and drought stressed plants using the COSTAT statistical computer software (Cohort Software, Berkely, California). The mean values were compared with LSD test (Least Significant Differences) following the Snedecor & Cochran (1980).
Experiment No. 4

In order to assess the protein expression under drought stress in maize cultivars (DTC, EV-78, and 6621), proteomic approach was used to better understand the metabolic pathways response implicated in water stress environment.

Seeds of maize (*Zea mays* L.) cultivars (DTC, EV-78 and 6621) got from the research center of AARI (Ayub Agriculture Research Institute), Faisalabad, Pakistan. Seeds were treated with 5% sodium hypochlorite solution for 5 minutes to sterilize before carrying out the experiment. Ten selected seeds of maize cultivars (DTC, EV-78 and 6621) were sown in plastic pots having the 24 cm diameter. Each replicate filled with 12 kg garden soil. The experimental design was randomized complete block design containing two treatments (control and drought stress) and four replications. After the germination of one week the plants are thinned to 4 in number in each replicate. When seedling was established (2 weeks old plants), four drought cycles were induced by withholding water irrigation up to wilting point and then rehydrate to the 100% field capacity with 3 L of full strength Hoagland’s nutrient solution. During this period of water withholding, control plants continued to be irrigated normally up to the maximum water holding capacity.

After the completion of four drought cycles, plants were harvested and separated into roots and shoots. Roots were thoroughly washed, blotted dry and the data for fresh biomass was recorded. Then samples were oven dried at 80°C for 72 hours and dry biomass accumulation was recorded.

Leaf protein extraction

Protein analysis was carried out on the youngest completely developed 3rd leaves of drought stressed maize plant and control plants, respectively. Leaf samples (about 5 g fresh weights/sample) were instantaneously grounded in pestle mortar in liquid N\textsubscript{2} and stored at -80 °C until protein extraction. Total leaf soluble protein was extracted by the method of phenol extraction (Hurkman and Tanaka, 1986; Sarvanan and Rose, 2004). Two grams fine powder of leaf tissue was suspended in 4 ml extraction buffer [Tris-HCl having pH 7.5 (0.5 M), sucrose (0.7 M), 0.1 M KCl, EDTA (50 mM), 2% β mercaptoethanol (2%) and 1mM PMSF]. Equal volume of phenol saturated with Tris-HCl (pH 7.5) was mixed then agitated for 30 minutes at
4°C and centrifuge at 10,000 rpm for ½ an hour at 4°C. The upper phenolic part was collected and an equal quantity of extraction buffer was added to this. This action was repeated and the upper phenolic phase was again collected. 0.1 M chilled ammonium acetate in methanol was added to the collected phenolic phase and was kept overnight at -20 °C for production of protein precipitates. The samples were again centrifuged at 10,000 rpm at 4°C for 30 minutes. The precipitated protein pellets was washed three times in chilled methanol and acetone and air dried for few minutes. Protein pellets was dissolved in 1 ml IEF buffer (20 mM DTT, 8 M urea and 4% SDS).

The protein extracts were profiled by SDS-PAGE.

**SDS-PAGE:**

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) used to analyze the proteins of different samples (E-VS10-SYS, Germany) (Laemmli, 1970). The basic components and standard procedure for polyacrylamide gel preparation and visualization of proteins bands are as follow.

**Composition of stock solutions for the extraction of protein**

1. **Acrylamide gel solution (30%):** To make the 30% acrylamide gel solution, 0.8 g methylenebisacrylamide and 30 g acrylamide mix together and raised the volume upto 100 ml by using distilled water.
2. **SDS (10%):** 10 g SDS dissolve in small quantity of distilled water and bring the volume upto 100 ml
3. **4x Resolving gel buffer (1.5 M Tris HCL maintain pH 8.8 with 0.4% SDS):** Mix 36.4 g tris base and 8 ml SDS (10%) dissolve in 110 ml of distilled water. Adjust the pH to almost 8.8 with 1 N hydrochloric acid. Then finalize the volume to 200 ml with distilled water
4. **4x Stacking gel buffer (0.5 M Tris HCL adjust the pH to 6.8 with 0.4% SDS):** 12.12 g of tris base and 8 ml 10 % SDS dissolve in 110 ml of distilled water. Maintain the pH to 6.8 by mixing the 1 N HCL and then finalize the volume upto 200 ml by using the distilled water.
5. 4x Tris-glycine running buffer without SDS: For making this buffer, 36.0 g of tris base and 172.8 g of glycine mix together and add distilled water to make 3L volume.

6. 1x Tris-glycine running buffer with SDS: 750 ml of 4x Tris-glycine buffer mix with 30 ml of 10% SDS solution. Then make the volume upto 3L by dissolving with distilled water.

7. Ammonium per sulphate solution (10%): 0.1 g of ammonium per sulphate dissolve in distilled water to make the volume one liter.

8. N,N,N,N Tetramethylethylene diamine (TEMED): Catalog number was T9281 and used as neat liquid.

9. 4x Sample buffer: For the preparation of 4x sample buffer dissolve 4 ml glycerol, 2 ml of β-mercaptoethanol, 1.2 g SDS, 4 ml of 4x stacking buffer and 0.03 g of bromophenol blue. Aliquot transferred in 1.5 ml microcentrifuge tube and stored at -20 °C.

10. Stainer: CBB R-250 is added to the ratios of 50, 10, and 40 of methanol, acetic acid, water solution, respectively to prepare the stain of CBB. Afterward, it was filtered through whatman filter paper Number 1.

11. Destainer: Mix methanol, acetic acid, and water solution in the ratios of 50, 10 and 40 ml to prepare the destainer.

Resolving gel solution (12%): This gel solution was prepared by mixing the 100 µl TEMED, 80 µl ammonium per sulfate solution and 5.5 ml resolving gel solution in the falcon tubes. After preparation pour this gel solution in the gel plates and then immediately spread 1 ml isopropanol on the top surface of gel. Polymerization of gel took the time of almost 15 minutes. Moreover, composition of resolving gel solution contained 6 ml of 30% acrylamide gel solution, 5.25 ml distilled water, 3.75 ml 4x resolving gel buffer, 100 µl 10% ammonium per sulfate solution and 60 µl TEMED.

Stacking Gel Solution (5%): For the preparation of stacking gel, 100 µl APS and 60 µl TEMED mixed with resolving gel solution. Gel was prepared carefully to avoid the bubble formation. The gel polymerization time was almost 40 minutes. Instantly, after pouring the solution comb was injected carefully to make sure that there was no air bubble beneath the comb ends. In addition, the composition of stacking gel solution consist of 0.8 ml of 30% acrylamide gel solution, 4.2 ml
distilled water, 3.75 ml 4x stacking gel buffer, 100 µl of 10% ammonium per sulfate solution and 60 ml TEMED.

**Reduced protein sample:** Aliquots of 21 µl protein extract and 7µl reducing buffer (heated at 95°C in water bath for 5 minutes) were loaded on the gel.

**Gel running:** SDS-PAGE apparatus (EEPS- 300700, Germany) was roofed by fixing the lid and switched to the power supply. Voltage power was adjusted to 125 V for the 12 % gel. When power supply was on, the bands were repressed moving downward looked as single line of dye. After this loading dye moved to the end of gel then turned off the power. Electrophoresis module removed carefully, the glass sandwiches were taken off and finally get the gel. Precautionary measure is that when gel pasted to the plates then primarily it was soaked in the buffer and then carefully remove with spatula.

**Staining and De-staining Process:** Gel was stained with CBB R-250 dye on electrical stirrer for one hour. Acetic acid (CH₃COOH) was used in the stainer for the fixation of protein bands in the gel. De-staining of the gel was carried out by placing the gel in de-stainer with slow shaking for 30-40 minutes slow shaking for 30-40 minutes.

Table 3.4. Chemicals used for the preparation of staining and de-staining solutions.

<table>
<thead>
<tr>
<th>Chemicals used in staining</th>
<th>Concentrations (100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBB R-250</td>
<td>0.25 g</td>
</tr>
<tr>
<td>Methanol</td>
<td>50 ml</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>10 ml</td>
</tr>
<tr>
<td>Distilled water</td>
<td>40 ml</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chemicals used in de-staining</th>
<th>Concentrations (100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>methanol</td>
<td>50 ml</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>10 ml</td>
</tr>
<tr>
<td>Distilled water</td>
<td>40 ml</td>
</tr>
</tbody>
</table>
Visualization of gel

After de-staining, gel was pictured and the protein bands of different samples were compared to the references band of standard marker (Invitrogen, 69079-3) as showed in the (Fig 3.3).

**Fig. 3.3.** Protein marker (61-98 kDa)

**Hydrogen per oxide determination** ($\text{H}_2\text{O}_2$): Hydrogen per oxide was measured following the method of Velikova *et al.*, (2000). 0.5 g fresh leaves was uniformly homogenized in 0.1% trichloroacetic acid (5 ml). This homogenization was done in pre-chilled pestle and mortar. Then centrifuge the homogenate at 12000 g for the time period of 15 minutes. Finally, add the 0.5 ml phosphate buffer (pH) and 1 ml potassium iodide (KI) to the 0.5 ml of supernatant. The mixture was vortexes and absorbance read at 390 nm.

**Malondialdehyde determination**: Drought induced oxidative damaged (membrane lipid peroxidation) was assessed by determining the amount of Malondialdehyde in the leaf tissue as stated by Carmak and Horst (1991), with minor modifications. 1.0 g fresh leaf tissue was homogenized in concentration of 3 ml of 0.1% of trichloroacetic acid in a chilled pestle and mortar. Centrifugation of homogenate was done and adjusted at 12000 g for the time period of 15 minutes. 3 ml of thiobarbituric acid (0.5%) prepared in 20% TCA was added to 0.5 ml supernatant. Then mixture was heated at 95 °C in water bath for 50 minutes. The reaction was
stabilized by cooling the sample tubes in ice water bath. Finally, samples were centrifuged at 10,000 g for 10 minutes and the absorbance read at 600 and 532 nm. The MDA concentration was calculated as the change in absorbance at 600 and 532 nm wavelength by applying the following formula.

\[
\text{MDA concentration (nmol)} = \Delta \text{ (absorbance 532 nm - absorbance 600 nm)}/1.56 \times 10^5
\]

**Activities of antioxidant enzymes:** For the extraction of antioxidant enzymes, 0.5g fresh leaves were ground using pestle and mortar in 5 ml of 50 mM cooled phosphate buffer (pH 7.8). After filtration through cheesecloth, the homogenate was centrifuged at 15000 g for 20 minutes at 4 °C and the supernatant used for enzyme assays.

**Peroxidases:** Peroxidases (POD) was assessed following (Chance Maehly, 1955) with minor revisions. The POD activity was determined by guaiacol oxidation process. 50 mM phosphate buffer having the pH (7.0), guaiacol (20 mM), \( \text{H}_2\text{O}_2 \) (40 mM) and 0.1 ml enzyme extract to make the final volume of reaction mixture up to 3 ml. change deviation in absorbance was read at 470 nm after every 20 seconds. one unit POD activity was defined as the change of 0.01 absorbance unit per minute per mg of protein.

**Statistical analysis:** Mean, standard deviation and standard error (+S.E) were calculated by using MS Excel 2010. The data for the malondialdehyde and hydrogen per oxide determination, and peroxidase activity were subjected to Two Way Analysis of Variance (2WCR ANOVA) using a COSTAT computer package (Cohort Software, Berkeley, California). The mean values were compared with the Least Significant Difference test (LSD) following Snedecor & Cochran (1980).
Experiment No. 5

Assessment of yield potential of selected maize cultivars at various moisture regimes under field conditions

Water deficiency is a main abiotic stressor component of the environment that severely reduced the yield of crops. So, Yield and yield attributes play a role of indicators for the selection of drought tolerant cultivars to decrease the impact of water deficit on yield productivity in breeding programs. In order to identify these indicators related to the drought tolerance, a field experiment was conducted on the fields of Botanical Garden, Bahauddin Zakariya University Multan, Pakistan during 22 March, 2013 to 7 July, 2013. Soil characteristics of the fields given in Table

Table 3.5. Soil properties of the field experiment

<table>
<thead>
<tr>
<th>Physio-chemical analysis of soil</th>
<th>values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand</td>
<td>38.25 %</td>
</tr>
<tr>
<td>Silt</td>
<td>46.26 %</td>
</tr>
<tr>
<td>Clay</td>
<td>15.27 %</td>
</tr>
<tr>
<td>Textural class</td>
<td>Loamy</td>
</tr>
<tr>
<td>Electrical conductivity</td>
<td>1.52 dS/m</td>
</tr>
<tr>
<td>pH</td>
<td>8.2</td>
</tr>
<tr>
<td>CO\textsuperscript{3-2}</td>
<td>nil</td>
</tr>
<tr>
<td>HCO\textsubscript{3}</td>
<td>8.7 mmolL\textsuperscript{-1}</td>
</tr>
<tr>
<td>Cl\textsuperscript{-1}</td>
<td>3.15 mmolL\textsuperscript{-1}</td>
</tr>
<tr>
<td>Ca\textsuperscript{2+} + Mg\textsuperscript{2+}</td>
<td>8.3 mmolL\textsuperscript{-1}</td>
</tr>
<tr>
<td>Na\textsuperscript{+1}</td>
<td>6.9 mmolL\textsuperscript{-1}</td>
</tr>
<tr>
<td>SAR</td>
<td>3.39 (mmolL\textsuperscript{-1}\textsuperscript{1/2})</td>
</tr>
<tr>
<td>Organic matter</td>
<td>0.60 %</td>
</tr>
<tr>
<td>Available P</td>
<td>5.0 ppm</td>
</tr>
<tr>
<td>Available K</td>
<td>140 ppm</td>
</tr>
<tr>
<td>Zinc</td>
<td>0.74 ppm</td>
</tr>
<tr>
<td>Copper</td>
<td>0.18 ppm</td>
</tr>
<tr>
<td>Iron</td>
<td>4.40 ppm</td>
</tr>
<tr>
<td>Manganese</td>
<td>0.84 ppm</td>
</tr>
</tbody>
</table>

In this experiment, seeds of maize cultivars (DTC, EV-78 and 6621) were placed as ridge sowing. The experiment was arranged in randomized complete-block design. Plot area was 12×12 ft\textsuperscript{2} (144 ft\textsuperscript{2}). Each plot area consisted of 6 rows, 12ft (360 cm) long with 60 cm spacing between rows and 20cm between plants. There are two irrigation treatments control and water
stressed; in water stressed treatment irrigation was withheld at the tassel forming stage. Watering was discontinued in water-deficit treatments up to wilting point and leaf rolling stage while the well-watered plants continued to receive irrigation to the field capacity each week till physiological maturity. Sowing was done manually and 2 seeds per hill were placed at 20 cm plant distance. Thinning of the plant was done to maintain one plant per hill.

Nitrogen was applied as urea (NH$_2$-CO-NH$_2$ (46% N) at the rate of 100 kg/ha, DAP at 60 kg/ha while 50kg/ha potassium sulphate applied at sowing, 60 cm height and tasseling stage. Maize cultivars were kept free of weeds by hoeing to avoid the weed crop competition.

Plot area (12×12 ft$^2$) of each treatment was harvested and 20 sub-samples of each maize cultivar were taken for the analysis of varying yield attributes. The following yield components were measured according to standard procedures:

1. **Weight of cob with sheath leaves (g):** Twenty cobs of each maize cultivar from plot area (12×12 ft$^2$) was taken at random and weighed by electric balance.
2. **Weight of cob without sheath leaves (g):** It was taken (20 cobs) at random from plot area (12×12 ft$^2$) of each maize cultivar and weighed by means of electric balance.
3. **Weight of kernels per cob (g):** This was selected at random from the grain lot of twenty cobs of plot area (12×12 ft$^2$) and weighed with the help of electric balance.
4. **Number of kernel rows per cob [Cob girth (Zhang et al., 2010)]:** Number of kernel rows of 20 cobs from each plot area (12×12 ft$^2$) were counted and then averaged.
5. **Number of kernels per cob:** Number of kernels of 20 cobs from each plot area were counted and taken average.
6. **1000-Kernel weight (g):** This was taken at random from the grain lot of each plot area and weighed by electric balance.
7. **Cob length [CB]:** The potential number of kernels per row of the twenty cobs was counted from each plot area (12×12 ft$^2$) and then calculated average.
8. **Cob size [CG× CB] =** The number of kernel rows [Cob girth] × the number of kernels per row [Cob length (CL)].
9. **Grain yield**: Grain yield was recorded by weighing the kernels shelled from the cob from the central four rows of each plot area \(12 \times 12 \text{ ft}^2\) or \(3.5676 \times 3.5676 \text{ m}^2\) and converted it into kg ha\(^{-1}\) using the formula:

\[
\text{Grain yield (kg ha}^{-1}) = \frac{\text{Grain yield (kg)}}{\text{harvested area (1.80 m} \times \text{1.80m})} \times 10000
\]

Then this mass of kernels/plot converted into kilograms/hectare (kg/ha.)

10. **Grain pith ratio (GPR)**: Pith ratio of grain was calculated as the ratio of grain weight to pith weight after shelling of cobs of maize (grain yield/yield of pith).

11. **Cob sheath ratio (CSR)**: Twenty maize cobs were weighed on electrical balance including their sheath leaves as well as without sheath leaves and the ratio of cob sheath was determined as the ratio of cob weight to sheath weight when cob of maize were shelled.

12. **Relative yield reduction\ ([RYR\%]) = 100(1 - \text{grain yield}_{\text{stressed}} / \text{grain yield}_{\text{non-stressed}})\)

**Statistical analysis**: Mean, standard deviation and standard error (±S.E) were calculated by using MS Excel 2010. The data for various yield and yield attributes were subjected to Two Way Analysis of Variance (2WCR ANOVA) using a COSTAT computer program (Cohort Software, Berkeley, California). The mean values were compared with the Least Significant Difference test (LSD) following (Snedecor & Cochran, 1980)
Experiment No. 1

Screening for water stress tolerance in maize cultivars at the juvenile stage

Seed germination of six maize cultivars declined significantly due to PEG\textsubscript{6000} induced water stress. Maize cultivars also differed significantly under both normal and PEG-induced water stress (Table 4.1). Among cultivars, DTC exhibited the maximum germination percentage (95%) at the highest concentration of PEG, whereas cv. 6621 was the lowest in this attribute at the highest level of PEG-induced water stress (Fig. 4.1). In addition, cvs. EV-77 and EV-78 were intermediate in seed germination percentage (Fig. 4.1).

PEG-induced water stress markedly reduced the rate of seed germination of all maize cultivars (P ≤ 0.001) (Table 4.1). Rate of seed germination of six maize cultivars was maximally inhibited at the highest level of PEG (9%) (Fig. 4.1). Off all maize cultivars, cv. DTC showed highest rate of seed germination at all concentrations of PEG. In contrast, cv. 6621 had the lowest germination rate at all levels of PEG-induced water stress (Fig. 4.1). Remainder cultivars were intermediate in this attribute at all levels of water stress (Fig. 4.1).

Simulated water stress reduced the energy of emergence of all maize genotypes particularly at the highest level of PEG (9%). Maize cultivars significantly varied (P ≤ 0.001) in energy of emergence at all levels of PEG-induced water stress (Table 4.1). Among cultivars, cv. DTC exhibited greater EG at all concentrations of PEG, whereas cvs. EV-77 and 6621 were the lowest in this seed germination attribute at the highest level of PEG-induced water stress (Fig. 4.1).

Germination tolerance index (GTI) decreased progressively in maize cultivars as the concentration of PEG increased in the growth medium. The cultivars varied in germination tolerance index at varying concentration of PEG (Fig. 4.1). Among cultivars, highest GTI was recorded in EV-78 and EV-79, whereas cv. 6621 was the lowest in this attribute at the 9% PEG-induced water stress (Fig 4.1).
Fig. 4.1. Germination percentage (GP), energy of emergence (EG), germination rate (GR) and germination tolerance index (GTI) of six maize (*Zea mays* L.) cultivars grown at varying PEG$_{6000}$ concentrations (0, 3, 6 and 9%) for 10 days.
Table 4.1. Mean square values of studied traits from analysis of variance (ANOVA) for germination percentage, germination rate (GR), energy of emergence (EG), and germination tolerance index of six maize lines/cultivars grown at varying PEG\textsubscript{6000} concentrations for 10 days.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>GP</th>
<th>GR</th>
<th>EG</th>
<th>GTI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cvs.</td>
<td>5</td>
<td>833.1***</td>
<td>227.1***</td>
<td>1.572***</td>
<td>740.8***</td>
</tr>
<tr>
<td>PEG</td>
<td>3</td>
<td>1017.9***</td>
<td>302.5***</td>
<td>6.182***</td>
<td>3576.2***</td>
</tr>
<tr>
<td>Cvs. × PEG</td>
<td>15</td>
<td>52.93***</td>
<td>6.219ns</td>
<td>0.197ns</td>
<td>36.95ns</td>
</tr>
<tr>
<td>Error</td>
<td>48</td>
<td>12.15</td>
<td>3.462</td>
<td>0.122</td>
<td>112.1</td>
</tr>
</tbody>
</table>

ns = non-significant; *, **, *** significant at 0.05, 0.01, and 0.001 probability levels.

Cvs.=Cultivars; PEG= Polyethylene glycol

Table 4.2. Mean square values from analysis of variance (ANOVA) for seedling vigor index, shoot fresh and dry weight (mg/seedling), root fresh and dry weight (mg/seedling) of six maize lines/cultivars grown at varying PEG\textsubscript{6000} concentrations for 10 days.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Seedling vigor index</th>
<th>Shoot fresh weight</th>
<th>Shoot dry weight</th>
<th>Root fresh weight</th>
<th>Root dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cvs.</td>
<td>5</td>
<td>1688870***</td>
<td>27284.7***</td>
<td>471.9***</td>
<td>18459***</td>
<td>282.8***</td>
</tr>
<tr>
<td>PEG</td>
<td>3</td>
<td>4384007***</td>
<td>38702.2***</td>
<td>230.6***</td>
<td>27333***</td>
<td>257.8***</td>
</tr>
<tr>
<td>Cvs. × PEG</td>
<td>15</td>
<td>128822***</td>
<td>969.2ns</td>
<td>7.491ns</td>
<td>1222*</td>
<td>5.654ns</td>
</tr>
<tr>
<td>Error</td>
<td>48</td>
<td>40822.7</td>
<td>971.8</td>
<td>8.597</td>
<td>588.8</td>
<td>10.66</td>
</tr>
</tbody>
</table>

ns = non-significant; *, **, *** significant at 0.05, 0.01, and 0.001 probability levels. Cvs.=Cultivars; PEG= Polyethylene glycol

Table 4.3. Mean squares from analysis of variance (ANOVA) of the data for shoot length, root length (cm), dry biomass tolerance index (DBTI), plant height tolerance index (PHTI), and root length tolerance index (RLTI) of six maize lines/cultivars grown at varying PEG\textsubscript{6000} concentrations for 10 days.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Shoot length</th>
<th>Root length</th>
<th>DBTI</th>
<th>PHTI</th>
<th>RLTI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cvs.</td>
<td>5</td>
<td>18.85***</td>
<td>51.47***</td>
<td>409**</td>
<td>1110***</td>
<td>543.3***</td>
</tr>
<tr>
<td>PEG</td>
<td>3</td>
<td>29.34***</td>
<td>213.8***</td>
<td>1721***</td>
<td>2801***</td>
<td>4673.3***</td>
</tr>
<tr>
<td>Cvs. × PEG</td>
<td>15</td>
<td>2.36***</td>
<td>7.529***</td>
<td>68.7ns</td>
<td>97.21ns</td>
<td>143.2ns</td>
</tr>
<tr>
<td>Error</td>
<td>48</td>
<td>0.285</td>
<td>2.162</td>
<td>82.74</td>
<td>0.6111</td>
<td>79.4</td>
</tr>
</tbody>
</table>

ns = non-significant; *, **, *** significant at 0.05, 0.01, and 0.001 probability levels.

Cvs.= Cultivars; PEG= Polyethylene glycol
Seedling vigor index of all maize genotypes was severely declined due to PEG-induced water stress. All cultivars had shown significant (P≤0.001) variation in SVI under PEG-induced water stress (Table 4.2). Off all maize genotypes, DTC had greater seedling vigor index at all levels of water stress induced by PEG (Fig. 4.2), whereas the reverse was true for cv.6621 at all levels of water stress. In addition, all remaining maize genotypes were intermediated in this attribute.

Imposition of water stress by PEG caused a drastic reduction in shoot fresh and dry biomass of seedlings of all maize cultivars. Among the cultivars, DTC was the highest in fresh and dry weight while the lowest was cv. 6621 at all level of water stress (Fig. 4.3). Moreover, cv. EV-78 was intermediated in this morphometric attribute at all levels of PEG-induced water stress.

A significant reduction in root fresh and dry biomass of maize cultivars was noticed due to PEG-induced water stress. The adverse effect of PEG-induced water stress on root fresh and dry biomass of all maize cultivars was maximal at 9% PEG. In addition, the reducing effect of PEG-induced water stress was minimal on the root fresh and dry weight of cv. DTC as compared to all other cultivars of maize (Fig. 4.3; Color plate 1).

Shoot and root lengths of all maize seedlings were also reduced with increasing level of PEG in the growth medium (Table 4.3; Fig. 4.3). Cultivars also differed significantly in these growth attribute (P≤0.001). Among cultivars, 6621 was the lowest in having in shoot and root length at the highest level of PEG-induced water stress (9%), whereas cv. DTC was the highest in these attributes (Fig. 4.3).

Since genotypic responses to varying levels of PEG in the growth medium in all these biometric markers varied significantly, these attributes were further assessed based on tolerance indices. It was found that dry biomass stress tolerance index (DBTI) was the lowest for cv. 6621 at 6% and 9% PEG concentration in the growth medium, whereas the all other cultivars were similar in this attribute at all levels of water stress (Table 4.3; Fig. 4.4). At low and moderate level of PEG-induced water stress, cv. EV-78 was the lowest in plant height stress tolerance index (PHTI) as compared to all maize cultivars, whereas at the highest level of water stress cvs. EV-78 and 6621 were the lowest in this attribute. In contrast, root length tolerance index was the highest in cv. Faisalabad mays followed by cv. EV-79 at 9% PEG level (Fig. 4.4), while the remainder cultivars were similar in RLTI at the highest level of PEG-induced water stress.
Fig 4.2. Seedling vigor index (SVI) of six maize (*Zea mays* L.) cultivars grown at varying PEG$_{6000}$ concentrations (0, 3, 6 and 9%) for 10 days.
Fig 4.3. Growth attributes of six maize (Zea mays L.) cultivars grown at varying PEG6000 concentrations (0, 3, 6 and 9%) for 10 days.
Fig 4.4. Dry biomass, plant height, and root length stress tolerance indices of six maize (Zea mays L.) cultivars grown at varying PEG$_{6000}$ concentrations (0, 3, 6 and 9%) for 10 days.
Color plate 1. Germination of six maize (*Zea mays* L.) cultivars grown at varying PEG$_{6000}$ concentrations (0, 3, 6 and 9%) for 10 days.
Experiment No. 2

Photosynthetic traits are the key indicators of physiological performance and tolerance of maize plants under drought cycles.

Fresh and dry weights of shoots and roots, shoot and root lengths of three maize cultivars (DTC, EV-78 and 6621) were significantly ($p \leq 0.001$) reduced due to imposition of four drought cycles (Table 4.4). Maize cultivars also differed significantly (Fig. 4.5; Color plate 2 & 3). Cultivar DTC exhibited the least reduction in fresh and dry weights of shoots and roots after four drought cycles, whereas the reverse was true for cv. 6621 maize. Moreover, shoot and root lengths were maximal in cv. DTC after 4 cycles of water stress than in other maize cultivars (Fig. 4.5). Similarly, cv. DTC had greater number of leaves per plant followed by EV-78, whereas the 6621 showed the minimum number of leaves after four drought cycles (Fig. 4.6).

Leaf water potential ($\Psi_L$) was significantly ($P \leq 0.001$) reduced in the three maize cultivars (DTC, EV-78 and 6621) due to 4 cycles of water stress (Table 4.5; Fig. 4.6). Drought resistant cultivar, DTC had greater $\Psi_L$ whereas cv. 6621 had minimal leaf water potential ($\Psi_L$) after four drought cycles (Fig. 4.6). Total free amino acids and total soluble proteins decreased in drought stressed plants of all maize cultivars examined in the present study (Table 4.5). Maize cultivars also differed significantly in both these biochemical attribute ($P \leq 0.001$) and cv. DTC was higher in both total soluble protein and free amino acids than the other two maize cultivars under water stress conditions (Fig. 4.7).

A marked reduction in total chlorophyll contents in all three maize cultivars was observed when subjected to four 4 cycles of water stress. Maize cultivars also differed in chlorophyll contents under normal or water stress condition, cv. DTC maize exhibited higher chlorophyll contents while the 6621 maize cultivar was the lowest in total chlorophyll contents (Fig. 4.6).

Net CO$_2$ assimilation rate ($A$), internal or sub-stomatal CO$_2$ ($C_i$) concentration and transpiration rate ($E$) decreased markedly ($p \leq 0.001$) due to drought stress in the three maize cultivars (Table 4.6). However, this reducing effect of water stress on $A$ and $C_i$ was minimal on cv. DTC. Maximum reduction in these gas exchange attributes was observed in cv. 6621 after 4 drought cycles (Fig. 4.8). In addition, drought tolerant cv. DTC had lower transpiration but higher $A$ and
$C_i$ values than the other maize cultivars (Fig. 4.8). Due to this reason, drought tolerant cv. DTC had greater water use efficiency ($WUE = A/E$) under severe water stress conditions as compared

![Graphs showing fresh and dry biomass, shoot length and root length of three maize (Zea mays L.) cultivars differing in drought tolerance when 3 week old plants were subjected to various (0, 1, 2 and 4) drought cycles.]

**Fig. 4.5.** Fresh and dry biomass, shoot length and root length of three maize (Zea mays L.) cultivars differing in drought tolerance when 3 week old plants were subjected to various (0, 1, 2 and 4) drought cycles.
Colour plate 2. Growth of three maize (*Zea mays* L.) cultivars differing in drought tolerance when 3 week old plants were subjected to various (0, 1, 2 and 4) drought cycles.
**Colour plate 3.** Comparison in the growth of three maize (*Zea mays* L.) cultivars differing in drought tolerance when plants were subjected to various (0, 1, 2 and 4) drought cycles.
Table 4.4. Mean squares values from analysis of variance (ANOVA) for fresh and dry weights of shoots and roots (g/plant), shoot and root length (Inch/plant) and number of leaves of three maize \((\text{Zea mays L.})\) cultivars differing in drought tolerance when 3 week old plants were subjected to various \((0, 1, 2 \text{ and } 4)\) drought cycles.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Shoot fresh weight</th>
<th>Shoot dry weight</th>
<th>Root fresh weight</th>
<th>Root dry weight</th>
<th>Shoot length</th>
<th>Root length</th>
<th>Number of leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drought cycles</td>
<td>3</td>
<td>72370***</td>
<td>1748***</td>
<td>2097***</td>
<td>70.21***</td>
<td>1447***</td>
<td>9.02***</td>
<td>4.80***</td>
</tr>
<tr>
<td>cvs</td>
<td>2</td>
<td>23908***</td>
<td>379***</td>
<td>3322.3***</td>
<td>43.58***</td>
<td>951.4***</td>
<td>10.59***</td>
<td>13***</td>
</tr>
<tr>
<td>DC*cvs</td>
<td>6</td>
<td>2097.9***</td>
<td>25.99**</td>
<td>27.95ns</td>
<td>3.41**</td>
<td>53.72***</td>
<td>1.40*</td>
<td>0.222ns</td>
</tr>
<tr>
<td>Error</td>
<td>36</td>
<td>67.263</td>
<td>6.72</td>
<td>39.41</td>
<td>0.81</td>
<td>6.030</td>
<td>0.47</td>
<td>0.486</td>
</tr>
<tr>
<td>Total</td>
<td>47</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ns=non-significant; *,**,*** significant at 0.05,0.01 and 0.001 probability levels, respectively.

Table 4.5. Mean squares values from analysis of variance (ANOVA) for total chlorophyll, leaf water potential \((\Psi)\), total free amino acids (mg/g fresh wt.), total soluble protein (mg/g fresh wt.) of three maize \((\text{Zea mays L.})\) cultivars differing in drought tolerance when 3 week old plants were subjected to various \((0, 1, 2 \text{ and } 4)\) drought cycles

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Total chlorophyll</th>
<th>(\Psi_l)</th>
<th>Total free amino acids</th>
<th>Total soluble protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drought cycles</td>
<td>3</td>
<td>131.6***</td>
<td>0.554***</td>
<td>4.476***</td>
<td>1.141***</td>
</tr>
<tr>
<td>cvs</td>
<td>2</td>
<td>378.6***</td>
<td>0.117***</td>
<td>3.788***</td>
<td>0.745***</td>
</tr>
<tr>
<td>Drought cycles*cvs</td>
<td>6</td>
<td>8.859ns</td>
<td>0.075***</td>
<td>0.247***</td>
<td>0.045**</td>
</tr>
<tr>
<td>Error</td>
<td>36</td>
<td>11.40</td>
<td>0.006</td>
<td>0.029</td>
<td>0.011</td>
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<tr>
<td>Total</td>
<td>47</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

ns=non-significant; *,**,*** significant at 0.05,0.01 and 0.001 probability levels, respectively.
Fig. 4.6. Total chlorophyll, leaf water potential (Ψ_L) and number of leaves/plant of three maize (Zea mays L.) cultivars differing in drought tolerance when 3 week old plants were subjected to various (0, 1, 2 and 4) drought cycles.
Fig. 4.7. Total free amino acids (mg/g fresh wt.) and total soluble protein (mg/g fresh wt.) of three maize (*Zea mays* L.) cultivars differing in drought tolerance when 3 week old plants were subjected to various (0, 1, 2 and 4) drought cycles.
Table 4.6. Mean squares values from analysis of variance (ANOVA) for gas exchange properties (Net assimilation of CO$_2$ (A), sub-stomatal CO$_2$ concentration, transpiration rate (E), water use efficiency (WUE) and leaf water potential (Ψ$_L$) of three maize (Zea mays L.) cultivars differing in drought tolerance when 3 week old plants were subjected to various (0, 1, 2 and 4) drought cycles.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>A</th>
<th>Ci</th>
<th>E</th>
<th>A/E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drought cycles</td>
<td>3</td>
<td>106.1***</td>
<td>1899***</td>
<td>0.47**</td>
<td>3.67*</td>
</tr>
<tr>
<td>cvs</td>
<td>2</td>
<td>71.50***</td>
<td>3738***</td>
<td>0.74***</td>
<td>26.68***</td>
</tr>
<tr>
<td>Drought cycles*cvs</td>
<td>6</td>
<td>22.15***</td>
<td>62.53ns</td>
<td>0.02ns</td>
<td>3.04**</td>
</tr>
<tr>
<td>Error</td>
<td>36</td>
<td>3.379</td>
<td>162</td>
<td>0.08</td>
<td>0.84</td>
</tr>
<tr>
<td>Total</td>
<td>47</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ns=non-significant; *, **, *** significant at 0.05, 0.01 and 0.001 probability levels, respectively.
**Fig. 4.8.** Gas exchange properties (net assimilation of CO\(_2\) (A), sub-stomatal CO\(_2\) concentration (Ci), transpiration rate (E), water use efficiency (WUE) differing in drought tolerance when 3 week old plants were subjected to various (0, 1, 2 and 4) drought cycles.

Overall cv. DTC had greater photosynthetic rate which is associated with greater \(C_i\), lower transpiration rate and higher WUE than EV-78 and 6621 maize cultivars.
Experiment No. 3

Chlorophyll a fluorescence of maize (*Zea mays* L.) cultivars differing in degree of drought tolerance

From the fluorescence transients, it is evident that chlorophyll fluorescence over 1 sec decreased in water stressed plants of maize cultivars cv. DTC and cv. EV-78, whereas it increased in cv. 6621 under water stress conditions (Fig 4.9). Since plants have different Fo and Fm values under both control and water stress conditions, chlorophyll fluorescence transients were double normalized and then plotted over log time scale, from where it is found that fluorescence level remained same at OJ and JI steps in all three maize cultivars examined in the present study. However, at IP step fluorescence decreased markedly in only water stressed plants of cv. DTC, whereas in cv. EV-78 and cv. 6621 fluorescence level was almost same. In order to comparative analysis for fluorescence among cultivars under water stress, difference kinetics of double normalized fluorescence was calculated by subtracting double normalized fluorescence of normal plants from double normalized fluorescence of water stressed plants and plotted over a log time scale as OP curve (Fig. 4.10). From OP curve, it is evident that fluorescence in water stressed plants of cv. DTC at OJ, JI and IP steps were minimal and cv. 6621 was maximal in all these steps representing different biophysical events occurring at the thylakoid membranes. In addition, cv. EV-78 remained intermediated in OP curve under water stress conditions (Fig 4.11-Fig. 4.14). To further analyze events at OJ, JI and IP steps, difference kinetics over 50-300 us as L band, 50-2000 µs as K band, 50-200000 µs OV and 30-180 ms as IP bands were calculated. Cultivar DTC had negative values in L band, whereas cv. EV-78 and cv. 6621 have positive values indicating that PSII units were more connected and more efficient in energy transfer from excited chlorophyll molecules to reaction center in cv. DTC than those in cv. EV-78 and cv. 6621. However, cv. EV-78 had lower values than that in cv. 6621 (Fig. 4.15). Similarly, cultivars cv. DTC and cv. EV-78 had negative values in K band, whereas cv. 6621 had positive values which suggest greater intactness of oxygen evolving complex (OEC) with PSII units in cv. DTC and moderately tolerant EV-78 cultivars than the drought sensitive cv 6621. Differential fluorescence at OI phase was highest in drought tolerant (DTC) and lowest in drought sensitive maize (*Zea mays* L.) cultivars (6621). Similarly, fluorescence in IP curves increased in drought sensitive (6621) as compared to drought tolerant (DTC) and moderately tolerant maize cultivars.
(EV-78). These results are indicative of water stress sensitivity in cv. 6621 could be due to more damages to PSII structure.

**Fig. 4.9.** The intensities of raw OJIP chlorophyll a fluorescence kinetics (log t scale) recorded between time interval (1-10⁶ µs) in dark adapted maize (Zea mays L.) cultivars (DTC, EV-78 and 6621) under control (D0) and four drought cycles (D4).

---

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Fig. 4.10. The OJIP chlorophyll a fluorescence transients (log t scale) were double normalized between the extreme phases \( F_O \) and \( F_M \) : 
\[
V_{OP} = \frac{(F_T - F_O)}{(F_M - F_O)}
\]
in dark adapted maize (Zea mays L.) cultivars (DTC, EV-78 and 6621) under control (D0) and four drought cycles (D4).
Fig. 4.11. The OJIP chlorophyll a fluorescence transients showing its intensities recorded between F₀ and Fₖ with time interval (30-300 µs): $V_{OK} = (F_t - F_0)/(F_K - F_0)$ in dark adapted maize (*Zea mays* L.) cultivars (DTC, EV-78 and 6621) under control (D0) and four drought cycles (D4).
Fig. 4.12. The OJIP chlorophyll a fluorescence transients were double normalized between $F_0$ and $F_j$ up to 2000 $\mu$s: $V_{OJ} = (F_t-F_0)/(F_M-F_0)$ in dark adapted maize (*Zea mays* L.) cultivars (DTC, EV-78 and 6621) under control (D0) and four drought cycles (D4).
Fig. 4.13. The OJIP chlorophyll a fluorescence transients were double normalized between $F_0$ and $F_1$ ($\geq 1$) and ($\leq 1$) phases: $V_{O1}(\geq 1) = (F_t-F_0)/(F_{11}-F_0)$ and $V_{O1}(\leq 1) = (F_t-F_0)/(F_{11}-F_0)$ in dark adapted maize (*Zea mays* L.) cultivars (DTC, EV-78 and 6621) under control (D0) and four drought cycles (D4).
Fig. 4.14. The OJIP chlorophyll a fluorescence transients were double normalized between $F_I$ and $F_P$: $V_{IP} = (F_t - F_I)/(F_P - F_I)$ in dark adapted maize (*Zea mays* L.) cultivars (DTC, EV-78 and 6621) under control (D0) and four drought cycles (D4).
Fig. 4.15. Kinetic differences of $V_{\text{OP}}$ [$\Delta V_{\text{OP}} = (F_t - F_O)/(F_M - F_O)$], $V_{\text{OK}}$ [$\Delta V_{\text{OK}} = (F_t - F_O)/(F_K - F_O)$], and $V_{\text{OJ}}$ [$\Delta V_{\text{OJ}} = (F_t - F_O)/(F_K - F_O)$] showing the L-band and the K-band recorded in dark adapted maize (*Zea mays* L.) cultivars (DTC, EV-78 and 6621) under control (D0) and four drought cycles (D4).
Chlorophyll a fluorescence kinetics with its biophysical parameters derived from OJIP analysis

In order to assess biophysical alterations in thylakoid membranes of the three selected maize cultivars due to drought stress a JIP test was performed using data obtained from OJIP curve and presented as percent of control in a radar plot (Fig. 4.16). The percent of control values of the three maize cultivars for chlorophyll fluorescence at O (Fo) and P steps (Fm), variable fluorescence (Fv) and relative variable fluorescence at “J” and “I” steps (V_J and V_I) were significantly higher (at p ≤ 0.05 and 0.001) in water stressed plants of drought sensitive cv. 6621 than in cv. DTC and cv. EV-78 (Fig. 4.16). The data for ratios of chlorophyll fluorescence variables (Fm/Fo, Fv/Fo, Fv/Fm), which depict PSII functional status, showed that Fm/Fo and Fv/Fo were significantly lower in water stress sensitive cultivar 6621 than in cv. DTC and cv EV-78. However, maize cultivars were similar in Fv/Fm or quantum yield of primary photochemistry (φPo or Fv/FM). The Mo, rate of accumulation of closed reaction centers, significantly increased in drought sensitive cv. 6621, whereas it was significantly lower in water stressed plants of cv. EV-78 and cv. DTC. Oxidation reduction of Quinone (QA) and electron carriers were assessed using single turnover (S_S), multiple turnover (S_M), and number of QA redox turnover until FM (N) and all these biophysical attributes significantly increased in water stress sensitive cv. 6621, whereas values of these biophysical attributes were lower in moderately water stress tolerant and water stress tolerant cultivars (Cv. EV-78 and cv. DTC).

All energy fluxes such as absorption fluxes (ABS/RC, ABS/CSo, ABS/CSm), trapping fluxes (TRo/RC, TRo/CSo, TR/CSm), electron transport fluxes (ETo/RC, ETo/CSo/ET/CSm) were increased due to imposition of four drought cycles in water stress sensitive cv. 6621, whereas all energy fluxes were decreased in moderately water stress tolerant and water stress tolerant cultivars cv. EV-78 and cv. DTC with respect to their controls (Fig. 4.17 and Fig. 4.18). Drought stress in terms of four drought cycles significantly (p ≤ 0.05) enhanced the dissipated energy flux per reaction center (DIo/RC) in all maize cultivars, and the greater increase in dissipated energy fluxes were found in drought sensitive maize cultivar (6621). In addition, moderately drought tolerant (EV-78) and tolerant maize cultivar (DTC) were lower in dissipated energy fluxes. Density of reaction centers (Active, inactive, and total) were assessed using RC/ABS, RC/CSo,
RC/CSm and these values decreased due to imposition of four drought cycles (D4). However, densities of active reaction

**Table 4.7.** Fluorescence values, ratios of fluorescence values, JIP-test derived parameters of maize (*Zea mays* L.) cultivars (DTC, EV-78 and 6621) under control and four drought cycles. All the measurements are deduced from the OJIP transients conducted on fully expanded 3<sup>rd</sup> leaf from the shoot apex of the potted plants.

<table>
<thead>
<tr>
<th>Attributes</th>
<th>DTC</th>
<th>EV-78</th>
<th>6621</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Drought</td>
<td>Control</td>
</tr>
<tr>
<td><em>F&lt;sub&gt;o&lt;/sub&gt;</em></td>
<td>3979±169</td>
<td>3352±62.38</td>
<td>3821±99.24</td>
</tr>
<tr>
<td><em>F&lt;sub&gt;J&lt;/sub&gt;</em></td>
<td>11022±521</td>
<td>9099±202</td>
<td>10664±361</td>
</tr>
<tr>
<td><em>F&lt;sub&gt;I&lt;/sub&gt;</em></td>
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<td><em>F&lt;sub&gt;M&lt;/sub&gt;</em></td>
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<td>18296±397</td>
<td>21404±648</td>
</tr>
<tr>
<td>V&lt;sub&gt;F&lt;/sub&gt;</td>
<td>17693±701</td>
<td>14943±358</td>
<td>17582±567</td>
</tr>
<tr>
<td>V&lt;sub&gt;I&lt;/sub&gt;</td>
<td>0.396±0.006</td>
<td>0.384±0.004</td>
<td>0.387±0.007</td>
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<td>V&lt;sub&gt;FM&lt;/sub&gt;/V&lt;sub&gt;F&lt;/sub&gt;</td>
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<td>5.463±0.088</td>
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<tr>
<td>V&lt;sub&gt;I&lt;/sub&gt;/V&lt;sub&gt;M&lt;/sub&gt;</td>
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<td>0.82±0.003</td>
</tr>
<tr>
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<td>948±2.35</td>
</tr>
<tr>
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<td>1.62±0.031</td>
<td>1.72±0.038</td>
</tr>
<tr>
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<td>1.32±0.022</td>
<td>1.41±0.028</td>
</tr>
<tr>
<td>DI&lt;sub&gt;O&lt;/sub&gt;/RC</td>
<td>0.88±0.015</td>
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<td>ABS/CS&lt;sub&gt;O&lt;/sub&gt;</td>
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<tr>
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<td>2738±49.16</td>
<td>3139±81.80</td>
</tr>
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<td>1684±26.07</td>
<td>1921±46.63</td>
</tr>
<tr>
<td>DI&lt;sub&gt;O&lt;/sub&gt;/CS&lt;sub&gt;O&lt;/sub&gt;</td>
<td>732±36.54</td>
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<td>682±21.94</td>
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<tr>
<td>ABS/CS&lt;sub&gt;M&lt;/sub&gt;</td>
<td>21672±858</td>
<td>18296±397</td>
<td>21404±648</td>
</tr>
<tr>
<td>TR&lt;sub&gt;O&lt;/sub&gt;/CS&lt;sub&gt;M&lt;/sub&gt;</td>
<td>17693±701</td>
<td>14943±358</td>
<td>17582±567</td>
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<tr>
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<tr>
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<td>PI&lt;sub&gt;A&lt;/sub&gt;</td>
<td>3.86±0.215</td>
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<td>4.19±0.199</td>
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<td>ψ&lt;sub&gt;o&lt;/sub&gt;/ψ&lt;sub&gt;o&lt;/sub&gt;</td>
<td>0.74±0.013</td>
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</table>
centers were minimal in drought tolerant maize cultivar (DTC), whereas these were maximal in
drought sensitive cv. 6621. Significant changes ($p \leq 0.01$) was observed in performance indexes
including $\text{PI}_{\text{ABS}}$ and $\text{PI}_{\text{csm}}$ by induction of four drought cycles (D4). Comparison among DTC,
EV-78 and 6621 maize ($\textit{Zea mays}$ L.) cultivars revealed that PI was maximal in drought tolerant
cv. DTC and it was minimal in drought sensitive cv. 6621. Moderately drought tolerant cultivar
cv. EV-78 was intermediate in this bio-physical attribute. A summary of phenomenological
fluxes is presented in Fig. 4.16.
Fig. 4.16. Radar plot depicting changes in chlorophyll a fluorescence transients in dark adapted maize (*Zea mays* L.) cultivars (DTC, EV-78 and 6621) under control (D0) and four drought cycles (D4). All the measurements are deduced from the OJIP analysis conducted on fully expanded 3\textsuperscript{rd} leaf from the shoot apex of the potted plants. Data are the mean ± S.E.
Fig. 4.17. Phenomenological leaf model of energy fluxes per excited cross section (at completely closed reaction centres) based on the derived equation reported by (Strasser et al., 2000) for the control and drought stressed leaves of maize (*Zea mays* L.) cultivars (DTC, EV-78 and 6621). The width of arrows represents the value of absorbance (ABS/CSm), trapping flux (TRo/CSm), electron transport (ETo/CSm) and heat dissipation of excess light (DI/CSm), all expressed as per leaf cross section. The black balls designates the ratio of inactive reaction centres.
Fig. 4.18. Energy pipeline models of specific energy fluxes per reaction centres (RC) in the control and drought stressed leaves of maize (Zea mays L.) cultivars (DTC, EV-78 and 6621). ABS, TRo, ETo and DI indicated the absorbance, maximum trapping flux beyond the QA, electron transport and dissipation. Active and inactive reaction centres indicated by the white and black circles.
Experiment No. 4

Assessment of antioxidant potential of selected maize cultivars and protein expression under drought

The banding pattern of total water soluble proteins in the leaves of three maize cultivars (DTC, EV-78 and 6621) subjected to four drought cycles as well as under controlled conditions were analyzed using one dimensional SDS-PAGE. Polypeptides of approximately 22, 33, 39, 55 and 98 kDa were present in all three maize cultivars (DTC, EV-78 and 6621) under control and stress conditions (Fig. 4.19). However, relative quantitative expression varied in three cultivars under both control and drought conditions. In cv. DTC, 22 kDa protein band has weakened under drought stress as compared to control and similarly, this particular band is present in higher concentrations in comparison with rest of the two cultivars i.e., EV-78 and 6621, under control conditions. Interestingly, the same two cultivars showed higher quantities of 22kDa band under drought conditions in comparison with their control plants contradictory to cv. DTC. Moreover, a similar banding pattern was observed again for cv. DTC subjected to water stress and re-watered, which showed a decrease in the abundance of 33 kDa proteins with respect to control conditions. In contrast, an increase in the amount of 33 kDa proteins was observed in both EV-78 and 6621 maize cultivars under the four drought cycles as compared to control condition.

Additionally, the cv. DTC have shown relatively higher amounts of a 39 kDa polypeptide under control conditions while its abundance decreased after the induction of the four drought cycles. On the contrary, cvs. EV-78 and 6621 have increased the abundance of 39 kDa band under the induction of water stress as compared to control condition. Another polypeptide of approx. 55 kDa has shown a different banding pattern both between cvs. as well as between treated and non-treated plants of the respective cvs. e.g., the intensity of this 55 kDa band was higher in cv. DTC than EV-78 and 6621 under control conditions. However, a comparison between control and drought condition clearly showed that cv. DTC maintained the significant amounts of 55 kDa protein even under the water stress conditions. In contrast, cvs. EV-78 and 6621 followed the previous trend of protein banding pattern showing higher amounts in cv. EV-78 and cv. 6621 under water deficit conditions than control.
Figure 4.19 showed the increased intensity of a high molecular weight (98 kDa) protein in cv. DTC than cvs. EV-78 and 6621 under the control conditions; whereas under water stress conditions, cv. 6621 exhibited the enhanced expression of 98 kDa protein band than control conditions. In addition, cv. EV-78 also showed the increased intensity of 98 kDa protein band under the imposition of four drought cycles than control. But protein band of cv. EV-78 was more in quantity than cv. 6621. Moreover, cv. DTC showed less reduction in quantity of 98 KDa protein band under drought stress with respect to control.

Fig. 4.19. One dimensional gel electrophoresis (SDS-PAGE): Comparative analysis of pattern in protein bands of three cultivars of maize under control and four drought cycles. M (marker), Lane 1-3 designates the comparison of three maize cultivars (DTC, EV-78 and 662) under control conditions and Lane 4-6 when cultivars subjected to four drought cycles. Molecular weights of the marker proteins are given in kDa.
Water stress caused an increase in accumulation of H$_2$O$_2$ and malondialdehyde (MDA) in all maize cultivars (Table 4.8; Fig. 4.20). However, this increase in H$_2$O$_2$ and MDA due to water stress was less in water stress tolerant cultivar DTC. Although accumulation of H$_2$O$_2$ due to water stress was higher in water stress sensitive cv. 6621, membrane damage measured as MDA was higher in water stressed plants of EV-78 (moderately water stress tolerant). Similarly an activity of peroxidase (POD) was significantly increased in maize cultivars due to water stress (Fig. 4.20). However, POD activity in water stressed plants of EV-78 and 6621 was greater than in water stressed plants of cv. DTC (Fig. 4.20).
Fig. 4.20. Hydrogen peroxide (H$_2$O$_2$), leaf malondialdehyde (MDA), and peroxidase activity (POD) of three maize (Zea mays L.) cultivars differing in drought tolerance when 3 week old plants were subjected to various (0, 1, 2 and 4) drought cycles.
Table 4.8. Mean square values from analysis of variance of the data for Hydrogen peroxide (H$_2$O$_2$), leaf malondialdehyde, and peroxidase activity (POD) of three maize (Zea mays L.) cultivars differing in drought tolerance when 3 week old plants were subjected to various (0, 1, 2 and 4) drought cycles.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Hydrogen per oxide (H$_2$O$_2$)</th>
<th>Malondialdehyde contents</th>
<th>Peroxidase (POD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drought cycles</td>
<td>3</td>
<td>72370***</td>
<td>1748***</td>
<td>2097***</td>
</tr>
<tr>
<td>cvs.</td>
<td>2</td>
<td>23908***</td>
<td>379***</td>
<td>3322.3***</td>
</tr>
<tr>
<td>Drought cycles*cvs</td>
<td>6</td>
<td>2097.9***</td>
<td>25.99**</td>
<td>27.95ns</td>
</tr>
<tr>
<td>Error</td>
<td>36</td>
<td>67.263</td>
<td>6.72</td>
<td>39.41</td>
</tr>
<tr>
<td>Total</td>
<td>47</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ns=non-significant; *,**,*** significant at 0.05,0.01 and 0.001 probability levels, respectively.
Experiment No. 5

Assessment of yield potential of selected maize cultivars at various moisture regimes under field conditions

Drought stress at the reproductive stage especially during tassel formation in maize (Zea mays L.) hindered the fertilization process because pollens remain immature and thus yield reduction occurs (Gambín and Borrás, 2010). In the present study, kernel yield (kg/ha) and kernel yield components of maize cultivars (DTC, EV-78 and 6621) such as 1000- kernel weight (g), number of kernel number/cob, kernel weight/cob (g), were significantly (p ≤ 0.001) reduced due water stress (Table 4.9). Maize cultivars (DTC, EV-78 and 6621) were also differed significantly in these attributes. Kernel yield was maximally reduced in water stress sensitive cultivar 6621 and relative yield reduction (RYR) in this water sensitive maize cultivar was 75%. In contrast, kernel yield was maximal in water stress tolerant cv. DTC in which relative yield reduction was 48%. In addition, cv. EV-78 remained intermediate in kernel yield and showed 64% relative yield reduction due to water stress (Fig. 4.21). To assess contribution of different yield components in degree of water stress sensitivity, different yield components were assessed.

Thousand-kernel weight (1000-kernel weight) is a significant yield contributing factor, which plays a decisive role in showing the potential of a cultivar under stress environment. Data regarding the 1000-kernel weight (g) revealed that drought cycles significantly reduced 1000-kernal weight in all maize cultivars (Table 4.9). However, the reducing effect of water stress on 1000-kernal weight (kernel size) was minimal in cv. DTC and water stressed plants of cv. 6621 had the lowest 1000-kernal weight (Fig. 4.21). Heavier kernels and broader in size were recorded in DTC followed by the EV-78 under drought stress. While the lighter kernels was observes in the case of 6621 maize cultivar.

Number of kernels per cob is an important materialistic character which contributes towards the final grain yield. Kernel number per cob significantly (p ≤ 0.001) decreased in maize cultivars (DTC, EV-78 and 6621) due to the imposition of drought. The maximum reduction in number of kernels per cob was found in cv. 6621, whereas the reverse was true for cv. DTC. Cultivar EV-78 remained intermediate in this yield attribute under water stress (Table 4.9 ; Fig. 4.21; Colour Plate ).
Table 4.9. Mean squares values from analysis of variance (ANOVA) for kernel weight/cob (g), grain yield (kg ha\(^{-1}\)), 1000-kernel weight (g), and kernel number/cob of three maize (Zea mays L.) cultivars (DTC, EV-78 and 6621) differing in drought tolerance when 3 week old plants were subjected to various (0, 1, 2 and 4) drought cycles a field experiment conducted during January to April, 2013.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Kernel weight/cob (g)</th>
<th>Kernel yield (kg ha(^{-1}))</th>
<th>1000 kernel weight (g)</th>
<th>Kernel number/cob</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drought cycles</td>
<td>1</td>
<td>51543***</td>
<td>103087***</td>
<td>3482***</td>
<td>510125***</td>
</tr>
<tr>
<td>cvs</td>
<td>2</td>
<td>956***</td>
<td>1912***</td>
<td>801***</td>
<td>14156***</td>
</tr>
<tr>
<td>Drought cycles*cvs</td>
<td>2</td>
<td>718**</td>
<td>1437***</td>
<td>238***</td>
<td>15191***</td>
</tr>
<tr>
<td>Error</td>
<td>234</td>
<td>116</td>
<td>113</td>
<td>12.70</td>
<td>1913</td>
</tr>
<tr>
<td>Total</td>
<td>239</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ns=non-significant; *,**,*** significant at 0.05, 0.01 and 0.001 probability levels, respectively.

Table 4.10. Mean squares values from analysis of variance (ANOVA) for kernel number/row of the cob (cob length), number of rows of kernels/cob (cob girth), and cob size (cob length×cob girth), weight of cob with sheath leaves (g), weight of cob without sheath leaves (g), and cob sheath ratio (CSR) of three maize (Zea mays L.) cultivars (DTC, EV-78 and 6621) differing in drought tolerance when 3 week old plants were subjected to various (0, 1, 2 and 4) drought cycles in a field experiment conducted during January to April, 2013.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Cob length (CL)</th>
<th>Cob girth</th>
<th>Cob size (CL × CG)</th>
<th>Wt. of cob with sheath leaves</th>
<th>Wt. of cob without sheath leaves</th>
<th>Cob sheath ratio (CSR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drought cycles</td>
<td>1</td>
<td>5717***</td>
<td>273***</td>
<td>1405312***</td>
<td>75840***</td>
<td>37932***</td>
<td>7653***</td>
</tr>
<tr>
<td>cvs</td>
<td>2</td>
<td>170***</td>
<td>77.7***</td>
<td>56556***</td>
<td>13652***</td>
<td>7543***</td>
<td>1216**</td>
</tr>
<tr>
<td>Drought cycles*cvs</td>
<td>2</td>
<td>63***</td>
<td>0.13ns</td>
<td>1055ns</td>
<td>236ns</td>
<td>6.37ns</td>
<td>116ns</td>
</tr>
<tr>
<td>Error</td>
<td>234</td>
<td>4.38</td>
<td>1.50</td>
<td>1243</td>
<td>163</td>
<td>121</td>
<td>193</td>
</tr>
<tr>
<td>Total</td>
<td>239</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ns=non-significant; *,**,*** significant at 0.05, 0.01 and 0.001 probability levels, respectively.
Fig. 4.21. Effect of four drought cycles on yield and yield components of maize cultivars (DTC, EV-78 and 6621) in a field experiment conducted during January to April, 2013.
Fig. 4.22. Effect of four drought cycles on kernel number/row of the cob (cob length), number of rows of kernels /cob (cob girth), and cob size (cob length×cob girth) of maize cultivars (DTC, EV-78 and 6621) in a field experiment conducted during January to April, 2013.
Fig. 4.23. Effect of four drought cycles on weight of cob with sheath leave, weight of cob without sheath leaves (g), cob sheath ratio (CSR) and grain pith ratio (GPR) of maize cultivars (DTC, EV-78 and 6621) in a field experiment conducted during January to April, 2013.
Data regarding the kernel number per row of the cob (cob length) and number of rows of kernels per cob (cob girth) indicated that drought significantly \((p \leq 0.001)\) reduced these yield attributes in all three maize cultivars (Table 4.10). Cultivars DTC and EV-78 had produced the maximum cob lengths and cob girth than that of 6621 maize cultivar (Fig. 4.22). Thus, derived cob size (cob length \times cob width) was also significantly \((p \leq 0.001)\) greater in cv. DTC and cv. EV-78 than in cv. 6621 under drought stress (Table 4.10; Fig. 4.22).

Similarly, cv. DTC maize cultivar had maximum cob weight including sheath leaves and without sheath leaves followed by EV-78 maize cultivar under drought stress. Whereas, weight of cob with sheath leaves and without sheath leaves was minimum in 6621 maize cultivar (Fig. 4.23). Cob sheath ratio was lower in water stressed plants of all three maize cultivars. However, reduction cob sheath ratio (CSR) was maximal in water stress sensitive (cv. 6621) and moderately water stress tolerant cultivar EV-78. Statistically maximum cob sheath ratio (CSR) was recorded in water stressed plants of cv. DTC (Fig. 4.23).
**Color plate 4.** Overview of the field experiment

**Color plate 5.** Overview of the field experiment
Color plate 6. Yield of drought tolerant maize cv. DTC under control and drought stress
Color plate 7. Yield of moderately drought tolerant maize cv. EV-78 under control and drought stress
Color plate 8. Yield of drought sensitive maize cv. 6621 under control and drought stress
Development of tolerant cultivars to drought stress is a burgeoning field of plant physiology and considered to be the most challenging one. Phenotypic characteristics linked with the physiological developmental traits confer the plants ability to function better under critical stress conditions (Guha et al., 2010; Pathan et al., 2004). Thus, the understanding of the complex physiological, biochemical basis of drought tolerance in plants is practical application in resolving the demand of increasing crop yield. So, the main objective of the present study is to find physiological selection criteria based on mechanism of stress tolerance in selected maize cultivars at different maize plant developmental stages.

Water scarcity greatly influences various stages of crop growth and development (Maes et al., 2009) but juvenile stage is the most sensitive to water stress. Seed germination and seedling growth governs overall success of a crop in terms of yield (Boureima et al., 2011; Lobell et al., 2008; Mahdid et al., 2011; Ribaut et al., 2009). Seed germination is limited by water deficit as water is crucially required for imbibition, subsequent cell division and development of embryonic axes (Govindaraj et al., 2010; Li et al., 2011). In the screening experiment from the present study, it is obvious that increasing water deficit induced by PEG$_{6000}$ reduced germination percentage, germination rate and energy of emergence in all maize cultivars (EV-77, EV-78, EV-79, Faisalabad maize, DTC and 6621). Among cultivars DTC appeared to possess tolerance potential to drought for germination attributes while 6621 seems to lack such ability. These results verified previous findings of various researchers that PEG$_{6000}$ lowers the availability of water for seed imbibition with subsequent adverse effects on embryonic growth. However, such adverse impact on seed germination and seedling growth varies among cultivars of same species and it depends on genetic potential of crop cultivar (Ghiyasi et al., 2008; Gholami et al., 2012; Patade et al., 2009; Rahimi, 2013). For example, in the present study, cvs. DTC and EV-79 had better germination and greater root length in DTC under severe moisture deficit. It is suggested that longer roots might helped in water absorption from the growth medium and thus supported in biomass accumulation under water deficit conditions. Thus, the variability among genotypes under water stress condition as observed earlier (Praba et al., 2009; Puangbut et al., 2010; Seghatoleslami et al., 2007) indicates that drought tolerance can be attained by the alteration
of only those growth attributes which are advantageous for stress hit environment but are under genetic control. Moreover, application of strong selection pressure can help in exploring innate genetic potential of diverse germplasm.

In addition to appropriate selection pressure, various physiological indices are likely to provide insights of sensitivity of crop to water stress. For example, root length tolerance index, shoot length tolerance index and use of other similar indices has widely been reported in the literature (Jajarmi, 2009; Zhang et al., 2010). Cultivar DTC had higher for seedling vigor and dry biomass tolerance indices, whereas root length tolerance index (RLTI) was higher in Faisalabad mays. Similarly, cultivar EV-77 was higher in shoot length tolerance index (SLTI). Thus, differential responses of the cultivars were noticed for various tolerances indices and it is difficult to discriminate cultivars based on single tolerance index. It’s pertinent to mention here that sensitivity of SVI is greater than other tolerance indices. Thus, based on GTI and SVI cultivars were discriminated for water stress tolerance. In previous studies, GTI and SVI were used as selection criteria at the seed germination and seedling growth stages in wheat (Bayoumi et al., 2008; Dhanda et al., 2004; Soltani et al., 2006), maize (de Abreu et al., 2014; Hussain et al., 2013; Ma et al., 2010), rice (Jiang and Lafitte, 2007; Sun et al., 2010), and soybean (Khajeh-Hosseini et al., 2003; Vieira et al., 1991).

Under harsh set of environmental conditions, germination success alone cannot guarantee successful seedling establishment and vice versa. Plant responses to water stress varied significantly at various growth and developmental stages depending upon the severity and duration of stress (Ksouri et al., 2007). In addition to this, the variation in the degree of drought tolerance is structured by the specie specific morpho-physiological response during the different growth stages (Ali et al., 2011; Shamim et al., 2014). Number of studies reported the variation in tolerance to drought act differentially at different growth stages (Guóth et al., 2009; Rajjou et al., 2012; Sečenji et al., 2010; Shamim et al., 2014; Thameur et al., 2012). In the present study, degree of water stress tolerance varied in some of maize cultivars but cv. DTC remained water stress tolerant in terms of biomass production. Similarly, cv. 6621 remained water stress sensitive at the adult vegetative growth stage. It highlights that it is possible to screen and select at the germination stage. From various studies on effect of drought on maize at different developmental stages, it was found that both the time and the degree of stress are also important in determining the degree of water stress sensitivity.
Water stress had a significant detrimental effect on growth of all maize cultivars, which might have been due to reduction in leaf water contents. Medrano et al. (2002) suggested that drought stress reduced the leaf area during leaf expansion period thus reduced photosynthetic performance ultimately reduced the growth. It is suggested that under water stress conditions, cell division and cell enlargement or plant growth become reduced by signal transmitted from root to shoot via xylem (Chaves et al., 2009). This happened even before the reduction in leaf water potential. Roots are mainly responsible for signal transmittance, water uptake and drought tolerance. A considerable amount of genetic variability in different crop cultivars for reduction in growth rate at very early stages has been well documented (Reynolds and Tuberosa, 2008; Tuberosa et al., 2014; Varshney and Tuberosa, 2013). This is also observed in the present study. For example, cv. DTC had greater biomass accumulation with deep root system under drought stress than other cultivars. Similarly, water stress sensitive maize cultivar 6621 showed lower biomass production with very early leaf rolling and wilting symptoms than other maize cultivars. In addition, cv. EV-78 was intermediate in growth, leaf rolling and wilting and root length. From these results, deep root system enabled plants of cv. DTC to explore and uptake more water from drying soil than the other maize cultivars. This deep root system in plants of cv. DTC helped in maintaining plant water status and delayed in leaf rolling and wilting. From the results leaf water potential, it is clear that water stress tolerant cultivar DTC had greater leaf water potential than the moderate or water stress sensitive maize cultivars. If we draw the parallels between leaf water potential and degree of water stress tolerance in maize cultivars, it is obvious that leaf water potential is associated with degree of water stress tolerance in maize cultivars. Deep root system was identified as a key trait in drought tolerance in different crops such as chickpea (Kashiwagi et al., 2015), maize (Grieder et al., 2014), and wheat (Ma et al., 2013). Upon tissue dehydration, plants generally accumulate organic compatible that lower osmotic potential and cause influx of water to maintain turgor potential. This phenomenon is called osmotic adjustment. Osmotic adjustment is one of the main mechanisms of water stress tolerance operating in different crop species. Although we did not measure leaf osmotic potential and turgor potential, it is hypothesized that lowering in leaf water potential in cv. DTC was due to operation of OA that might be helpful in maintaining the growth under water stress condition. These results are similar with some of earlier studies on Jatropha curcas seedlings, an efficient osmotic adjustment occurred at lower $\Psi_w$ (-1.0 MPa) (Silva et al., 2010).
A number of water stress tolerant mechanisms have been evolved in plant species, which include osmotic adjustment (OA). The accumulation of compatible solutes such as soluble sugars, soluble proteins, and amino acids favors OA. Soluble proteins and amino acids play a role in the regulation of osmotic potential of the plant cell (Wang et al., 2004). In the present study, total soluble protein contents and free amino acids increased in drought stressed plants of maize cultivars (DTC, EV-78 and 6621). However cv. DTC was higher in both total soluble protein and free amino acids than the other two maize cultivars. Accumulation of soluble proteins and amino acids are positively associated with leaf water status. Thus, this cultivar maintained its hydrated status by increasing the protein concentration and considered as drought tolerant maize cultivar.

Crops are mainly cultivated for food to feed increasing population of the world. Therefore, the major focus of crop stress tolerance is on yield production in stressful environment. However, crop yield is mainly determined by plant vegetative growth or plant photosynthetic tissue. For example, increase in grain yield in rabi sorghum under drought conditions was positively correlated to increase in growth characters like increase in panicle length and breadth, panicle weight and dry matter (Ghorade et al., 2013). Similarly, morphological traits such as number of leaves and shoot biomass contribute in determining the grain yield of maize and wheat (Anwar et al., 2011; Grzesiak et al., 2013). Similarly, reduced plant height to an optimal limit could be associated to increase the yield potential and concurrently reduce the risk of lodging (Araus et al., 2008). This limitation in plant height may also lead to enhanced partitioning towards the developing cob which in turn might result in increasing number of kernels per cob. From the results of the present study, it is obvious that biomass of shoots, plant height, total number of leaves were higher in drought tolerant cultivar DTC than the other maize cultivars. A positive association between these vegetative characteristics and total yield was also found (Table 5.1) which indicates that higher plant growth determining traits favors higher yield in water stress tolerant maize cultivar DTC than the other. This statement can be supported by the fact that stem length and stem dry weight determined the stem carbohydrate reserves that are important source of carbon during grain filling (Richards et al., 2002). Thus, the final yield is an integral of the growth over the whole season, a trait that influences the ability of the plant to grow during or survive a period of moisture stress may be relatively important in the context of the whole life cycle of plants.

Among various attributes, yield potential is the foremost attribute in assessing drought tolerance. As described earlier, various morphometric attributes and physiological processes are directly translated in yield. However, extent of reduction in yield due to drought
stress depends on duration and intensity of drought stress, plant developmental stage at which plant experiences drought stress and plant genetic potential to cope with drought stress. However, the most damaging impact of drought stress on yield potential of crop plants occurs when crop plants experiences water deficit during reproductive phase as is recently observed in chickpea (Pushpavalli et al., 2015). From the results of the present study, water stress imposed at the vegetative or at the reproductive growth stage reduced maize kernel yield. However, water stress tolerant cv. DTC had higher maize kernel yield and cv 6621 was the lowest in maize kernel yield. These results suggested that greater biomass production favors the higher yield under water limited conditions. A positive correlation between maize yield and each of shoot biomass and number of leaves has been observed (Table 5.1).

A number of studies have demonstrated that vegetative growth of maize determine maize yield by affecting number of kernels and kernel weight. These studies also identified that environmental factors and genotypic potential also influenced the number of kernels and kernel weight (Borrás et al., 2004; Gambín and Borrás, 2010; Gambín et al., 2007; Schoper et al., 1987; Schussler and Westgate, 1995). Drought stress reduced both kernel number and weight, but the kernel number was most affected due to drought. However, mild water deficit at the vegetative growth stage reduce the kernel weight by affecting mobilization of carbohydrate reserve in stem and leaf sheath and it did not reduce the kernel numbers (Farré and Faci, 2009). Whereas, severe water deficit reduced the kernel numbers from 20 to 50% compared to full irrigation (Gambín and Borrás, 2010). In the present study, both number and weight of kernel reduced in the three maize cultivars due to water deficit. These results can be explained in view of the fact that maize is more sensitive to drought stress at the reproductive than other cereals because anthers and the silks are separated at a distance of about one meter and there is more chance of exposure of pollens and stigmas with their surroundings (Bolaños and Edmeades, 1993; Monneveux et al., 2008). Water deficit conditions cause delay in silk growth, increase in anthesis-silking interval (ASI) and reduction of pollen viability that resulted in reduced number of kernels fertilized (Bolaños and Edmeades, 1993). Moreover, adverse effects of water stress on dry matter partitioning to reproductive tissues also resulted in development of lesser number of ovules with subsequent fertilization (Edreira et al., 2014; Gambín and Borrás, 2010; Ji et al., 2010). From the results of the present study and reports published in the literature as mentioned above, it is suggested that differential yield potential of maize genotypes examined in the present study due to one of the above mentioned reasons or combination of these factors. Moreover, cultivar DTC was higher in all these yield attributes which can be attributed to its better genetic potential.
Because degree of adverse effect of water deficit on yield depends on development of number and/or size of ovule depends on number of endosperm cells, metabolic processes involved in endosperm cell division, number of amyloplasts in endosperm cells, starch deposition process, physiological state of vegetative growth, and type of genotype (Commuri and Jones, 2001; Edreira et al., 2014; Farré and Faci, 2009; Gambín and Borrás, 2010). Our results also showed that kernel number per cob decreased due to drought stress and maximum reduction in this yield attribute was found in cv. 6621. Although we did not measure degree of floral abortion, metabolic activity of developing kernels, the conceptual framework allows us to speculate that there was a larger decrease in kernel set (Color plate 9) due to greater sensitivity of fertilization of flowers to develop kernels or ovule development in water stress sensitive cultivar 6621 than the other maize cultivars, whereas in water stress tolerant cv DTC there was a lesser decrease in number of kernel set with better supply of assimilates from the source (photosynthetic tissue) to sink.

Color plate 9. Yield comparison of three maize cultivars under control and drought stress
The amounts of photosynthetic pigments along with amount of photosynthetic tissue in plants are also contributed in plant yield. Photosynthetic pigments i.e., chlorophyll contents transform sunlight energy into useful biochemical energy as ATP and NADPH that are utilized in Calvin cycle to produce carbohydrates (Jaleel et al., 2009). Drought not only degrades chlorophyll directly, but it also reduces chlorophyll content by damaging thylakoid membranes (Anjum et al., 2011; Huseynova et al., 2009; Kannan and Kulandaivelu, 2011). The decrease in Chl content is a commonly observed phenomenon under drought stress (Bijanzadeh and Emam, 2010; Din et al., 2011; Mafakheri et al., 2010). In contrast, Kulshrethta et al. (1987) found no significant effect of drought stress on Chl content in wheat. There are also some reports, which show an enhanced accumulation of Chl under drought stress (Estill et al., 1991; Hamada and MA; Pirzad et al., 2011). From the results of the present study it is clear that water stress reduced the chlorophyll contents in the three cultivars and maize cultivars also differed in chlorophyll contents. Water stress tolerant maize cultivar DTC exhibited higher chlorophyll contents while the maize cultivar 6621 the lowest in total chlorophyll contents. Maximum reduction in chlorophyll content in water stress sensitive cultivar 6621 might have been due reduced protein synthesis or disruption of protein of pigment protein complex encoded by cab gene family or light harvesting pigment protein complex which protects photosynthetic apparatus (Allakhverdiev et al., 2003). These results can be supported in view of already published literature that water stress reduced the chlorophyll contents in plants either by increase in degradation of chlorophyll content or by decrease in rate of biosynthesis of chlorophyll or by combination of these. Water stress degrade the chlorophyll content through singlet oxygen production (Krieger-Liszkay, 2005) entailing the bleaching of chlorophyll. So, higher chlorophyll contents in water stressed plants of water stress tolerant cv. DTC might have been due to better protective mechanism from degradation or higher rate of chlorophyll biosynthesis than the other cultivars.

Photosynthesis is a key physiological process which contributes substantially to plant growth and development by using its chemical form of energy (Flexas et al., 2014; Taiz and Zeiger, 2010). Decline in net CO$_2$ assimilation rate (A) due to imposition of drought stress in maize cultivars (DTC, EV-78 and 6621) is a consequence of stomatal limitations that directly affect the biochemical mechanism. Response of gas exchange characteristics of three maize cultivars were varied under the induction of water stress but the maximum rate of CO$_2$ assimilation (A), transpiration (E) and WUE (A/E) were retained by the stress tolerant cv. DTC. It was stated by the number of studies that closing of stomata is the earliest response to drought, limits water loss through stomates. However, stomatal closure also limits CO$_2$
assimilation rate due to water potential impairment particularly in water stress sensitive plants. Moreover, decline in CO$_2$ assimilation occurs to adjust the mesophyll capacity to the lower supply of CO$_2$ as well as to increase the WUE due to stomatal closure (Anjum et al., 2011). However, different studies stated that drought stress affect the photosynthesis through the stomatal and non-stomatal limitations (Campos et al., 2014; Ghannoum et al., 2003; Petcu et al., 2014). It was suggested that stomatal limitations in A are crucial during the early stages of stress while non-stomatal regulation to photosynthesis linked to the regulation as well as imbalance at the metabolic level and become limiting over a longer period of drought stress (Ramalho et al., 2014). From these reports it is suggested that reduced photosynthetic capacity of maize plants via stomatal limitations due to water stress might have been a strategy to improve water use efficiency, particularly in cv DTC. This is further supported by the fact that crop plants with high water use efficiency during drought stress gave the more yield (Araus et al., 2008).

Reduced CO$_2$ fixation due to stomatal closure under water stress conditions may cause imbalance between photosystem II photochemical efficiency and photosynthetic redox reactions that leads to photo-damage of PSII (Chaves et al., 2009; Gajanayake et al., 2014; Karimi et al., 2014; Sicher et al., 2014). Chlorophyll fluorescence transient is considered as the early responsive quantitative indicator of structure and function of photosystems under stress conditions in plants. Raw chlorophyll a fluorescence transients exhibited typical OJIP polyphasic rise in maize plants under control and water stress conditions. However, these transients varied in terms of variable chlorophyll fluorescence. Moreover, chlorophyll fluorescence at O (Fo) and P steps (Fm), variable fluorescence (Fv) and relative variable fluorescence at “J” and “I” steps ($V_J$ and $V_I$) were higher in water stressed plants of maize plants. Of the maize cultivars, these attributes had greater values in drought sensitive cv. 6621 than in cv. DTC or cv. EV-78. It was seemed that water stress caused a decrease in photosynthetic electron transport beyond QA (Stirbet and Govindjee, 2011). Moreover, it could also be due inactivation of reaction center due to water stress which could slow down the reduction of plastoquinone (QA). PSII structural damage in maize cultivars due to water stress might have been due to reduced energy transfer from the antenna complex to the reaction centers (Kalaji et al., 2011). In the present study, increase in Fo and Vj was much higher in drought sensitive cv. 6621 than the other maize cultivars. These results can be explained in view of the argument of (Redillas et al., 2011) that drought stress caused a greater reduction in electron transport by inactivating more reaction centre of PSII of water
stress sensitive cultivar cv.6621 which leads to increase in accumulation of $Q_A^-$ consequently increase in fluorescence level at J step.

From OP curve, it is evident that fluorescence in water stressed plants of cv. DTC at OJ, JI and IP steps were minimal and cv. 6621 was maximal in all these steps representing different biophysical events occurring at the thylakoid membranes. In addition, cv. EV-78 remained intermediated in OP curve under water stress conditions. The results of negative L band in cv. DTC showed the tolerance potential to prevent the dissociation of LHCII of the PSII complex and maintained the persistence of energetic connectivity under water stress. While the positive L band in cvs. EV-78 and 6621 indicated the loss in energy transfer from the excited chlorophyll molecules to reaction centers under drought stress. These results are in agreement with the findings of (Guha et al., 2013; Oukarroum et al., 2009). Similarly the difference kinetics of $V_{OJ}$ showed the negative K band in cvs. DTC and EV-78 suggesting the ability of these maize cultivars to resist the imbalance of electrons between the donor and acceptor side of PSII due to drought stress. Thus, this exhibited the stability of oxygen evolving complex (OEC) and considered as the drought tolerant (DTC) and moderately tolerant (EV-78) cultivars. While the appearance of positive K band in cv. 6621 indicated the lack of intactness in OEC under induced water stress and considered as the drought sensitive cultivar. As reported earlier that induction of stress condition was the cause of attenuation of K band (Gomes et al., 2012; Strasser et al., 2004).

The increase in IP phase gives an indication of higher electron pool of the final electron acceptors of PSI (Ceppi et al., 2012). This reflects the efficiency of increment with which the electron is transferred the acceptor side of PSI. Similar finding was reported by the (Redillas et al., 2011) and depicted the higher Fe concentration was in the pool of final electron acceptors of the PSI. In contrary to other report of (Jiang et al., 2008) on Citrus grandis L. decrease in IP phase. It may be seemed that under stress conditions increase in capacity of the final electron acceptors to retrieve the more electrons for NADPH production. Moreover it may also possible that all final electron acceptors do not reach to ferredoxin to form the NAPDH in drought sensitive cv.6621. Another work reported that electrons releasing from the reduced ferredoxin coincide to the PSI interact with the ferredoxin dependent enzymes to fix the N$_2$ and regulate the assimilation of CO$_2$ (Fukuyama, 2004). This lowering the electron flux to produce the NADPH. Furthermore, due to overloading of electron transport, there is a diversion of electron flow from the ferredoxin to molecule of oxygen reducing to superoxide ions (Mehler reaction) (Gill and Tuteja, 2010). The OI phase described the kinetic properties give and take of the electrons from the plastoquinone pool.
The ratios of chlorophyll fluorescence variables (Fm/Fo, Fv/Fo, Fv/Fm), which depict PSII functional status, showed that Fm/Fo and Fv/Fo were significantly lower in water stress sensitive cultivar 6621 than in cv. DTC and cv EV-78. However, maize cultivars were similar in Fv/Fm or quantum yield of primary photochemistry (φPo or Fv/Fm) revealed as an important trait to improve the performance of photosynthesis and showed as expeditious tool in screening and the identification of drought resistant genotypes (Silvestre et al., 2014). These results (qualitative and semi-quantitative analysis of OJIP curves, JIP test results) indicated that water stress tolerant cv DTC have better ability to down-regulate photosynthetic electron transport to balance the energy partitioning between photosystems which may cause photo-oxidation. The typical symptoms of photo-oxidation are chlorophyll degradation and reduction in chlorophyll biosynthesis.

Chloroplasts are the major source of production of reactive oxygen species (ROS) in plants (Iturbe-Ormaetxe et al., 1998). Fortunately, chloroplasts are the organelles that have the highest antioxidative protection due to presence of carotenoids, tocopherols, and antioxidative enzymes that scavenge ROS and help to maintain the integrity of photosynthetic membranes thereby minimizing oxidative damage (Foyer et al., 2012; Foyer and Shigeoka, 2011; Noctor and Foyer, 1998). In the present study, activities of antioxidant enzymes increased due to water stress. Moreover, drought tolerant cultivar DTC had relatively higher antioxidant capacity and protection of its photosynthetic apparatus.

All macro and micro nutrients are required for various physiological and biochemical processes which include maintenance of plant water status, osmotic adjustment, photosynthetic activity, protein synthesis etc. In the present study, accumulation of K+ decreased in maize cultivars due to water stress, but the differential accumulation of potassium in maize cultivars cannot be related to their degree of water stress tolerance.

In conclusion, differences in water stress tolerance in maize cultivars was associated with photosynthetic tissue (leaves/shoot biomass), and root growth. Reduction in growth and yield of maize cultivars due to water stress was associated with reduced photosynthetic capacity which in turn depends on non-stomatal factors such as thylakoidal reactions. Plant thylakoidal reactions are better protected by photoprotective mechanisms like down-regulation of electron transport and safer dissipation of absorbed energy as heat. To maintain energy balance between photosystems and Calvin cycle, activities of antioxidant enzymes might have also played a key role in protecting photosynthetic pigments from photo-oxidation. Photoprotective mechanism in maize cultivars is given in phenomenological models.
Identification of traits associated with yield potential and stress adaptation are necessary for plant breeding. The main traits identified in cv DTC is deep root system, better water use efficiency, better protective mechanism from photo-oxidation, better photosynthetic capacity and better utilization or partitioning of food reserves which are translated in better growth and grain yield under limited water resources.
Table 5.1. Correlation among various growth, yield and physiological attributes of three maize (*Zea mays* L.) cultivars (DTC, EV-78, and 6621) when 3 week old plants were subjected to various (0, 1, 2 and 4) drought cycles.

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<th>RDW</th>
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<th>RL</th>
<th>Total chl. leaves no.</th>
<th>A</th>
<th>E</th>
<th>WUE</th>
<th>TFAA</th>
<th>TSP</th>
<th>Ψ&lt;sub&gt;L&lt;/sub&gt;</th>
<th>1000-SW</th>
<th>CSR</th>
<th>KNRC</th>
<th>Yield</th>
<th>KNC</th>
<th>NRKC</th>
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SFW= Shoot fresh weight, SDW= Shoot dry weight, RFW= Root fresh weight, RDW= Root dry weight, SL= Shoot length, RL= Root length, A= Net assimilation of CO2, E= transpiration rate, WUE= water use efficiency, TFAA= total free amino acid, TSP= total soluble protein, Ψ<sub>L</sub>= leaf water potential, 1000-SW= 1000-seed weight, CSR= Cob sheath ratio, KNRC= Kernel number per row of the cob, KNC= Kernel number per cob, NRKC= Number of rows of kernels per cob, WCSL= weight of cob with sheath leaves, WCWSL= weight of cob without sheath leaves.
CONCLUSIONS

The extensive genetic variations were observed in seedling traits of maize cultivars for tolerance and sensitivity to drought stress.

It was found that greater biomass production and root length are the potential indicators to describe the drought tolerance capacity.

Cultivar DTC has greater genetic potential of tolerance against drought stress, whereas cv. 6621 the sensitive to water stress.

Germination and seedling growth stages were more important to determine the drought tolerance capacity in maize. Moreover, degree of water stress tolerance in selected cultivars of maize remained same.

Drought tolerance of maize cultivars at the adult vegetative growth stage was associated to maintenance of plant water status by osmotic adjustment and photosynthetic capacity. Plant photosynthetic capacity of maize cultivars under drought stress was mainly contributed by photosynthetic pigments, photosynthetic rate and water use efficiency (WUE).

Maintenance of plant water status might have been advantageous for maintaining the structural and functional integrity of PSII in tolerant cv. DTC that stabilize the photosynthetic CO$_2$ fixation even under the water limited environment.

Greater efficiency of PSII in drought tolerant cultivar DTC was linked with better photoprotective mechanism of down-regulation of electron flux through PSII.

It was also concluded that performance index (PI$_{ABS}$) could be used as good indicator of drought tolerance in maize cultivars.

The chlorophyll a fluorescence is a rapid and non-invasive technique to understand the changes in thylakoidal reactions under drought stress as well as to use in breeding program for drought tolerance.
The field evaluation confirmed that cv. DTC was also more tolerant to drought stress at the reproductive growth stage than the other cultivars (EV-78 and 6621).

Kernel yield in the three maize cultivars under drought stress is determined by number and size of the kernel. Since number of kernels represents successful fertilization and size of kernel represents grain filling, drought tolerant cultivar DTC was advantageous in these attribute over other cultivars.

Overall, photosynthetic performance of maize cultivars related to growth and yield under drought stress, which will be valuable for future characterization as tool for screening, selection and breeding program.
FUTURE PERSPECTIVES

Drought is one of the major abiotic stresses impairing plant growth and productivity. In view of increasing human population, increase in crop productivity under water limited conditions is a daunting task. Although considerable progress has been made in developing high yielding crop cultivars under water deficit conditions, success in this regard is still far below than target. This will further aggravate the problem of crop productivity. It is therefore necessary to increase crop productivity to feed ever-expanding world population by 2025. The major problem in improving crop productivity under water deficit conditions is lack of understanding of complex mechanism of drought tolerance. With the advent of new physiological and molecular biology based techniques, it is now possible to find out the exact places of damages in plant tissue caused by water stress. In the present study, it was found that root development and plant water status are important determinants for degree of drought tolerance in the examined maize cultivars. However, it is not known whether root length or root branching pattern helped plants of drought tolerant cultivar DTC in maintaining plant water status or simply better root hydraulic conductivity favored in this regard. Recently, it is established that root hydraulic conductivity is associated with water transporters i.e., aquaporins. Thus it is suggested that further studies should be conducted to ascertain whether or not aquaporins based root hydraulic conductivity is greater in cv DTC under water deficit conditions.

The plant water status favors photosynthesis as plant uptake water through roots, translocate it to leaves via xylem, transpire it through stomates while it influx CO$_2$ through stomates. Under water deficit conditions, plants tend to conserve water by stomatal closure at the expense of reduced CO$_2$ fixation and carbohydrate production. In the present study, drought tolerant cultivar DTC had greater ability to maintain leaf water potential by accumulating osmolytes (osmotic adjustment) and fix CO$_2$ at a bit higher rate than the other maize cultivars. Thus, maintenance of plant water status plays a pivotal role in adapting water deficit conditions. Since role of organic and inorganic osmolytes in osmotic adjustment varies, it is suggested that future studies will focus in identifying potential osmolytes such as trehalose, polyol sugars, proline and glycinebetaine that contributes in osmotic adjustment in maize under drought conditions.

In the present study, it is also found that drought tolerant cultivar DTC had a greater potential to fix CO$_2$ which is partially associated with stomatal factors and water use efficiency. A few years back it is established that water use efficiency is controlled from some genomic regions or specific genes. In view
of this information, it is suggested that genomic regions or genes responsible for water use efficiency in said maize cultivar can be identified - a promising avenue for improving water stress tolerance in maize.

In the present study, it was found that kernel yield in maize cultivars determined by number and size of kernels under water stress. The size of the kernel depends on translocation photo-assimilates to sink and sink strength. Both translocation of photoassimilates and sink strength are influenced by available energy and hormonal signaling. Further studies in this area of research using integrated physiological and molecular biology based techniques will be of great help in increasing crop productivity.
Chapter 6
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