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Review Article

Physiological and biochemical basis of drought tolerance in vegetables

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Abstract Among all the abiotic stresses to which plants may be exposed, drought-stress is probably the most limiting on plant distribution and productivity, both in low and high rainfall areas. Understanding the physiological and biochemical responses to drought is essential for a holistic perception of plant resistance mechanisms to water-limited conditions and also to design screening techniques for drought tolerance that may be employed in crop breeding. Plants can respond and adapt to water deficit situation by altering their cellular metabolism and invoking various defence mechanisms. Survival under this stressful condition depends on the plant's ability to perceive the stimulus, generate and transmit the signals, and initiate various physiological and biochemical changes. Responses of different genotypes to water deficit condition have been studied for a long time, and several morphological, physiological and biochemical characters have been suggested to be responsible for drought tolerance in vegetables. In this paper, an endeavour has been made to review the important physiological and biochemical traits that are influenced by drought stress, and may be important indices for identification/screening of drought tolerant genotypes in vegetable crops.

Introduction

Adverse environmental conditions such as drought, high soil salinity and temperature extremes are important abiotic stresses causing severe yield loss to agricultural crops. Environmental stress is the primary cause of crop losses worldwide, reducing average yields for the major crops by more than 50 per cent (Bray *et al.*, 2000). Drought is an inevitable feature of climate that occurs in virtually all climate regimes. Although global figures for the trends in economic losses associated with drought do not exist, an UNDP Bureau of Crisis Prevention and Recovery (2004) indicates that annual losses associated with natural disasters increased from US$75.5 billion in the 1960s to nearly US$660 billion in the 1990s. Agricultural regions affected by drought can experience yield loss up to 50% or more. Over 35% of the world's land surface is considered to be arid or semiarid, experiencing precipitation that is inadequate for most agricultural uses. The threat of global warming may further increase the frequency and severity of extreme climate events in the future (IPCC, 2001). Vegetables are succulent in nature and generally consist of greater than 90% water. Thus, drought stress, particularly at critical growth period may drastically reduce productivity and quality of vegetables (Table 1). Many physiological and biochemical processes essential for plant growth and development are affected by water deficit condition, and plant exhibit various defense mechanisms against drought stress at the molecular, cellular and whole plant levels.

Several mechanisms have been adopted by drought-tolerant plants to adapt water stress including reduction in water loss by increasing stomatal resistance, increases of water uptake by developing large and deep root systems and accumulation of osmolytes. The osmolytes accumulated include amino acids such as proline, glutamate, glycine-betaine and sugars (mannitol, sorbitol and trehalose). These compounds play a key role in preventing membrane disintegration and enzyme

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Table 1: Critical stages of drought stress and its impact on vegetable crops

Vegetable	Critical stage of water requirement	Impact of water deficit
Tomato	Flowering and period of rapid fruit enlargement	Flower shedding, lack of fertilization, reduced fruit size, fruit splitting and development of calcium deficient disorder i.e. blossom end rot (BER)
Eggplant	Flowering and fruit development	Reduces yield with poor colour development in fruits
Chilli and Capsicum	Flowering and fruit set	Shedding of flowers and young fruits, reduction in dry matter production and nutrient uptake
Cabbage and cauliflower	Head/ curd formation and enlargement	Tip burning and splitting of head in cabbage; browning and buttoning in cauliflower
Carrot, radish and turnip	Root enlargement	Distorted, rough and poor growth of roots, strong and pungent odour in carrot, accumulation of harmful nitrates in roots
Cucumber	Flowering as well as throughout fruit development	Deformed and non-viable pollen grains, bitterness and deformity in fruits
Onion	Bulb formation and enlargement	Splitting and doubling of bulb, poor storage life
Okra	Flowering and pod development	Considerable yield loss, development of fibres, high infestation of mites
Melons	Flowering and evenly throughout fruit development	Poor fruit quality in muskmelon due to decrease in TSS, reducing sugar and ascorbic acid, increase nitrate content in watermelon fruit
Lettuce	Consistently throughout development	Toughness of leaves, poor plant growth, tip burning
Pea	Flowering and pod filling	Reduction in root nodulation and plant growth, poor grain fill
Potato	Tuberization and tuber enlargement	Poor tuber growth and yield, splitting
Leafy vegetables	Throughout growth and development of plant	Toughness of leaves, poor foliage growth, accumulation of nitrates

Source: Kemble and Sanders (2000)

inactivation in the low water activity environment. Plants display a variety of physiological and biochemical responses at cellular and whole-organism levels towards prevailing drought stress, thus making it a complex phenomenon. The identification of suitable plant characters for screening large numbers of genotypes in a short time at critical stages of crop growth, with the aim of selecting drought tolerant cultivars, remains a major challenge to the plant breeder.

Physiological parameters

The screening of more number of genotypes for drought tolerance would be accelerated with a greater understanding of physiological traits related to water stress. Many physiological characters responsible for continued growth under water stress have been identified. For example, osmotic adjustment is considered to be an adaptation to drought stress by which an increase in the solute content of cells can lead to maintenance of turgor and turgor-related processes at low water potential (Kumar and Elston, 1992). Variation in water use efficiency (WUE), leaf area, specific leaf area (SLA), leaf area ratio (LAR) and leaf gas exchange (i.e. carbon assimilation (A_N), transpiration, stomatal conductance (gs) and internal CO_2 concentration) in response to water deficit are important parameters to serve as an index for drought tolerance (Anyia and Herzog, 2004). Drought avoidance by maintaining high leaf water content is negatively associated with leaf area as well as SLA. High assimilation rate under water deficit is associated with high relative leaf water content (RWC). Decline in assimilation rate are mainly due to stomata closure, however, evidences of non-stomatal regulation were also found. Instantaneous water use efficiency (iWUE, a molar ratio of assimilation to transpiration) and leaf internal CO_2 (Ci) are negatively correlated, while Ci is moderately related with SLA. Drought tolerant genotypes had the higher leaf water retention, cell membrane stability (CMS), RWC, and the lower Relative Water Loss in comparison to drought sensitive genotypes. The interaction effect of all above parameters depends on growth strategy of the species considered, and their ability to adjust during drought stress. Some of the physiological parameters which are noticeably affected by water deficit conditions are discussed below:

Stomatal conductance and photosynthesis

One of the basic mechanims for reducing the impact of drought is early stomatal closure at the beginning of the period of water deficit. Stomatal closure not only reduces water loss, but also reduces the gas exchange between the plant and the ambient air. The reduced CO_2 intake then results in reduced photosynthesis (Chaves *et al.*, 2002). As plant water potential falls due to water deficit, the sensitivity of stomatal conductance and photosynthesis rate reduced (Table 2). Water deficit causes reduction in photosynthesis mainly due to decreased stomatal conductance. Stomatal closure has been reported in tomato at leaf water potential (ψ_{leaf}) between -0.7 to 0.9 MPa (Duniway, 1971), in pepper -0.58 to -0.88 MPa (Srinivasa Rao and Bhatt, 1988), however eggplant can withstand a greater drought than the most other vegetables. Srinivasa Rao and Bhatt (1990) observed that drop in photosynthesis of eggplant with decreasing ψ_{leaf} was less than tomato and capsicum. Bahadur *et al.* (2009) observed significant reduction in photosynthesis rate and stomatal conductance in spring-summer okra when water stress were imposed for 10 or 12 days (Table - 3). Stomatal conductance is the major limitation to photosynthesis under drought conditions in cowpea; however, a pronounced non-stomatal limitation can occur under severe drought stressed conditions that may also lead to impairment of photosynthetic activity (Singh and Reddy, 2011). If perpetual decline in photosynthesis is more than the transpiration, then non-stomatal factors contribute more to the reduction of photosynthesis than stomatal effects. This is because stomatal resistance accounts for a smaller portion of total resistance in CO_2 pathway. During this non-stomatal control of photosynthesis intercellular resistance for CO_2 from the intercellular space of the chloroplasts plays an important role. Thus, a decrease of the photosynthesis rate under water deficit condition can be attributed to both stomatal and non-stomatal limitations. Non-stomatal photosynthesis limitation has been attributed to the reduced carboxylation efficiency, ribulose-1,5-bisphospate (RuBP) regeneration, amount of functional Rubisco, or to the inhibited functional activity of PSII. Flexas *et al.* (2002) have shown that drought induced changes in many photosynthetic parameter are more related to variations in maximum daily stomatal conductance than to variations in the most commonly used water status parameters, like leaf water potential or relative water content. The drought-tolerant species control stomatal function to allow some carbon fixation at stress, thus improving water use efficiency, or open stomata rapidly when water deficit is relieved. In fact

Table 2: Physiological and biochemical parameters of pea leaves subjected to water deficit (adopted from Iturbe-Ormaetxe *et al.*,1998)

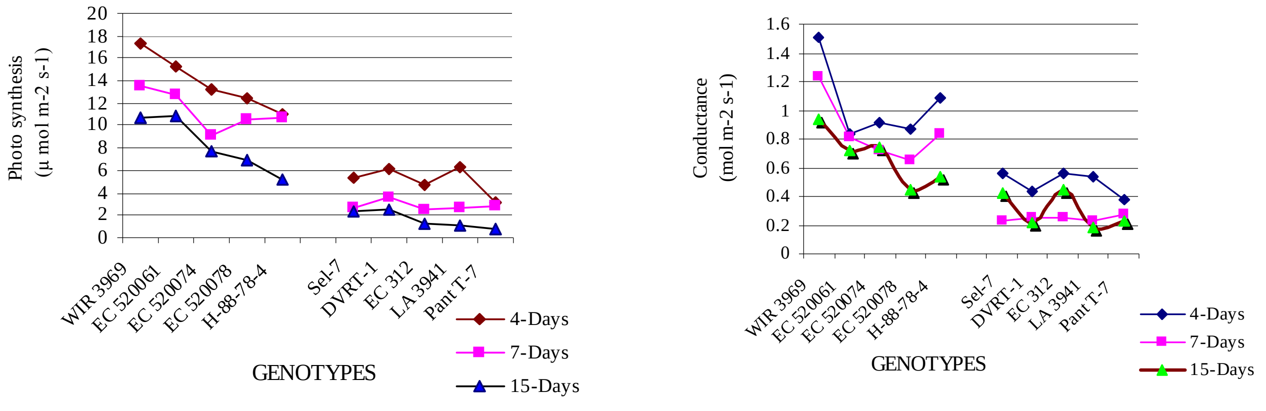
Sl No.	Parameter	Control	Mild drought stress (ψ_{leaf} = -1.3MPa)	Severe drought stress (ψ_{leaf} = -1.9MPa)
Physiological indices				
1	Photosynthesis (µmol CO_2 m^{-2} s-^{1})	8.82^{a}	2.2^{b}	0.18^{c}
2	Stomatal conductance (cm s^{-1})	0.35^{a}	0.05^{b}	0.03^{c}
3	Transpiration (mmol H_2O $m^{-2}s^{-1}$)	2.71^{a}	0.56^{b}	0.43^{b}
4	Relative water content (%)	85.7^{a}	84.1^{b}	78.0^{c}
5	Soluble protein (mg g^{-1} dry wt)	113.5^{a}	105.2^{a}	80.5^{b}
Photosynthetic pigments (µgcm^{-2})				
1	Chlorophyll *a*	25.93^{a}	21.46^{b}	18.14^{b}
2	Chlorophyll *b*	9.82^{ab}	8.93^{bc}	7.78^{c}
3	β-carotene	3.19^{a}	2.4^{bc}	1.98^{c}
4	Neoxanthin	1.65^{a}	1.37^{b}	1.18^{b}
5	Lutein	5.78^{a}	5.23^{ab}	4.45^{b}
6	Violaxanthin	1.51^{a}	0.89^{b}	0.59^{c}
7	Zeaxanthin	0.07^{a}	0.28^{b}	0.4^{c}
Non-enzymatic antioxidants and Pyridine nucleotides (units nmol g^{-1} dry wt)				
1	ASC	18900^{a}	13100^{b}	11400^{b}
2	GSH	2250^{a}	1530^{b}	700^{c}
3	GSSG	100^{a}	110^{a}	90^{a}
4	NAD^{+}	194.7^{ab}	177.4^{c}	157.4^{c}
5	NADH	17.2^{ab}	21.0^{c}	20.2^{c}
6	$NADP^{+}$	56.9^{a}	48.7^{c}	43.2^{b}
7	NADPH	51.2^{a}	38.4^{b}	21.4^{c}
Antioxidant Enzymes (activities in units $min^{-1}g^{-1}$dry wt)				
1	APX (µmol ASC)	61.4^{a}	60.1^{a}	36.8^{b}
2	DR (µmol ASC)	3.21^{a}	3.03^{a}	1.43^{b}
3	MR (µmol NADH)	27.4^{a}	26.1^{ab}	25.3^{b}
4	GR (µmol NADPH)	6.67^{a}	5.8^{b}	3.14^{c}
5	Catalase (mmol H_20_2)	7.03^{a}	5.54^{b}	4.01^{c}
6	SOD (units g^{-1}dry wt)	778^{a}	987^{b}	845^{a}

stomatal conductance can be used as an integrative parameter to reflect the severity of water stress. It is well established that in conditions of moderate water deficit, the photosynthetic apparatus is not damaged but continue to function, however, under severe water deficit, the photosynthetic capacity is reduced which could be reflected in enhancement of the internal CO_2. Under mild water stress, it has been found that grapevine photosynthesis is depressed almost exclusively by stomatal closure, as indicated by increased water use efficiency (i.e. the ratio of photosynthesis to transpiration or stomatal conductance to water vapour) (Cifre *et al.*, 2005). Under moderate drought stress conditions, reduced stomatal conductance (g_s) is the primary cause of photosynthetic inhibition from reduced supply of CO_2 to the intercellular space (Lawlor *et al*, 2002). However under severe water stress situations, non-stomatal inhibition of photosynthesis has been described (Flexas *et al.*, 2004).

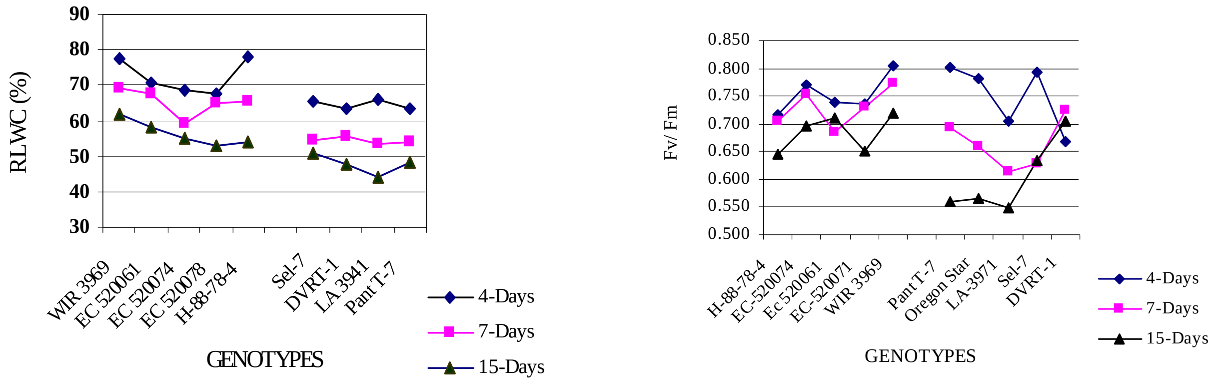
Using stomatal conductance as an integrative parameter for the degree of drought, three phases of photosynthetic response can be differentiated along a water stress gradient, that are shared by different grape cultivars (Flexas *et al.*, 2002).

(1) A phase of mild water stress is defined for a decreasing range of stomatal conductance from 0.5-0.7 to 0.15 mol H_2O $m^{-2}s^{-1}$ in case of grapevine (Cifre *et al.*, 2005). This is characterized by a relatively small decline of net CO_2 assimilation, which results in a progressive increase of intrinsic water-use efficiency and a decline of sub-stomatal CO_2 availability in the mesophyll, the rate of photorespiration increases, which enables the maintenance of the thylakoid electron transport rate (ETR) (Flexas *et al.*, 2002). At this stage, stomatal closure is probably the only limitation to photosynthesis.

(2) A moderate drought stress is characterised by intermediate stomatal conductance values (0.05-0.15mol H_2O $m^{-2}s^{-1}$). During this phase a further reduction in net CO_2 assimilation occurs and water use efficiency (WUE) usually increases however, Naor *et al.*, 1994 reported to decrease in WUE. Ci Naor & Wample (1994) still decreases, but ETR



a) Photosynthesis b) Stomatal conductance



c) Relative leaf water content d) Chlorophyll fluorescence (PS II activity)

Fig. 1 Effect of imposing mild (7 days) and severe stress (15-days) in tomato genotypes under pot experiment (adopted from Bahadur *et al.*, 2010)

Table 3: Effect of imposing drought stress on physiological parameters of spring-summer okra (adopted from Bahadur *et al.*, 2009)

Treatment	ψ_{leaf} (- MPa)	RWC (%)	T Leaf (°C)	A_N (µmole CO_2 /m^2 /s)	g_s (mmole H_2O /m^2 /s)
Irrigation intervals					
4-day	0.91 ^{d}	83.0^{a}	39.2^{c}	14.10^{a}	351.9^{a}
7-day	1.36 ^{c}	78.6^{b}	40.2^{b}	12.35^{b}	293.2^{b}
10-day	1.99 ^{b}	73.5^{c}	40.5^{b}	9.41^{c}	261.1^{b}
12-day	2.68^{a}	66.1^{d}	41.9^{a}	5.69^{d}	86.9^{c}
CD $_{(P\,=\,0.05)}$	0.17	3.68	0.60	1.22	38.52

and the carboxylation efficiency characteristically decline during this phase (Flexas *et al.*, 2002). The decline of carboxylation efficiency is dominated by decreased mesophyll conductance at this stage (Flexas *et al.*, 2002) since the activity of photosynthetic enzymes, such as Rubisco, is mostly unaffected (Bota *et al.*, 2004). Non-photochemical quenching (NPQ), a chlorophyll fluorescence parameter indicative of thermal dissipation in the antenna of PSII, increases under these conditions, and steady state chlorophyll fluorescence drops under high light (Flexas *et al.*, 2002).

(3) A phase of severe drought stress takes place when stomatal conductance is very low (<0.05 mol H_2O $m^{-2}s^{-1}$). During this phase, steeper reduction of net CO_2 assimilation, WUE, ETR and carboxylation efficiency occur. NPQ further increases and the excitation capture of PSII (Fv/Fm) is eventually reduced, especially during very hot days (Flexas *et al.*, 2002). Further, WUE decreases and Ci steeply increases (Flexas *et al.*, 2002), indicating that non-stomatal limitation to photosynthesis become dominant resulting in non-recovery of photosynthesis even after irrigation (Quick *et al.*, 1992).

Mesophyll conductance (g_m) and biochemical limitation (b_l) (often termed as non-stomatal limitations) to photosynthesis mainly under severe water stress has also gained importance in the recent years and their relative importance to photosynthesis limitation has been subjected to longstanding debate. (Keenan *et al.*, 2010) In drought stress, solute potential (g_s) has been shown to relate well and exhibit a specific pattern over almost all the important photosynthetic parameters similarly (Rouhi *et al.*, 2007). Bahadur *et al.* (2010) found promising results for tomato cultivars/genotypes that maintained relatively higher photosynthesis, stomatal conductance, RWC and chlorophyll fluorescence at mild and severe drought stress (Fig. 1). Earlier, Srinivasa Rao *et al.* (1999) also reported that cultivar Arka Meghali has better ability to cope with water stress at various crop stages exhibiting better osmotic adjustment, photosynthesis, RWC and other physiological traits under mild and severe drought stress.

Osmotic adjustment and maintenance of cell turgor

Osmotic adjustment (OA) has been considered as an important physiological adaptation character associated with drought tolerance and it has drawn much attention during the past years. Osmotic adjustment (OA) is defined as the active accumulation of organic solutes in plant tissues in response to an increasing water deficit. It is considered as useful process for maintaining cell turgor when tissue water potential declines. OA has been shown to maintain stomatal conductance and photosynthesis at lower water potentials, delayed leaf senescence and death, reduced flower abortion, improved root growth and increased water extraction from the soil as water deficit develops (Turner *et al.*, 2001). OA involves the net accumulation of solutes in a cell in response to fall in water potential of the cell's environment. As a consequence, the cell's osmotic potential is diminished which in turn attracts water into the cell by tending to maintain turgor pressure. According to Martinez *et al.* (2004) compatible solutes like sugars, glycerol, amino acids such as proline or glycinebetaine, polyols, sugar alcohols (like mannitol and other low molecular weight metabolites) would also contribute to this process. In addition, Hessini *et al.* (2009) argued that these compounds benefit stressed cells in two ways: (1) by acting as cytoplasmic osmolytes, thereby facilitating water uptake and retention and (2) by protecting and stabilizing macromolecules and structures (i.e. proteins, membranes, chloroplasts, and liposomes) from damage induced by stress conditions. Osmotic adjustment allows the cell to decrease osmotic potential and, as a consequence, increases the gradient for water influx and maintenance of turgor. Osmotic adjustment has been assessed as a capacity factor (rate of change in solute potential (ψ_s) with RWC), as described by Kumar *et al.* (1984). Physiological indices such as leaf water potential (ψ_{leaf}), solute potential (ψ_s), relative water content, turgor potential (ψ_p), osmotic adjustment, leaf diffusive conductance (K_l), difference between canopy and air temperature (T_c-T_a) and water loss from excised leaves can be used as a screening tool. A study conducted by Kumar and Singh (1998) on *Brassica* genotypes revealed that higher osmotic adjustment extracted relatively more water from the deep soil layer (90-180 cm) than genotypes with lower osmotic adjustment (ranging from 50 mm to 69 mm). High-osmotic adjustment genotypes maintained full turgor down to a ψ_{leaf} of -2.4 MPa, but turgor potential (ψ_p) fell more rapidly with decreasing ψ_{leaf} in genotypes showing low osmotic adjustment. The decrease in ψ_{leaf} with RWC was smaller in low than high-osmotic adjustment genotypes of *Brassica* species. Osmotic adjustment was linearly, but negatively, related to water loss from leaves and positively related to K_l and T_c -T_a. Plants with higher osmotic adjustment transpired more water (greater K_l) and therefore, had cooler canopies (lower canopy temperature and greater T_c-T_a difference) than the plants with lower osmotic adjustment (Kumar and Singh, 1998). ψ_s in low-osmotic adjustment plants fell linearly and more rapidly with decrease in ψ_{leaf}, whereas it was not related to ψ_s in high osmotic adjustment genotypes (Kumar *et al.*, 1984). The relationship between $Ø_s$ and RWC revealed that high-osmotic adjustment genotypes maintained higher RWC as water deficits increased, with a greater decrease in $Ø_s$. Even at low water potential the leaves maintained greater turgor and this may have contributed to the maintenance of higher K_l and photosynthetic activity. High-osmotic adjustment genotypes maintained higher K_l and transpirational cooling (higher T_c- T_a) but showed lower water loss than low-osmotic adjustment genotypes.

Osmotic adjustment could play a significant role in maintaining turgor potential and turgor-related processes, such as opening of the stomata, photosynthesis, shoot growth and extension of roots in deeper soil layers. Continued root growth leads to greater exploration of soil volume and an enhanced water supply to the plant. Genotypic variability for osmotic adjustment exists in vegetable crops. Srinivasa Rao and Bhatt (1992) noticed better OA in tomato cvs. Arka Saurabh, Pusa Early Dwarf and Sioux, thereby relatively higher yields in these cultivars under moisture deficit condition. Furthermore, Srinivasa Rao *et al.* (1999) reported that OA in four cultivars of tomato did not show any significant variation during first week of drought stress, but after three weeks of stress the maximum OA of ~ 0.17 MPa during flowering stage and ~ 0.47 MPa during fruiting stage was observed in Arka Meghali. During the vegetative stage, better recovery of osmotic potential was observed in RFS-1 followed by Arka Meghali and Pusa Ruby, however, during the fruiting stage, recovery was better in cvs. Pusa Ruby, Arka Meghali and RFS-1.

Chlorophyll fluorescence

Drought stress is known to inhibit photosynthetic activity in tissues due to an imbalance between light capture and its utilization (Foyer and Noctor, 2000). The decrease in the maximum quantum yield of PSII photochemistry F_V/F_M implies a decrease in the capture and conversion rate of excitation energy by PSII reaction centres and so, a reduction in PSII photochemical efficiency indicating the disorganisation of PS II reaction centres under water stress conditions. In general, the harvested energy in excess of that consumed by the Calvin Cycle must be dissipated to avoid oxidative stress and may lead to decreased PSII performance (Wilhelm and Selmar, 2011). F_V/F_M was not affected by drought in Calluna, but a small (1.5%) yet significant decrease was seen in *Deschampsia* across season. Photosystem II (PSII) is highly sensitive to light and down regulation of photosynthesis under drought stress causes an energy imbalance in the PSII reaction centre leading to photoinhibition (Pastenes *et al.*, 2005). Mechanisms have evolved in the plant to protect from photoinhibition, such as non photochemical quenching, transport to molecules other than CO_2, particularly to oxygen, which leads to photorespiration and/or Mehler reaction (Flexas *et al.*, 2002), non-radiative energy dissipation mechanisms (Souza *et al.*, 2004) and chlorophyll concentration changes (Pastenes *et al.*, 2005). However, these processes ultimately lead to the lower quantum yield of PSII (Govindjee *et al.*, 1999). Measurements of F_V/F_M may provide rapid indication of change in current plant productivity in response to water change, and may be a good tool in genetic improvement or programmes enabling genotypes with particular characteristics to be selected at an early stage, but further work is required to examine its potential. Chlorophyll fluorescence measurements allow the discrimination among the tolerant and sensitive genotypes. Under water deficit condition, the tolerant genotypes maintain a higher photosynthetic activity than the sensitive. Studies conducted in tomato by Srinivasa Rao *et al.* (1999) and Bahadur *et al.* (2010) indicated that PSII activity (F_V/F_M) of drought tolerant genotypes was less decreased with imposing water stress than susceptible genotypes.

Water use efficiency (WUE)

WUE is traditionally defined either as the ratio of dry matter accumulation to water consumption over a season or as the ratio of photosynthesis (A) to transpiration (E) over a period of time. It is among one of traits that has been studied a lot because it can give an idea of the variation amongst genotypes in ability to utilize water efficiently under limited water supply. The large assemblage of literature on crop WUE as derived from research on carbon isotope discrimination allows some conclusions on the relations between WUE on the one hand, and drought tolerance and yield potential (YP) on the other. Briefly, apparent genotypic variations in WUE are expressed mainly due to variations in water use. Higher WUE is generally achieved by specific plant traits and environmental responses that reduce YP. Under most dryland situations where crops rely on unpredictable seasonal rainfall, the maximisation of soil moisture use is a crucial component of drought resistance (avoidance), which is generally expressed in lower WUE (Blum, 2005). It is now well documented that high yield potential and high yield under water-limited conditions are generally associated with reduced WUE mainly because of high water use. Features linked to low yield potential, such as smaller plants (Martin and Ruiz-Torres, 1999) or short growth duration (Lopezcastaneda and Richards, 1994) ascribe high WUE because of reduced water use. Genotypic variation in WUE was driven mainly by variations in water use rather than by variations in plant production or assimilation per unit of water use. If low water use is the breeder’s target, it is highly probable that selection for the same can be achieved by directly selecting for these plant traits, without measuring WUE (Blum, 2005).

The enhancement of biomass production under drought stress can be achieved primarily by maximizing soil water capture while diverting the largest part of the available soil moisture towards stomatal transpiration. This is defined as effective use of water, and it is the major engine for agronomic or genetic enhancement of crop production under a limited water condition. High WUE is a critical characteristic of drought-tolerant species, and is a water-saving strategy of plants in arid regions. However, there are many relative physiological traits affecting leaf WUE expressing wide variations in leaf WUE under normal and water stress conditions. Intrinsic water use efficiency (iWUE) estimated as a ratio of photosynthesis/transpiration has been recognized as a measure of carbon gain per unit of water loss and found to be inversely proportional to the ratio of intercellular and ambient CO_2 concentrations (C_i/C_a) (Martin *et al.*, 1992). Large variability in WUE has been reported among several species as well as cultivars within a species including cowpea (Condon *et al.*, 2002). Because higher rates of leaf photosynthesis are often associated with faster crop growth rates, a combination of higher photosynthesis and improved WUE may play a vital role for yield enhancement of crops under drought stress conditions (Parry *et al.,* 2005).

Transpiration as a direct measure of crop water loss

The onset of stress may initially cause a loss of cell turgor which in turn reduces gaseous exchange and leaf elongation since both are turgor-dependent processes. Evapotranspiration (ET) is known to positively correlated with yield of the crops, since it is a direct measure of crop water loss. Water stress causes a decrease in transpiration, an increase in foliage temperature and closure of stomata. Canopy temperature is dependent on climatic factors and internal plant water status. There seems to be a positive link between yield and transpiration rate. Important increases in crop yield might be possible if irrigation water is applied at the most appropriate time to prevent excessive and nutrient leaching. In order to improve irrigation efficiency, it is necessary to adjust the water application rate based on crop ET. Stomata regulated reduction in transpiration is a common response of plants to drought stress which also provides an opportunity to increase plant water-use efficiency. Bahadur *et al.*, (2010) reported that mild or severe water stress in tomato significantly reduced the transpiration rate and increase the leaf temperature.

Dehydration (desiccation) tolerance

Dehydration tolerance is defined as the relative capacity of leaf to sustain or conserve its function in a dehydrated state. It is assumed to be second defence line after dehydration avoidance. A legitimate measure of genetic variation in desiccation tolerance is the comparative function at low tissue RWC. Dehydration tolerance as an effective drought-resistance mechanism in crop plants is rare. It exists in the seed embryo, but once germinated the plant loses its tolerance. Dehydration tolerance requires that the plant enter a quiescent or a dormant state. The limited studies of dehydration tolerance in crop plants revealed that genotypic variation in plant recovery from dehydration, as a measure of tolerance, was positively correlated with plant water status (e.g. RWC) retained during desiccation rather than with a capacity to retain function at a dehydrated state. Chaves *et al.* (2002) investigated Mediterranean vegetation and concluded that differences among species can be traced to different capacities for water acquisition, rather than to differences in metabolism at a given water status. If all the available literature on crop drought resistance is taken together it can be suggested that both natural selection and selection by man have given preference to dehydration avoidance over dehydration tolerance as the major strategy for coping with drought stress, with the exception of resurrection (Blum, 2005). Bahadur *et al.*, (2009 and 2010) noticed significant reduction in leaf RWC in okra and tomato, respectively with imposing drought tolerance.

Cell membrane stability (CMS)

A major impact of plant environmental stress is cellular membrane modification, which results in its perturbed function or total dysfunction. The cellular membrane dysfunction due to stress is well expressed in increased permeability and leakage of ions, which can be readily measured by the efflux of electrolytes, and may be used as a tolerance index for drought stress (Sayar *et al.*, 2008; Yang *et al.*, 2008). The degree of cell membrane injury caused by stress can be assessed using this technique. Electric conductivity of solution containing the electrolytes leaking from leaf segment is used to assess the degree of drought tolerance. The tolerant genotypes show less electrolyte leakage due to maintenance of integrity of cell membrane.

Plant canopy temperature or transpirational cooling

It has been long recognised that leaf or canopy temperature is highly dependent on the rate of transpiration and therefore, can be used as an indicator of stomatal opening. Accordingly, infrared thermometry has been developed as a means for irrigation scheduling. Plant canopy temperature is directly correlated to stomatal conductance and transpiration. As long as the plants continue to transpire through open stomata the canopy temperatures could be maintained at metabolically comfortable range otherwise higher temperature would destroy the vital enzyme activities. Stomatal closures for a considerable period of time are known to increase the leaf temperature. The thermal imagery system is a powerful tool as it can capture the temperature differences of plant canopies fairly quickly and instantly. The trend in canopy temperature and differences in temperatures between canopy and air (T_c–T_a) is an indicator of the plant water stress. The relationships between canopy temperature, air temperature and transpiration is not simple, involving atmospheric conditions (vapor pressure deficit, air temperature and wind velocity), soil (mainly available soil moisture) and plant (canopy size, canopy architecture and leaf adjustments to water deficit). These variables are considered when canopy temperature is used to develop the crop water stress index (CWSI) which is gaining importance in scheduling irrigation in crops. Relatively lower canopy temperature in drought stressed crop plants indicates a relatively better capacity for taking up soil moisture and for maintaining a relatively better plant water status by various plant constitutive or adaptive traits. Besides, it should be noted that canopy temperature is dependent on climatic parameters and internal plant water status. High crop canopy temperature in water-stressed plants may also be related to decreased transpiration rate and leaf water retention capacity values. Drought resistant genotypes show higher values for T_c-T_a. There seems to be a positive link between yield and transpiration rate.

Biochemical parameters

Plants can respond to abiotic stress by altering the biochemical profile of their tissues and producing a diverse array of secondary metabolites. It is widely believed that the synthesis of many secondary metabolites in plants is part of the defence response to stress conditions. Shohael *et al.*, (2006) has shown that lipid peroxidation may be involved in the initiation of secondary metabolites. The accumulation of secondary metabolites arises from a need to protect membrane lipids from oxidative stress, and ROS serve as mediators in the biosynthesis of certain secondary metabolites (Zhu *et al.*, 2009). Oxidative damage generated by drought stress in the plant tissue is alleviated by a concerted action of both enzymatic and non-enzymatic antioxidant systems. These include β -carotenes, ascorbate(ASC), α -tocopherol (α -toc), reduced glutathione (GSH), carotenoids, enzymes including superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidise (APX), catalase (CAT), polyphenol oxidase (PPO) and glutathione reductase (GR). Several studies have been done on the activities of antioxidant

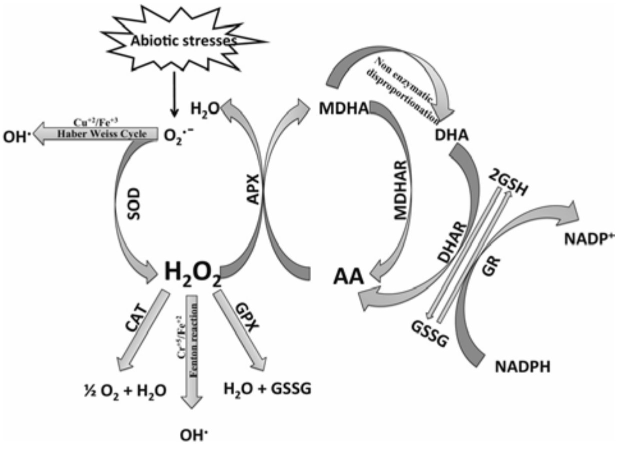


Fig 2. ROS and antioxidants defence mechanism
Source: Gill and Tuteja (2010)

system against drought stress, however, more thorough investigations are required to capitalise the same and evaluate new scope of research.

Reactive oxygen species (ROS)

ROS are partially reduced forms of atmospheric oxygen. They typically result from the excitation of O_2 to form singlet oxygen (O_2^1) or from the transfer of 1, 2 or 3 electrons to O_2, for superoxide radical (O_2^-), hydrogen peroxide H_2O_2 or a hydroxyl radical ($OH^.$), respectively. The cells are normally protected against ROS by the operation of the antioxidant defence system comprising enzymatic (SOD, CAT, GR, APX POD, PPO) and non-enzymic (ascorbate, α-tocopherol, carotenoids, glutathione) components. The activities of enzymes of the antioxidant system in plants under stress are usually regarded as an indicator of the tolerance of genotypes against stress conditions (Iturbe-Ormaetxe *et al*., 1998). Overall, the involvement of ROS in various metabolic processes in plant cells might have general implications.

Drought stress enhances the production of ROS in cellular compartments such as chloroplasts, peroxisome and mitochondria. ROS causes the peroxidation of membrane lipids, the denaturation of proteins and damage to nucleic acids (Mittler, 2002). If drought stress is prolonged, ROS production will overwhelm the scavenging action of the antioxidant system, resulting in extensive cellular damage and death. ROS are highly deleterious by-products of stress, and are likely to be important secondary messengers that trigger adaptation responses to the changing environment (Cruz de Carvalho and Contour-Ansel, 2008). Drought stress induces the formation of active oxygen species by misdirection of electrons in the true photosystems.

Superoxide dismutases (SOD)

The SODs catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. In higher plants, SOD isozymes are present in different cell compartments. Mn-SOD is present in mitochondria and peroxisomes, Fe-SOD mainly in chloroplasts, and Cu/Zn-SOD in cytosol, chloroplasts, peroxisomes and the apoplast. In green leaves, the majority of the SOD activity is present in plastids. Superoxide radicals are considered to be formed in various physiological processes under conditions of water stress. One important process is photosynthesis. An increased SOD activity by drought stress is considered to antagonize harmful actions of superoxide radicals and this indicates that higher activities of SOD are important for drought tolerance. Drought-tolerant cultivars of tomato have higher (30-40%) SOD activities under drought stress conditions (Lutfor *et al*., 2002). Increased SOD activities were observed early in plant development, even in 5-day old seedlings of tomato. Studies indicated that tomato cultivar difference of the increase in SOD activities by drought stress is remarkable and generally independent of plant age and ambient temperatures. Even at the early seedling stages, simple water stress treatment induces cultivar differences. This suggests that the defence system against superoxide radicals becomes active at a very young growth stage in tomato (Lutfor *et al*., 2002). This may enable the screening procedure for SOD to be initiated at earlier stages of plant development, which will save time, space and labour. SOD activities under short-term water stress can be taken as an extremely useful step in a breeding for drought tolerance. In addition, it has been suggested that there is a possibility for using SOD activities for plants grown under normal, non-stress conditions as a criterion for initial screening for tomato drought tolerance.

Catalases (CAT)

CATs are tetrameric heme containing enzymes with the potential to directly dismutate H_2O_2 into H_2O and O_2 and are indispensable for ROS detoxification during stressed conditions. CAT has one of the highest turnover rates for all enzymes: one molecule of CAT can convert 6 million molecules of H_2O_2 to H_2O and O_2 per minute. CAT is important in the removal of ROS generated in peroxisomes by oxidases involved in β-oxidation of fatty acids, photorespiration and purine catabolism. The CAT isozymes have been studied extensively in higher plants. CAT isozymes have been shown to be regulated temporally and spatially and may respond differentially to light (Skadsen *et al*., 1995). It has also been reported that apart from reaction with H_2O_2, CAT also react with some hydroperoxides such as methyl hydrogen peroxide (MeOOH) (Ali *et al*., 2006).

Ascorbate peroxidases (APX)

APX is assumed to play the essential role in scavenging ROS and protecting cells. APX is involved in scavenging of H_2O_2 in water stress and ASH-GSH cycles and utilizes ASH as the electron donor. The APX family consists of at least five different isoforms including thylakoid (tAPX) and glyoxisome membrane form (gmAPX), chloroplast stromal soluble form (sAPX), cytosolic form (cAPX) (Noctor *et al*., 1998). APX has a higher afinity for H_2O_2 (μM range) than CAT and POD (mM range) and it may have a more crucial role in the management of ROS during stress. Enhanced expression of APX in plants has been demonstrated during different stress conditions. Sharma and Dubey (2005) found that mild drought stressed plants had higher chloroplastic-APX activity than control grown plants but the activity declined at the higher level of drought stress.

Glutathione reductases (GR)

GR is a flavo-protein oxidoreductase, found in both prokaryotes and eukaryotes (Romero-Puertas *et al.*, 2006). It is a potential enzyme of the ASH-GSH cycle and plays an essential role in defence system against ROS by sustaining the reduced status of GSH. It is localized predominantly in chloroplasts, but small amount of this enzyme has also been found in mitochondria and cytosol (Edwards *et al.*, 1990). GR catalyzes the reduction of glutathione (GSH), a molecule involved in many metabolic regulatory and antioxidative processes in plants, e.g., GR catalyses the NADPH dependent reaction of disulphide bond of oxidized glutathione (GSSG) and is thus, important for maintaining the GSH pool (Chalapathi Rao and Reddy, 2008). Actually, GSSG consists of two GSH molecules linked by a disulphide bridge which can be converted back to GSH by GR. GR is involved in defence against oxidative stress, whereas, GSH plays an important role within the cell system including participation in the ASH-GSH cycle, maintenance of the sulfhydryl (eSH) group and a substrate for GSTs (Reddy *et al.*, 2006). It was suggested that GR plays an important role in the regeneration of GSH and thus protects against oxidative stress also by maintaining the ASH pool (Ding *et al.*, 2009).

Peroxidases (PODs)

PODs are a large family of enzymes that detoxify hydrogen peroxide, organic hydroperoxides or lipid peroxides to generate alcohols. PODs contain a heme cofactor in their active sites synthesized in the plastid. Heme is also coupled to the iron homeostasis, which may play an important role in plant/microbe interaction. In addition, PODs contain redox-active cysteine residues, which directly measure the redox potential in the cell or organelle. The most important organelle in a green leaf that controls the redox potential in the cell is the plastid. Peroxidases are involved in the defence against drought stresses by means of their role in the detoxification of AOS in the apoplast of lignifying tissues (Passardi *et al.*, 2004). The production of AOS, through modification of cell wall structure, could play a role in cell volume and turgor regulation after drought stress. It should be noted that POD are also responsible for cell wall lignification and other cell wall stiffening processes which conclude in the maturation of the cell wall. POD activity in shoots of the *Juniperus oxycedrus* was enhanced by drought stress by about 250% higher than under well-watered conditions (Roldan *et al.*, 2008).

Polyphenol oxidases (PPOs)

Polyphenol oxidases (PPOs) catalyze the O_2-dependent oxidation of mono- and o-diphenols to o-diquinones, highly reactive intermediates whose secondary reactions are believed to be responsible for the oxidative browning which accompanies plant senescence, wounding, and responses to pathogens (Friedman, 1997). Mehler reaction activity helps prevent over-reduction of components of linear electron transport under conditions of water stress when carbon assimilation, a major sink for the products of linear electron transport, declines. Under drought stress where photosynthesis is reduced, the Mehler reaction may provide a non destructive sink for absorbed light energy not used in photochemistry. If so, drought-stressed plants with suppressed PPO are expected to exhibit photooxidative damage and plants with elevated PPO may show increased stress tolerance.

Glutathione (GSH)

Glutathione (GSH, g-glutamyl-cysteinyl-glycine) is an abundant low-molecular-weight thiol metabolite and a major antioxidant in plant cells. Glutathione is present in most eukaryotic organisms, where it performs multiple functions. These include storage and transport of sulphur (Macnicol and Bergmann, 1984) and control of the redox status. GSH is a strong reductant that can scavenge toxic reactive oxygen species (ROS) directly or in cooperation with other antioxidants and ROS-processing enzymes. It seems likely that GSH levels are used as a cue in the coordination of mechanisms for both the supply of cysteine and the maintenance of the cellular NADPH pool. Coordination of these responses, merely by lowering the GSH pool, provides plants with a simple mechanism to respond defensively to a wide range of stresses through a coordinated upregulation of the capacity for GSH biosynthesis and its redox cycling.

Ascorbate (ASC)

Ascorbic acid is the most abundant, powerful and water soluble antioxidant acts to prevent or in minimizing the damage caused by ROS in plants. It occurs in all plant tissues, usually being higher in photosynthetic cells and meristems. Its concentration is usually higher in mature leaves with fully developed chloroplast and highest chlorophyll. It has been reported that ASC mostly remain available in reduced form in leaves and chloroplast under normal physiological conditions (Smirnoff, 2000). About

30 to 40% of the total ascorbate is in the chloroplast and stromal concentrations; upto the level of 50 mM have been reported (Foyer and Noctor, 2005). As an antioxidant, ascorbic acid has an important role in protecting against oxidative stress. Ascorbate eliminates ROS through multiple mechanisms. ASC has the capacity to directly eliminate several different ROS including singlet oxygen, superoxide and hydroxyle radicals. It also maintains the membrane-bound antioxidant α -tocopherol in the reduced state and indirectly eliminates H_2O_2 through the activity of APX. The function of the lipophilic antioxidant, α -tocopherol, is to protect membranes from oxidative damage. It is located primarily in thylakoid membranes and is therefore directly involved in the defence of chloroplast to oxidants.

α -*Tocopherols*

Tocopherols, a lipid soluble antioxidant are considered as potential scavengers of ROS and lipid radicals (Czytko *et al*., 2005). Tocopherols are considered as a major antioxidant in biomembranes, where they play both antioxidant and non-antioxidant functions. Tocopherols are considered general antioxidants for protection of membrane stability, including quenching or scavenging ROS like $^1O_2$. They are localized in plants in the thylakoid membrane of chloroplasts. Out of four isomers of tocopherols (α -, β -, γ -, δ -) found in plants, α -tocopherol has the highest antioxidative activity due to the presence of three methyl groups in its molecular structure. It is synthesized from ã-tocopherol in chloroplasts by γ -tocopherolmethyltransferase. Increased levels of α - tocopherol and ASH have been found in tomato following trizole treatment which may help in protecting membranes from oxidative damage and thus chilling tolerance in tomato plants (Shao *et al*., 2007).

Hydrogen peroxide (H_2O_2)

H_2O_2 plays a dual role in plants, at low concentrations, it acts as a signal molecule involved in acclamatory signal triggering tolerance to various biotic and abiotic stresses and, at high concentrations, it leads to program cell death. H_2O_2 has also been shown to act as a key regulator in a broad range of physiological processes, such as senescence (Quan *et al*., 2008) photorespiration and photosynthesis (Noctor and Foyer, 1998) stomatal movement (Bright *et al*., 2006) cell cycle (Mittler, 2002) and growth and development (Foreman, 2003). H_2O_2 is starting to be accepted as a second messenger for signals generated by means of ROS because of its relatively long life and high permeability across membranes. Yushi *et al*., (2011) considered two mechanism by which H_2O_2 can help soybean plant to sustain under water deficit. First mechanism was the rapid stomatal closure after H_2O_2 spraying. Over the past several years, considerable progress has been made towards understanding the essential role of H_2O_2 in ABA-induced stomatal closure. Stomatal closure significantly decreases the transpiration rate and thus contributes to maintaining positive turgor pressure of the leaf cells. The second mechanism considered was the induction of compatible-solute synthesis by H_2O_2 spraying. It is assume that H_2O_2 directly regulates the expression of numerous genes involved in plant defence and the related pathways such as antioxidant enzymes, defence proteins and transcription factors.

Malondialdehyde (MDA)

The peroxidation of lipids is considered as the most damaging process known to occur in every living organism. Membrane damage is sometimes taken as a single parameter to determine the level of lipid destruction under various stresses. Now, it has been recognized that during lipid peroxidation products are formed from polyunsaturated precursors that include small hydrocarbon fragments such as ketones, MDA, etc and their related compounds (Garg and Manchanda, 2009). MDA is synthesized due to degradation of polyunsaturated lipids by ROS. The production of this aldehyde is used as a biomarker to measure the level of oxidative stress (Moore and Roberts, 1998). Increased MDA accumulation has been correlated with reduction of RWC and photosynthetic pigment content under prolonged drought. According to Smirnoff (1993), low water availability is often associated with increased levels of reactive oxygen species (ROS) that cause the peroxidation of membrane lipids, the denaturation of proteins and damage to nucleic acids. Lower MDA displays higher anti-oxidative ability, reflecting higher resistance to drought.

Carotenoids

Carotenoids are a large class of isoprenoid molecules, which are *de novo* synthesized by all photosynthetic and many non-photosynthetic organisms. They are divided into the hydrocarbon carotenes, such as lycopene and β -carotene or xanthophylls, typified by lutein. Carotenes form a key part of the plant antioxidant defence system, but they are very susceptible to oxidative destruction. β -carotene, present in the chloroplasts of all green plants is exclusively bound to the core complexes of PSI and PSII. Protection against damaging effects of ROS at this site is essential for chloroplast functioning. In addition to the function of an accessory pigment, β-carotene acts as an effective antioxidant and plays a unique role in protecting photochemical processes and sustaining them. A major protective role of β-carotene in photosynthetic tissue may be through direct quenching of triplet chlorophyll, which prevents the generation of singlet oxygen and protects from oxidative damage. Unyayar *et al.* (2005) found decreased chlorophyll *a*/*b* ratio in drought tolerant *Lycopersicon peruvianum* and concluded that this trait indicates better protection of PSII against drought stress. An increase in carotenoids/chlorophyll ratio might be of a protective value as carotenoids are known to be potent quenchers of ROS, particularly singlet oxygen.

Proline

Proline accumulation in leaves of drought-stressed plants and its role as an osmolyte or osmoprotectant has been abundantly documented (Pagter *et al.*, 2005). Although proline has long been considered as a compatible osmolyte, recent results highlight its multiple functions in stress adaptation, recovery and signalling. Proline accumulation due to drought stress results from a stimulated synthesis, inhibited degradation or an impaired incorporation of proline into proteins (Heuer, 1999). Moreover, proline plays a more complex role in conferring drought tolerance than in acting as a simple osmolyte (Szabados and Savoure, 2009). It may protect proteins structure by maintaining their structural stability (Rajendrakumar *et al.*, 1994), act as free radical scavenger (Reddy *et al.*, 2004), and be involved in the recycling of $NADPH^+$ H^+ via its synthesis from the glutamate pathway (Hare and Cress, 1997) involving g-glutamyl kinase, glutamyl phosphate reductase and D–pyrroline-5-carboxylate synthetase in tomato. Proline synthesis may provide some protection against photoinhibition under adverse conditions by restoring the pool of the terminal electron acceptor of the photosynthetic electron transport chain (Szabados and Savoure, 2009). In *Laurus*, beside its implication in osmotic adjustment, the stress-induced accumulation of proline in some population could also be related to drought-induced modification of cell wall proteome (Maatallah *et al.*, 2010). It may also function as a protein-compatible hydrotrope, alleviating cytoplasmic acidosis, and maintaining appropriate NADP/NADPH ratios compatible with metabolism (Hare & Cress 1997). Also, rapid breakdown of proline upon relief of stress may provide sufficient reducing agents that support mitochondrial oxidative phosphorylation and generation of ATP for recovery from stress and repairing of stress-induced damages.

Glycinebetaine (GB) and other osmolytes

Free amino acid accumulation is more important to account for most of the changes in osmotic potential. The accumulation of free amino acids under stress at all the growth stages indicates the possibility of their involvement in osmotic adjustment. Aliphatic QAC (quaternary ammonium compound) such as GB, stachydrine, homostachydrine, trigonelline have been found to accumulate in a large number of plants exposed to drought stress. Glycinebetaine is synthesized in chloroplasts with the help of two enzymes, choline monoxygenase and betaine aldehydehydrogenase,. The accumulation of GB might serve as an intercellular osmoticum of GB and could be closely correlated with elevation of osmotic pressure. GB may maintain the osmoticum, provided that the basal metabolism of the

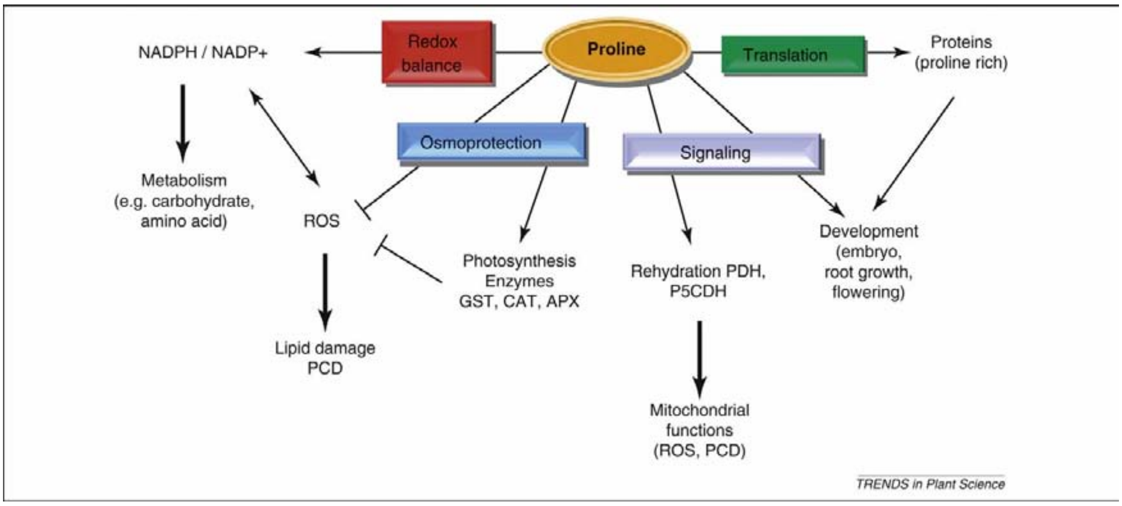


Fig 3: Multiple functions of proline in plants. (Adopted from Szabados and Savoure, 2009)

plant can sustain a high rate of synthesis of these compounds to facilitate osmotic adjustment for tolerance to drought stress (Kavi Kishore *et al.*, 1995).

Soluble sugars

The accumulation of soluble sugars (sucrose, glucose and fructose) is strongly correlated to the acquisition of drought tolerance in plants. It is well known that sugars protect the cells during drought by two mechanisms. First, the hydroxyl groups of sugars may substitute for water to maintain hydrophilic interactions in membranes and proteins during dehydration. Thus, sugars interact with proteins and membranes through hydrogen-bonding, thereby preventing protein denaturation. Secondly, sugars are a major contributing factor to vitrification, which is the formation of a biological glass in the cytoplasm of dehydrated cells. These intracellular glasses, by virtue of their high viscosity, drastically reduce molecular movement, impede the diffusion of reactive compounds in the cell, and may maintain the structural and functional integrity of macromolecules. It is by this property that glasses are thought to prolong the longevity of desiccated tissues by slowing down degradation processes during storage. Soluble sugars, especially sucrose, accumulate in seeds, pollen and in drought-tolerant vegetative tissues. For example, in *Craterostigma plantagineum*, 2-octulose stored in the hydrated leaves is converted to sucrose during drying to such an extent that in the dried state it comprises about 40% of the dry weight (Norwood *et al.*, 2000). In many higher plants under dehydration stress, carbohydrate metabolism is shifted to favour the conversion of other sugars to sucrose (Whittaker *et al.*, 2001). Trehalose, a non-reducing sugar, is also a potential organic osmoticum which has a substantial role in the protection of plants against drought stresses. Mannitol, a polyol, is one of the most important osmoprotectants that play a vital role in plant stress tolerance. Attempts have been made to achieve improved drought tolerance by the over-expression of mannitol in plants by engineering genes involved in the biosynthesis of mannitol and trehalose (Karakas *et al.*, 1997; Karim *et al.*, 2007).

LEA Proteins

Late embryogenesis abundant (LEA) proteins may accumulate in response to drought stress in plants and play an important role in plant protection against the adverse effects caused by drought stress (Gosal *et al.*, 2009). The putative role of LEA proteins in plant drought tolerance has been suggested to be due to their involvement in the maintenance of cell membrane structure and ion balance, binding of water, and their action as molecular chaperones.

Abscisic acid (ABA)

Plant hormones regulate stress responses not via linear pathways, but through complex molecular networks. Abscisic acid (ABA), a terpenoid phytohormone, is involved in the regulation of many aspects of plant growth and development including in seed maturation processes, acquisition of desiccation tolerance and dormancy, leaf senescence and stomatal aperture (Wasilewska *et al.*, 2008). ABA is also the key hormone that confers tolerance to environmental stresses such as drought, thereby permitting plants to cultivate where water availability is limited or sporadic. Endogenous ABA concentration is influenced by environmental conditions. ABA concentration was reported to increase up to 30-fold during drought stress (Outlaw, 2003). ABA synthesized in response to drought stress, is known to induce stomatal closure and to reduce transpirational water loss. Levels of endogenous ABA increase in tissues subjected to osmotic stress due to desiccation. ABA activates the synthesis of ROS in guard cells by a membrane-bound NADPH oxidase, and ROS mediate stomatal closure by activating (through hyperpolarization) plasma membrane Ca^{2+} channels. It can result in the increased generation of active oxygen species (AOS), enhances the activities of antioxidant enzymes such as SOD, CAT, APX and GR. However, details about the interaction between ABA, AOS and antioxidant response remain to be determined. ABA can vary the activities of antioxidant enzymes in response to drought stress as demonstrated by Unyayar *et al.*, (2004) in tomato crop.

Future thrust

Moisture stress is one of the greatest factors in reducing yield in the arid and semi-arid tropics. Since the period of drought stress under variable environments is unpredictable, generalization on the effects of stress on grain yield is difficult. Increased efficiency of water use within agricultural systems is an important priority in arable lands of many regions in the world. The growing threat of dwindling water resources demands germplasm that carry drought tolerance and water-use efficiency, as for most areas, both of these traits are desirable. Research must combine the latest genomics resources including quantitative genetics, genomics along with physiological and biochemical understandings of the interactions between crop plant genotypes and the growing environment to better inform crop improvement.

Screening for drought tolerance under naturally occurring drought stress may not be always reliable. Selections exercised for physiological traits under controlled environments inducing drought may be more to distinguish between tolerant and susceptible genotypes, particularly at flowering, fruiting or grain filling stages in crop plants. Greenhouse methods may also be applied for screening genotypes at early stages of plant development in many crop species. Efforts have been focused on the genetic analysis of drought tolerance through identification of markers/quantitative trait loci (QTL) with effects on traits related to drought tolerance. However, QTLs with effects on drought tolerance have not yet been identified in many important vegetable crops and thus, it is of utmost importance to analyse genomic regions responsible for drought stress and manipulate accordingly to develop tolerant genotypes applying modern breeding approaches such as marker assisted selection (MAS). The application of new technologies such as DNA arrays and proteome analysis should be employed to reveal possible functions of different biochemical indices involved in water stress conditions.

References

Anyia AO and Herzog H (2004) Water-use efficiency, leaf area and leaf gas exchange of cowpeas under mid-season drought. Eur. J. Agron. 20: 327-339.

Bahadur A, Kumar R, Mishra U, Rai A and Singh M (2010) Physiological approaches for screening of tomato genotypes for moisture stress tolerance. National Conference of Plant Physiology (NCPP- 2010) BHU, Varanasi during Nov. 25-27, 2010.142pp.

Bahadur A, Singh KP, Rai A, Verma A and Rai M (2009) Physiological and yield response of okra (*Abelmoschus esculentus* Moench) to irrigation scheduling and organic mulching. Indian J. Agric. Sci. 79: 813-815.

Blum A (2005) Drought resistance, water-use efficiency, and yield potential-Are they compatible, dissonant, or mutually exclusive? Aust. J. Agric. Res. 56: 1159-1168.

Bota J, Medrano H and Flexas J (2004) Is photosynthesis limited by decreased Rubisco activity and RuBP content under progressive water stress? New Phytol. 162: 671-681.

Bray EA, Bailey-Serres J and Weretilnyk E (2000) Responses to abiotic stresses. In: *Buchanan E*, *Gruissem W*, *Jones R* (Eds.), Biochemistry and Molecular Biology of Plant. 1158-1249 pp.

Bright J, Desikan R, Hancock JT, Weir IS and Neill SJ (2006) ABA-induced NO generation and stomatal closure in *Arabidopsis* are dependent on H_2O_2 synthesis. The Plant J. 45: 113-122.

Chalapathi Rao ASV and Reddy AR (2008) Glutathione reductase: a putative redox regulatory system in plant cells. In: Khan N A, Singh S, Umar S (Eds.). Sulfur Assimilation and Abiotic Stresses in Plants. Springer. 111-147.

Chaves MM, Pereira JS, Maroco J, Rodrigues ML, Ricardo CPP, Oserio ML, Carvalho I, Faria T and Pinheiro C (2002) How do plants cope with water stress in the field? Photosynthesis and growth. Ann. Bot. 89: 907-916.

Cifre J, Bota J, Escalona JM, Medrano H and Flexas J (2005) Physiological tools for irrigation scheduling in grapevines under field conditions: an evaluation of stomatal and mesophyll limitations. Funct. Plant Biol. 29: 461-471.

Condon AG, Richards RA, Rebetzke GJ and Farquhar GD (2002) Improving intrinsic water-use efficiency and crop yield. Crop Sci. 42: 122-131.

Cruz de Carvalho MH and Contour-Ansel D (2008) Drought stress and reactive oxygen species: production, scavenging and signaling. Plant Signal Behav. 3: 156-165.

Czytko H H, Grabowski J, Sandorf I, Weckermann K and Weiler EW (2005) Tocopherol content and activities of tyrosine aminotransferase and cystine lyase in *Arabidopsis* under stress conditions. J. Plant Physiol. 162: 767-770.

Ding S, Lu Q, Zhang Y, Yang Z, Wen X, Zhang L and Lu C (2009) Enhanced sensitivity to oxidative stress in transgenic tobacco plants with decreased glutathione reductase activity leads to a decrease in ascorbate pool and ascorbate redox state. Plant Mol. Biol. 69: 577-592.

Duniway JM (1971) Water relation of Fusarium wilt of tomato. Physiol. Plant 15: 10-21.

Edwards EA, Rawsthorne S and Mullineaux PM (1990) Subcellular distribution of multiple forms of glutathione reductase in leaves of pea (*Pisum sativum* L.). Planta 180: 278-284.

Flexas J, Bota J, Escalona JM, Sampol B and Medrano H (2002) Effects of drought on photosynthesis in grapevines under field conditions: an evaluation of stomatal and mesophyll limitations. Funct. Plant Biol. 29: 461-471.

Flexas J, Bota, J, Loreto F, Cornic G and Sharkey TD (2004) Diffusive and metabolic limitations to photosynthesis under drought and salinity in C3 plants. Plant Biol. 6: 1-11.

Foreman J, Demidchik V, Bothwell JH, Mylona P, Miedema H, Torres MA, Linstead P, Costa S, Brownlee C, Jones JD, Davies JM and Dolan L (2003) Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. Nature 422: 442-446.

Foyer CH and Noctor G (2005) Redox homeostis and antioxidant signalling: a metabolic interface between stress perception and physiological responses. Plant Cell 17: 1866-1875.

Foyer CH and Noctor G (2000) Oxygen processing in photosynthesis: regulation and signalling. New Phytol. 146: 359-388.

Friedman M (1997) Chemistry, biochemistry, and dietary role of potato polyphenols. J. Agric. Food Chem. 45: 1523-1540.

Garg N and Manchanda G (2009) ROS generation in plants: boon or bane? Plant Biosys. 143: 8-96.

Gill SS and Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol. Biochem. 48: 909-930.

Gosal SS, Wani SH and Kang MS (2009) Biotechnology and drought tolerance. J. Crop Impov. 23: 19-54.

Govindjee (1999) On the requirement of minimum number of four versus eight quanta of light for the evolution of one molecule of oxygen in photosynthesis: a historical note. Photosynthesis Res. 59: 249-254.

Hare PD and Cress WA (1997) Metabolic implications of stress induced proline accumulation in plants. Plant Growth Reg. 21: 79-102.

Hessini K, Martínez J P, Gandour M, Albouchi A, Soltani A and Abdelly C (2009) Effect of water stress on growth, osmotic adjustment, cell wall elasticity and water-use efficiency in *Spartina alterniflora*. Environ. Exp. Bot. 67: 312-319.

Heuer (1999) Osmoregulatory role of proline in plants exposed to environmental stressed. In: Pessarakli, M. (Eds.), Handbook of Plant and Crop Stress. Marcel Dekker, New York 2: 675-695.

IPCC-2001 Climate Change The Scientific Basis. Contributions of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change. Houghton JT, Ding Y, Griggs DJ, Noguer M, van de Linden PJ, Dai X, Maskell K and Ohnson CAJ (eds). U.K. and New York: Cambridge University Press.

Iturbe-Ormaetxe I, Escuredo PR, Arrese-IC and Becana M (1998) Oxidative damage in pea plants exposed to water deficit or paraquat. Plant Physiol. 116: 173-181

Karakas B, Ozias-Akins P, Stushnoff C, Suefferheld and Rieger M. (1997) Salinity and drought tolerance mannitol accumulating transgenic tobacco. Plant Cell Environ. 20: 609-616.

Karim S, Aronsson H, Ericson H, Pirhonen M, Leyman B, Welin B, Mantyla E, Palva ET, Van Dijck P and Holmstrom KO (2007) Improved drought tolerance without undesired side effects in transgenic plants producing trehalose. Plant Mol Biol. 64: 371-386.

Kavi Kishore PB, Sangam S, Amrutha RN, Laxmi PS, Naidu KP, Rao KRSS, Rao S, Reddy KJ, Theriappan P and Sreenivasulu N (2005) Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: its implications in plant growth and abiotic stress tolerance. Curr. Sci. 88: 424-438.

Keenan T, Sabate S and Gracia C (2010) The importance of mesophyll conductance in regulating forest ecosystem productivity during drought periods. Global Change Biol. 16: 1019-1034.

Kemble JK and Sanders DC (2000) Basics of vegetable crop irrigation. Alabama Co-operative Extension System ANR-1169.

Kumar A and Elston J (1992) Genotypic differences in leaf water relations between *Brassica juncea* and *B. napus*. Ann. Bot. 70: 3-9.

Kumar A and Singh DP (1998) Use of physiological indices as a screening technique for drought tolerace in oilseed *Brassica species*. Ann. Bot. 81: 413-420.

Kumar A, Singh P, Singh DP, Singh H and Sharma HC (1984) Differences in osmoregulation in *Brassica* species. Ann. Bot. 54: 537-541.

Lawlor DW (2002) Limitation to photosynthesis in water-stressed leaves: stomata vs. metabolism and the role of ATP. Ann. Bot. 89: 871-885.

Lopezcastaneda C and Richards RA (1994)Variation in temperate cereals in rainfed environments. 3. Water use and water-use efficiency. Field Crops Res. 39: 85-98.

Lutfor Rahman SM, Mackay Wayne A, Quebedeaux B, Nawata E, Sakuratani T and Uddin ASM (2002) Superoxide dismutase activity, leaf water potential, relative water content, growth and yield of a drought-tolerant and a drought-sensitive tomato *(Lycopersicon esculentum* Mill.) cultivars. Subtrop. Plant Sci. 54: 16- 22.

Maatallah S, Ghanem ME, Albouchi A, Bizid E and Lutts E (2010) A greenhouse investigation of responses to different water stress regimes of *Laurus nobilis* trees from two climatic regions. J. Arid Environ. 74: 327-337.

Macnicol PK and Bergmann L (1984) A role for homoglutathione in organic sulfur transport to the developing mung bean seed. Plant Sci. 36: 219-23.

Martin B and Ruiz-Torres NA (1992) Effects of water-deficit stress on photosynthesis, its components and component limitations, and on water use efficiency in wheat (*Triticum aestivum* L.). Plant Physiol. 100: 733-739.

Martin B, Tauer CG and Lin RK (1999) Carbon isotope discrimination as a tool to improve water-use efficiency in tomato. Crop Sci. 39:1775-1783.

Martinez JP, Lutts S, Schanck A, Bajji M and Kinet JM (2004) Is osmotic adjustment required for water stress resistance in the Mediterranean shrub (*Atriplex halimus* L.)? Plant Physiol. 161: 1041-1051.

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 7: 405-410.

Moore K and Roberts LJ (1998) Measurement of lipid peroxidation. Free Radic. Res. 28: 659-71.

Naor A and Wample RL (1994) Gas exchange and water relations of field-grown concord (*Vitis labruscana* Bailey.) grapevines. Am. J. Enol. Viticult. 45: 333-337.

Noctor G and Foyer CH (1998) A re-evaluation of the ATP: NADPH budget during C3 photosynthesis. A contribution from nitrate assimilation and its associated respiratory activity J. Exp. Bot. 49: 1895-1908.

Noctor G, Arisi ACM, Jouanin L, Kunert KJ, Rennenberg H and Foyer CH (1998) Glutathione: biosynthesis, metabolism and relationship to stress tolerance explored in transformed plants. J. Exp. Bot. 49: 623-647.

Norwood M, Truesdale MR, Richter A and Scott P (2000) Photosynthetic carbohydrate metabolism in the resurrection plant *Craterostigma plantagineum*. J. Exp. Bot. 51: 159-165.

Outlaw WH (2003) Integration of cellular and physiological functions of guard cells. Crit. Rev. Plant Sci. 22: 503-529.

Pagter M, Bragato C and Brix H (2005) Tolerance and physiological responses of *Phragmites australis* to water deficit. Aquat. Bot. 81: 285-299.

Parry MAJ, Flexas J and Medrano H (2005) Prospects for crop production under drought: research priorities and future directions. Ann. Appl. Biol. 147: 211-226.

Passardi F, Penel C and Dunand C (2004) Performing the paradoxical: how plant peroxidases modify the cell wall. Trends Plant Sci. 9: 534-540.

Pastenes C, Pimentel P and Lillo J (2005) Leaf movements and photoinhibition in relation to water stress in Yield-grown beans. J. Exp. Bot. 56: 425-433.

Quan LJ, Zhang B, Shi WW and Li HY (2008) Hydrogen peroxide in plants: a versatile molecule of the reactive oxygen species network. J. Integr. Plant Biol. 50: 2-18.

Quick WP, Chaves MM, Wendler R, David M, Rodrigues ML, Passaharinho JA, Pereira JS, Adcock MD, Leegood RC and Stitt M (1992) The effect of water stress on photosynthetic carbon metabolism in four species grown under field conditions. Plant Cell Environ. 15: 25-35.

Rajendrakumar CSV, Reddy BVB and Reddy AR (1994) Proline-protein interactions: protection of structural and functional integrity of M_4 lactate dehydrogenase. Biochem. Biophys Res. Commun. 2: 957-963.

Reddy AR, Chaitanya KV and Vivekanandan M (2004) Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. J. Plant Physiol. 161: 1189-1202.

Reddy AR, Raghavendra AS, Rao Madhava KV, Raghavendra AS and Reddy KJ (2006) Photooxidative stress. In: Physiology and Molecular Biology of Stress Tolerance in Plants. Springer- The Netherlands. 157-186.

Roldan A, Diaz-vivancoz P, Hernandez J A, Carrasco L and Caravaca F (2008) Superoxide dismutase and total peroxidise activities in relation to drought recovery performance of mycorrhizal shrub seedlings grown in an amended semiarid soil. Plant Physiol. 165: 715-722.

Romero-Puertas MC, Corpas FJ, Sandalio LM, Leterrier M, Rodriguez-Serrano M, del Rio LA and Palma JM (2006) Glutathione reductase from pea leaves : response to abiotic stress and characterization of the peroxisomal isozyme. New Phytol. 170: 43-52.

Rouhi V, Samson R, Lemeur R and Damme PV (2007) Photosynthetic gas exchange characteristics in three different almond species during drought stress and subsequent recovery. Environ. Exp. Bot. 59: 117-129.

Sayar R, Kemira H, Kameli A and Mosbahi M (2008) Physiological tests as predictive appreciation for drought tolerance in durum wheat (*Triticum durum* Desf.). Agron. Res. 6: 79-90.

Shao HB, Chu LY, Wu G, Zhang JH, Lu ZH and Hu YC (2007) Changes of some antioxidative physiological indices under soil water deficits among 10 wheat (*Triticum aestivum* L.) genotypes at tillering stage. Colloids Surfaces B: Biointerfaces 54: 143-149.

Sharma P and Dubey RS (2005) Modulation of nitrate reductase activity in rice seedlings under aluminium toxicity and water stress: role of osmolytes as enzyme protectant. J. Plant Physiol. 162: 854-864.

Shohael AM, Ali MB, Yu KW, Hahn EJ, Islam R and Paek KY (2006) Effect of light on oxidativestress, secondary metabolites and induction of antioxidant enzymes in *Eleutherococcus senticosus* somatic embryos in bioreactor. Process Biochem. 4: 1179-1185.

Singh S and Reddy K Raja (2011) Regulation of photosynthesis, fluorescence, stomatal conductance and water-use efficiency of cowpea (*Vigna unguiculata* [L.] Walp.) under drought. J. Photochem. Photobio. B Bio. 105: 40-50.

Skadsen RW, Schulz-Lefert P and Herbt JM (1995) Molecular cloning, characterization and expression analysis of two classes of catalase isozyme genes in barley. Plant Mol. Biol. 29: 1005-1014.

Smirnoff N (1993) The role of active oxygen in the response of plants to water deficit and desiccation. New Phytol. 125: 27-58.

Smirnoff N (2000) Ascorbic acid: Metabolism and functions of a multifaceted molecule. Curr. Opin. Plant Biol. 3: 229-235.

Souza RP, Machado EC, Silva JAB, Lagôa AMMA and Silveira JAG (2004) Photosynthetic gas exchange, chlorophyll fluorescence and some associated metabolic changes in cowpea (*Vigna unguiculata*) during water stress and recovery. Environ. Exp. Bot. 51: 45-56.

Srinivasa Rao NK and Bhatt RM (1988) Photosynthesis, transpiration, stomatal diffusive resistance, and relative water content of *Capsicum annum* L. Grossum (bell pepper) grown under water stress. Photosynthetica 22: 377-382.

Srinivasa Rao NK and Bhatt RM (1990) Response of photosynthesis to water stress in two eggplant cultivars (*Solanum melongena* L.). Photosynthetica 24: 506-513.

Srinivasa Rao NK and Bhatt RM (1992) Response of tomato to moisture stress: Plant water balance and yield. Plant Physiol. Biotech. 19: 36-41.

Srinivasa Rao NK, Bhatt RM and Naren A Mascarenhas JBD (1999) Influence of moisture stress on leaf water status, osmotic potential, chlorophyll fluorescence and solute accumulation in field grown tomato cultivars. Veg. Sci. 26: 129-132.

Szabados L and Savoure A (2009) Proline: a multifunctional amino acid. Trends Plant Sci. 2: 89-97.

Tan CS (1993) Tomato yield-evapotranspiration relationships, seasonal canopy temperature and stomatal conductance as affected by irrigation. Canadian J. Plant Sci. 73: 257-264.

Turner NC, Wright Graeme C and Siddique KHM (2001) Adaptation of grain legumes (pulses) to water-limited environments. Adv. Agron. 71: 193-231.

Unyayar S Y, Keles F and Çekiç Ö (2005) The antioxidative response of two tomato species with different drought tolerances as a result of drought and cadmium stress combinations. Plant Soil Environ. 51: 57-64.

Wasilewska A, Vlad F, Sirichandra C, Redko Y, Jammes F, Valon C, Frey NFD and Leung J (2008) An update on abscisic acid signaling in plants and more. Mol. Plant 1: 198-217.

Whittaker A, Bochicchio A, Vazzana C, Lindsey G and Farrant J (2001) Changes in leaf hexokinase activity and metabolite levels in response to drying in the desiccation- tolerant species *Sporobolus stapfianus* and *Xerophyta viscosa*. J. Exp. Bot. 52: 961-969.

Wilhelm C and Selmar D (2011) Energy dissipation is an essential mechanism to sustain the viability of plants: The physiological limits of improved photosynthesis. J. Plant Physiol. 168: 79-87

Yang Y, Han C, Liu Q, Lin B and Wang J (2008) Effect of drought and low light on growth and enzymatic antioxidant system of *Picea asperata* seedlings. Acta Physiol. Plant. 30: 433-440.

Yushi I, Haruka Y, Takashi Y, Mari I I, Susumu A and Shao H Z (2011) Hydrogen peroxide spraying alleviates drought stress in soybean plants. Plant Physiol. 168: 1562-1567.

Zhu Z, Liang Z and Han R (2009) Saikosaponin accumulation and antioxidative protection in drought-stressed *Bupleurum chinense* DC plants. Environ. Exp. Bot. 66: 326-333.