

Review

Assessment of drought stress tolerance in root and tuber crops

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Plant growth and productivity is adversely affected by environmental abiotic and biotic stresses. Drought is one of abiotic stresses that cause serious damage to plants. Therefore, understanding the extent of drought stress and assessing the mechanisms of drought tolerance in plants is very crucial to devise different coping mechanisms with the stresses. Brief discussions have been given on some of the terminologies such as drought tolerance vs. resistance; potential yield vs. yield potential, and water use efficiency. Mechanisms of adaptation to drought stress, inheritance of drought tolerance and the contribution of biotechnology to develop methods of coping up with the problems have been discussed. Finally, methods of screening for drought tolerance have been outlined. The integration of both agricultural practices and conventional and contemporary breeding strategies are necessary in developing crop varieties that are tolerant to abiotic stresses including drought.

Key words: Drought stress, resistance, root and tuber crops, tolerance.

INTRODUCTION

Plant growth and productivity is adversely affected by various abiotic and biotic stresses. Some of the abiotic factors such as low temperature, salt, flooding, drought, heat, oxidative stress and heavy metal toxicity (these stresses) induce various biochemical and physiological responses in plants, which respond and adapt in order to survive and reproduce even under sub-optimal conditions (Barnabás et al., 2008; Mahajan and Tuteja 2005; Seki et al., 2003).

Drought is an extended period of dry weather characterized by a shortage of water supply to plants (Acquaah, 2007). Drought begins when the readily available soil water in the root zone is exhausted (Taiz and Zeiger, 2006). Drought can be permanent, periodic, or random, occurring early, late, or in the middle of the crop season. Drought can also be cumulative or specific and short. Drought in conjunction with coincident high temperature and radiation poses the most important

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environmental constraints to plant survival and to crop productivity (Chaves et al., 2003; Neumann 2008; Zheng et al., 2009). Further, they are a menace for plants and prevent them from reaching their full genetic potential (Mahajan and Tuteja, 2005). Drought can either be terminal or intermittent (Neumann, 2008). While terminal drought, the availability of soil water decreases progressively and can lead to premature plant death, intermittent drought results from finite periods of inadequate irrigation occurring at one or more intervals during the growing season and is not necessarily lethal. The plant may adjust to short-term water stress by closing stomata and thereby reducing water loss through the leaves. Drought is an extended period of dry weather characterized by a shortage of water supply to plants (Acquaah, 2007). In drought conditions, water potential (Ψ_w) of soil becomes very negative due to concentration increase of soil solutes. The movement of cell water is determined by the water potential gradient ($\Delta\Psi_w$) that acts as a driving force for transport through a permeable cell membrane (Taiz and Zeiger, 2006). A plant can continue to absorb water only if its Ψ_w is lower than that of the soil. Indeed, drought stress causes changes in plant cells and tissues to adapt to drought stress condition and continue to acquire little available water from the soil (Bartels and Sunkar, 2005). Symptoms of drought stress start when crop has used between 50 and 80% of available soil moisture (Acquaah, 2007). In fact, the failure of plant to absorb the soil water has severe consequences.

Water plays a crucial role in the life of plant and its availability is a main factor that determines the plant population in the environment (Coley et al., 2009). Water is the main constituent of plant tissues but its quantity varies within plant tissues and plant species. The water content was estimated at 80 to 95% in mass of growing tissues, 85 to 95% in vegetative tissues, 35 to 75% in wood with dead cells, and at 5 to 15% in dried seeds (Taiz and Zeiger, 2006). The distribution of plant species in the environment is associated with their tolerance to environmental stresses (Brenes et al., 2009). Most widespread plant species are drought tolerant (Baltzer et al., 2008; Brenes et al., 2009). A low temperature was suspected to be the main limiting factor of life in the Antarctic environment; however, it was found that the water deficit is the major cause and a positive correlation was observed between the soil moisture and the abundance of plant species (Kennedy, 1993).

Drought is the primary abiotic stress that affects crop production and food availability. In many countries, agriculture depends on rain which in many cases does not meet the crop need (Ober, 2008). The fluctuation of rain affects the growth and production of crop and this causes famines in many semi-arid countries (Acquaah, 2007). Drought can cause the big loss of the production as compared to other isolated biotic or abiotic stress (Boyer, 1982; Ober, 2008). It affects production by

reducing the genetic production potential of a plant (Mitra, 2001). Consequently, it is responsible for the difference between the mean yield and the yield potential of a crop and the cause of yield instability in time (Sorrells et al., 2000).

Drought induces physiological, biochemical and molecular changes that have consequences on a plant growth and production (Reddy et al., 2004). The drought osmotic stress causes the removal of water from the cytoplasm to the extracellular space and cell dehydration (Bartels and Sunkar, 2005). Water deficit affects the photosynthetic ability of plants by changing the content and components of chlorophyll, reducing the net CO_2 uptake by leaves, or by decreasing activities of enzymes in the Calvin cycle (Becana et al., 1998; Cornic, 2000; Gong et al., 2005; Lawlor and Tezara, 2009). The osmotic stress of water deficit inhibits strongly the growth of leaves and stem of plants. This has negative effects on the crop yield potential (Westgate and Boyer, 1985). However, the degree of growth inhibition and yield potential reduction depends on the duration and intensity of drought stress, and the genotype of plant species (Bartels and Sunkar, 2005; Monakhova and Chernyad'ev, 2002).

The major cause of reduction of photosynthesis ability and plant growth under drought stress is the disequilibrium between the production of reactive oxygen species (ROS) and their scavenging systems (Becana et al., 1998). Plants under abiotic stresses generate ROS that cause oxidative reactions (Lin et al., 2006). The main ROS are hydrogen peroxide (H_2O_2) and superoxide (O_2^-). These ROS are routinely produced in different cellular reactions catalyzed by various enzymes such as lipoxygenase, peroxidase, NADPH oxidase and xanthine oxidase, but the main source of these molecules is the Fenton and Haber-Weiss reactions (Blokina et al., 2003; Debarry et al., 2005; Lin et al., 2006). The ROS damage lipids, carbohydrates and proteins of cell membrane and cell nucleic acids (Blokina et al., 2003; Fu and Huang, 2001; Sairam et al., 1997; Zhang and Kirkham, 1996). When a plant is under a serious stress condition, there is an accumulation of ROS because its ROS scavenging and repairing mechanisms of ROS damages are surpassed (Lin et al., 2006). Therefore, a plant must have efficient mechanisms of defense against ROS to survive a severe drought osmotic stress and adapt to drought condition. This paper review concepts of drought stress and methods for assessing drought tolerance in root and tuber crops.

DROUGHT RESISTANCE VERSUS DROUGHT TOLERANCE

Drought resistance refers to the ability of a plant to maintain favorable water balance and turgidity even when exposed to drought condition and its consequences.

When a genotype yields better than another under a severe strain of drought, it is relatively more drought resistant (Blum, 2002). The strain of drought is developed when crop demand for water is not met by the supply, and plant water status is reduced. Plants can resist drought by either dehydration avoidance or dehydration tolerance. Physiological responses of drought resistance depend on magnitude of stress and plant growth stage. For example, drought resistance in seedlings grown in a pot has nothing to do with drought resistance during grain filling in the field (Blum, 2011). Drought tolerance is defined as the relative capacity of plants to maintain functional growth under low leaf water status.

A genotype is drought resistant when it produces an economic crop, within the limits of its production potential under conditions of limited water availability. A genotype can be drought resistant due to the following mechanisms: drought escape, drought tolerance, drought avoidance and drought recovery (Barnabás et al., 2008; Chaves et al., 2003; Ekanayake, 1990). These mechanisms are not mutually exclusive and provide the crop with the ability to resist drought at any given period during its growth cycle (Chaves et al., 2003; Ekanayake, 1990). Plant escape drought by exhibiting high degree of developmental plasticity by being able to complete their life cycle before physiological water deficit occurs; this relies on successful reproduction prior to the onset of severe water stress such as by short life cycle, high rate of growth or the efficient storage and use of reserves for seed production (Barnabás et al., 2008; Chaves et al., 2003). Also, plants endure drought conditions by avoiding dehydration while maintaining tissue water potential as high as possible or tolerating low tissue water potential. This can be achieved either by minimizing water loss or maximizing water intake (Chaves et al., 2003). Water loss can be minimized by closing stomata, reducing light absorbance through leaf rolling, increasing reflectance by dense trichome layer, steep leaf angles or by decreasing canopy leaf area through reduced growth and shedding older leaves (Ehleringer and Cooper, 1992; Larcher, 2000).

Water use efficiency

Water use efficiency is defined by the relationship between grams of water transpired by a crop per gram of dry matter produced (Zangerl and Bazzaz, 1984). Therefore, crops that use water more efficiently produce more dry matter per gram of water transpired, so the efficient use of water is directly correlated to time of stomata opening and stomatal conductance (Zangerl and Bazzaz, 1984). As the plant absorbs CO₂ for photosynthesis, water is lost by transpiration, with variable intensity depending on the potential gradient between leaf surface and the atmosphere, following a stream of water potentials (Shwani et al., 2010).

Yield potential and potential yield

Yield potential is defined as the yield of a cultivar when grown in environments to which it is adapted, with nutrients and water non-limiting and with pests, diseases, weeds, lodging and other stresses effectively controlled (Acquaah, 2007). As such, it is distinguished from potential yield, which is defined as the maximum yield which could be reached by crop in given environments, as determined, for example, by simulation models with plausible physiological and agronomic assumptions (Evans and Fischer, 1999).

Mechanisms of adaptation to drought stress

The interaction and adaptation of plants to environmental signals and stresses is a complex network model (Shwani et al., 2010). Figure 1 highlights the probable physiological, biochemical and molecular responses to drought-stress in higher plants. Plants can withhold the drought stress by dehydration tolerance, dehydration avoidance or drought escape (Ludlow, 1989). In the strategy of dehydration tolerance, it was reported resurrection plants that can survive an extreme internal water deficit. These plants can be still alive when there is 95% of leaf water loss (Scott, 2000). The dehydration avoidance consists of maximizing the water absorption and minimizing the water loss under water deficit conditions. This model is mainly observed on succulent and C4 plant species. The strategy of drought escape is based on a short life cycle. Plants grow and reproduce before appearance of a drought season (Mckay et al., 2003; Mitra, 2001; Passioura, 1996; Richards, 1996). Even though there are these strategies, plants present various changes to resist drought stress. Perennial and deciduous plants reduce their foliage in drought seasons. Plants that are always green present sometimes thick leaves with solid cuticle, deep roots, highly sclerophyllous and reduced size leaves, and effective water use and control of evapotranspiration (Ain-Lhout et al., 2001; Lebreton et al., 1995; Sanguineti et al., 1999; Sorrells et al., 2000; Taiz and Zeiger, 2006). These strategies are usually assisted by others such as accumulation of compatible solutes and increased expression and production of antioxidants. Accumulation of compatible solutes is one of biochemical processes that help plants to survive under drought condition (McCue and Hanson, 1990). Compatible solutes play an adaptive role by osmotic adjustment and protection of cellular compounds (Ain-Lhout et al., 2001; Hare et al., 1998). The compatible solutes are mainly nitrogen containing molecules such as amino acids and polyamines, and hydroxyl compounds. Types of these compatible solutes and the level of their accumulation vary with plant species (McCue and Hanson, 1990). These molecules work together with antioxidants which intervene to eliminate ROS and repair

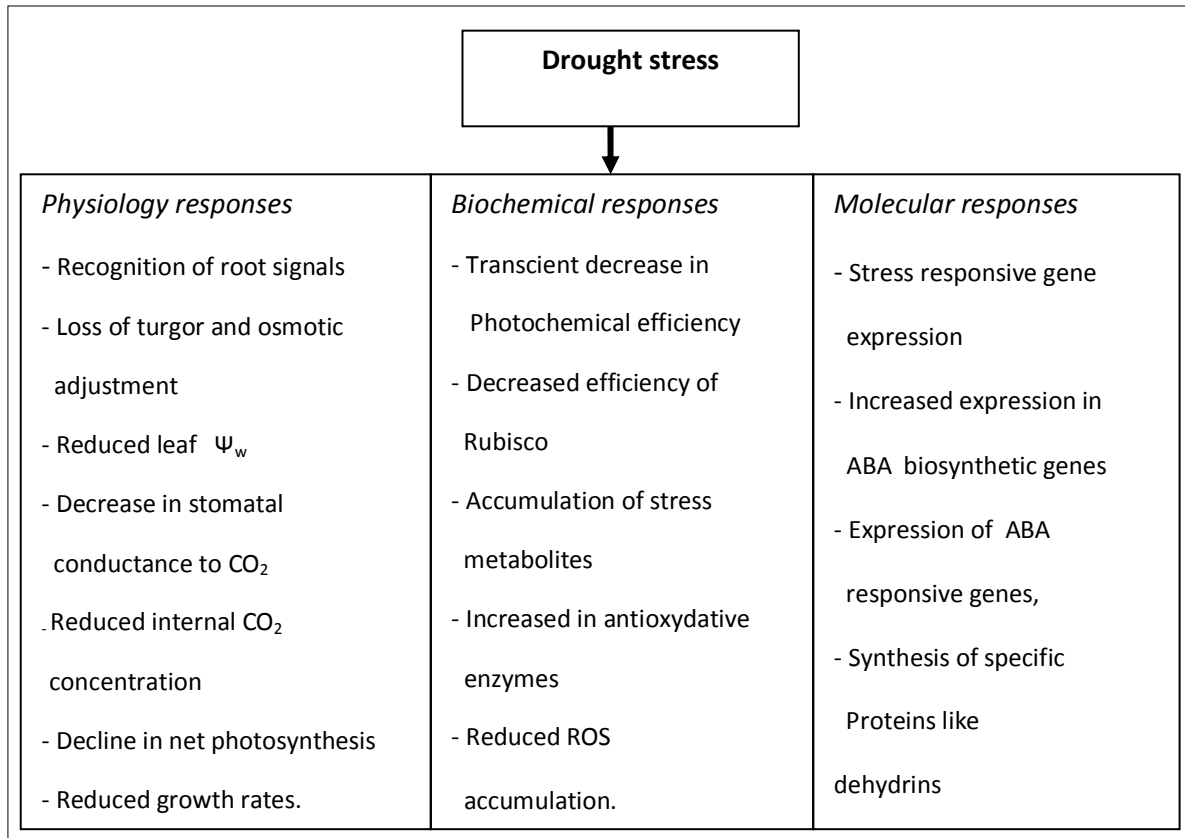


Figure 1. Physiological, biochemical and molecular responses to drought stress in plants (Reddy et al., 2004).

damages of ROS.

Plants produce different antioxidants that have abilities to scavenge ROS. Antioxidants are molecules with small molecular mass such as ascorbic acid, glutathione, tocopherols, phenolic compounds, ROS-interacting enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) (Blokina et al., 2003; Brosché et al., 2010). These molecules play an important role in controlling the equilibrium between the production and the elimination of free radicals. Moreover, they work in cohesive network reactions and use mainly redox reactions (Lin et al., 2006). Plant varieties that are drought resistant or tolerant express a higher quantity of antioxidants than sensitive varieties (Herbinger et al., 2002; Lin et al., 2006). The quality and the quantity of these molecules are plant species dependent and their expression is affected by plant environmental conditions (Blokina et al., 2003; Herbinger et al., 2002; Lin et al., 2006). Therefore, understanding the expression mechanisms of these molecules and their function models can assist to identify and develop of drought tolerant varieties.

Adaptation mechanisms to drought stress incriminate plants. Varieties of short life cycle can escape a drought period; but produce low yield (Acquaah, 2007). Mechanisms of dehydration avoidance such as stomatal

closure and a decrease of leaf area reduce the assimilation of light and atmospheric CO_2 necessary for photosynthesis (Cornic, 2000; Lawlor and Tezara, 2009). Dehydration tolerance with the accumulation of compatible solutes, the synthesis of antioxidants and the process of ROS scavenging requires too much plant energy. Consequently, these mechanisms reduce the ability of plants to synthesize organic products for stock organs (Mitra, 2001). Thus, the development of a drought tolerant variety such as sweet potato needs to balance all drought tolerance mechanisms without sacrificing the crop productivity (Mckay et al., 2003; Mitra 2001; Passioura, 1996; Richards, 1996).

Inheritance of drought tolerance

Drought tolerance is a complex trait because of the heterogeneity of drought stress in the time and space, and unexpected characteristics of drought stress (Sorrells et al., 2000). The drought tolerance involves actions and interactions of various biochemical, morphological and physiological mechanisms that are controlled by products expressed by different genes (Acquaah, 2007; Mitra, 2001). Moreover, it is difficult to study isolated single gene and to understand its role of drought tolerance in

plant (Mitra, 2001).

Contradiction cases of quantitative nature of drought tolerance were reported in various studies. Ekanayake et al. (1985) have observed that root characteristics are controlled by a qualitative inheritance under a drought condition. The leaf rolling, the osmotic adjustment and the number of roots were identified to be qualitative traits (Mitra, 2001). Study on water deficit mediator genes has indicated that plant species vary in symptoms and reactions to water deficit (Sorrells et al., 2000). The genes responsible for earliness of stem reserves, leaf persistence and dwarfing were identified to be associated with drought tolerance (Foulkes et al., 2007). A drought resistance gene linked with genes for plant height and pigmentation that has pleiotropic effects on a root system was identified in rice and crowpea (Agbicodo et al., 2009; Mitra, 2001; Morgan, 1995). Other proposed candidate genes that are involved in drought tolerance are genes coding for dehydrin proteins that protect cellular components under dehydration condition (Shinozaki and Yamaguchi-Shinozaki, 2007), proteins controlling the equilibrium and damages of ROS (Foyer and Noctor, 2005), proteins involving in osmotic adjustment and plant morphology (Moinuddin et al., 2005; Ober, 2008) and enzymes involved in the accumulation of compatible solutes (Mitra 2001). Indeed, drought tolerance involves many genes which code for products working in a highly coordinated network.

DROUGHT AND BIOTECHNOLOGY

Good genetic maps based on molecular marker technologies are now available for major crops. Many of the traits determining abiotic stress tolerance and the quality and quantity of yield are controlled by a large number of genes, which have only minor individual effects but which act together (quantitative trait loci, QTL) (Barnabás et al., 2008). In crop species with large, complex genomes such as sweet potato, QTL analysis is an important tool in the identification of genetic markers to assist breeding efforts. Barnabas et al. (2008) suggested that correlating genetic information with physiological and morphological traits related to drought tolerance and other traits such as yield will allow the development of new varieties with improved yield safety under water-limited conditions using molecular marker-assisted breeding. The same author highlighted the use of proteomics in investigating the effect of drought and/or heat stress on protein composition as an important step towards understanding the link between environmental factors and plant development. On the other hand, the introduction of isolated individual genes into the germplasm through genetic engineering offers a variety of opportunities to increase environmental stress tolerance in crops (Barnabás et al., 2008). Wahid et al. (2007) applauded the significant contribution of

biotechnology in a better understanding of the genetic basis of heat tolerance.

Recent advances in plant genomics have led to the identification of a vast number of potentially beneficial water-stress-related genes, plus technologies for gene overexpression or silencing. Moreover, these can be introduced into transgenic plants under the control of appropriate promoters and are transmitted to subsequent generations (Neumann, 2008).

Screening for drought tolerance

Environmental conditions of study

Study of drought tolerance in plants can be carried out under field or controlled environmental conditions (Acquaah, 2007). The field condition consists of conducting trials under natural conditions. These trials are carried out in the real environments of a plant but it has some limitations of fluctuation of water availability caused by unexpected rainfall. Moreover, environmental factors such as temperature, air humidity and light are variable. Therefore, the screening for drought tolerance is complicated by difficulties of field management (Lafitte et al., 2004). The rainout shelter and *in vitro* techniques were proposed to overcome the limitations of selection for drought tolerance under field condition (Acquaah, 2007). The rainout shelter is a mobile infrastructure that protects plants under experiment from rain. This method controls the uniformity of water supply to plants (Blum, 2002). The *in vitro* approach consists of growing cells or tissues of plant or plantlets on a defined drought stressing culture media under an aseptic and controlled environment (Ahloowalia et al., 2004; Wang et al., 1999). The *in vitro* technique provides precise results but the working environment differs from the natural environment of plant. Therefore, the combination of *in vitro* screening with selection under the natural condition or under the rainout shelter could improve the quality of results.

Screening methods for drought tolerance

Drought stress tolerance is a complex quantitatively inherited and controlled trait. These stresses affect the plant in different ways and induce different genetic responses. Therefore, very careful strategies and powerful methods are required to assess the level of genetic resistance and/or tolerance.

Drought tolerance can be identified by quantifying phenological, morphological, physiological and biochemical characteristics and using molecular tools (Blum, 2002). Phenological and morphological characteristics are mostly used in breeding for drought tolerance. In these approaches data collection consists of measurement of plant growth (size of roots, stem and leaf

Table 1. A scale of visual symptoms of leaf wilting.

Score	Estimation (in %) of turgid leaf area	Description of the symptoms
9	> 95	All leaves turgid
8	80	Lower leaves wilted
7	70	
6	60	Lower half of the plant wilted
5	50	
4	40	
3	30	Top leaves still turgid
1	<5	Plant completely wilted; leaves show necrosis
0	0	

area, gain of fresh and dry weights and yield loss), growth stage (days to flowering and maturity), senescence and leaf rolling (Cheema and Sadaqat, 2004; Spitters and Schaapendonk, 1990). The water content and water potential of plant are indicators used to identify drought tolerant varieties. A variety that maintains its internal water status under a drought stress is considered as drought tolerant. Drought tolerance is also determined by quantifying plant biochemical products such as compatible solutes, chlorophyll, antioxidants and other proteins produced by plant as responses to drought stress (Kasukabe et al., 2006; Reddy et al., 2004; Wang et al., 1999). Diffusion porometry for leaf water conductance, root penetration, distribution and density in the field and infrared aerial photography for dehydration and leaf temperature are used commonly in studies for drought tolerance (Mitra, 2001). Practical methods to measure the drought tolerance are mainly based on phenological, morphological and physiological traits and on biotechnological approaches.

Phenological traits

Growth rate and yield are main phenological traits for determining drought tolerance in crops. The yield reduction due to drought stress, the number of surviving plants and/or a count of plants with tubers are often practiced to study drought tolerance in root crops (Ekanayake, 1990). Data of growth and yield reduction due to drought stress are collected from trial carried out under control and drought stress conditions. These data are transformed into drought tolerance indices such as drought resistance index (DRI) = $(Y_s/Y_n)/(M_s/M_n)$, mean productivity (MP) = $(Y_s+Y_n)/2$, stress tolerance index (STI) = $(Y_s)(Y_n)/(M_p)$, stress susceptible index (SSI) = $(1-(Y_s/Y_n))/(1-(M_s/M_p))$ and tolerance (TOL) = Y_n-Y_s . Where Y_n : yield of a given genotype in non-stressed environment, Y_s : yield of a given genotype in drought stressed condition, M_s : mean yield over all genotypes in stressed environment, M_n : mean yield over all genotype in non-stressed environment (Golabadi et al., 2006;

Talebi et al., 2009). These drought tolerances indices assist to identify drought tolerant genotypes within germplasm collections

Morphological traits

Leaf wilting

Visual symptoms of leaf wilting (Table 1) are recorded on a scale base and the measurement unit is expressed in cm s^{-1} . But this might be very subjective since it is observation from visual symptoms.

Senescence

The slower the process of leaf senescence, the better is the genotype. It is measured using scores on a scale from 0 to 10, dividing the percentage of estimated total leaf area that is dead. The description of this scoring is presented in Table 2.

Leaf rolling

The leaf rolling is a good indicator of dehydration avoidance mechanism. The best genotypes show the low scores. It is measured using scores from 1 to 5 (Table 3), when leaves are still more upright at the hottest time of the day.

Physiological traits

Canopy temperature

Canopy temperature measurements have been widely used to study the drought response of various crops. This approach is based upon the close, inverse relationship between leaf temperature and transpirational cooling. When stress develops and water status in low

Table 2. A scale of visual symptoms of leaf senescence.

Score	Description	Score	Description
1	10% dead leaf area	6	60% dead leaf area
2	20% dead leaf area	7	70% dead leaf area
3	30% dead leaf area	8	80% dead leaf area
4	40% dead leaf area	9	90% dead leaf area
5	50% dead leaf area	10	100% dead leaf area

Table 3. A scale of visual symptoms of leaf rolling.

Score	Description
1	Unrolled, turgid
2	Leaf rim starts to roll
3	Leaf has a the shape of a V
4	Rolled leaf rim covers part of leaf blade
5	Leaf is rolled like an onion

transpiration is reduced and canopy temperature rises. Blum et al. (1989) found a positive correlation between drought susceptibility of wheat genotypes and canopy temperature in stressed environments. Genotypes that suffered greater relative yield losses under drought stress tended to have warmer canopies at midday. Stark et al. (1991) also reported that the most drought-resistant potato genotypes usually had the lowest canopy temperatures during periods of drought. Canopy temperature is measured by a hand infrared thermometer (IRT) which is designed to sense long-wave infrared radiation emitted from its target and convert it to average temperature display which can be related to transpiration. Measurements ($^{\circ}\text{C}$) will be made from 9:00 to 10:00 ideally on clear (cloudless), windless and sunny days.

Relative leaf water content (RWC)

Relative water content (RWC) is determined by weighing the entire leaves or leaf discs immediately after cutting, following prolonged immersion in water and after oven drying by the method described by Okono (2010). Three topmost fully expanded leaves were sampled from a plant. Six random replications were taken from a single plot. Each sample was represent a different plant within the genotype. The leaf sampling was taken in the afternoon after 2:00 pm, when the change in atmospheric condition and solar radiation slow in order to reduce change in water relation with time. Each sample was be placed in plastic bag. The plastic bag was immediately be placed in a cooler (at around 10-15 $^{\circ}\text{C}$). In the laboratory, each leaf in a plastic bag was weighed to obtain the leaf sample weight (fresh weight, FW), after which the sample was immediately placed hydrated to full turgidity for four hours under normal room light and temperature

conditions. Samples were re-hydrated by floating on deionised water in a covered petri dish. After four hours, the samples were taken out of the water and will be thoroughly dried of any surface moisture quickly and lightly with paper towel and immediately weighed to obtain the fully turgid weight (TW). Samples are then oven dried at 80 $^{\circ}\text{C}$ for 24 h and weighed (after being cooled down in a desiccator) to determine the dry weight (DW). All weighing was done to the nearest mg. The relative water content is calculated as followings:

$$\text{RWC (\%)} = \left(\frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \right) \times 100 ,$$

where FW: = sample fresh weight, TW: sample turgid weight; and DW: sample dry weight.

Stomatal conductance

Stomatal responses to environmental drought have a substantial influence on plant adaptation to arid climates (Bates and Hall, 1982). Stomata regulate water use and the development of water stress, and they influence plant growth rates through effects on carbon dioxide assimilation. Stomatal conductance is measured by using diffusion porometer. It can be calculated by the following empirical formula by Ball-Berry et al. (1987) model as modified by Xu and Baldocchi (2003):

$$g = g_0 + m \frac{ARH}{C_a}$$

Where, g is stomatal conductance, RH is the relative humidity at the leaf surface, C_a is the CO_2 concentration at the leaf surface, and g_0 and m are the intercept and slope, respectively.

Tuzet et al. (2003) suggested another model for determining stomatal conductance which incorporated the stomatal response to leaf water potential as follows:

$$g_{\text{CO}_2} = g_0 + \frac{aA}{ci - \Gamma} \cdot f\psi_v$$

Where, g_{CO_2} is the stomatal conductance for CO_2 , g_0 is the residual conductance, A is assimilation rate, ci is the CO_2 concentration in the intercellular spaces, Γ is the CO_2 compensation point and a is an empirical coefficient. Stomatal conductance varies with leaf irradiance, leaf temperature, atmospheric water vapour pressure deficit and CO_2 concentration (Tuzet et al., 2003). On the other hand, stomatal conductance depends on guard cell and epidermal turgor.

Cell membrane thermo-stability (CMS)

The CMS is highly correlated with yield under drought stress, therefore, this trait can assist to identify drought

tolerance in crops (Blum, 2005). Identification of cell membrane stability consists of measurement of electrolyte using the conductimetric method as described by Blum (2011). 15 to 20 cm² leaf materials are sampled from 5 plants of each genotype grown on control and drought stress conditions and placed in tubes. These leaves samples are washed three times with 20 ml desionised water. Clean samples are soaked in 20 ml of desionised water and incubated at 10°C for 24 h. The conductivity (T1) of the liquid of each tube is measured after 1 h of equilibration at room temperature. Each tube is covered and autoclaved for 15 min to kill the leaf tissues. The conductivity (T2) of each autoclaved sample is measured again after equilibration of one hour at room temperature. The CMS is determined with the following formula:

$$CMS (\%) = \frac{1 - (1 - T_1/T_2)}{1 - C_1/C_2} \times 100$$

Where T1 and T2: Treatment conductivities before and after autoclaving, and C1 and C2 = control conductivities before and after autoclaving.

Leaf chlorophyll content

Strong correlations between higher chlorophyll and carotenoids contents and stress tolerance in plants were reported. It was observed that the chlorophyll content is different between drought resistant and susceptible genotypes. Drought resistant genotypes showed the highest chlorophyll content. This characteristic was suggested to be used as screening tool for drought tolerance (Ganji Arjenaki et al., 2012). Houman et al. (2011) reported that genotypes with high chlorophyll index under drought stress conditions revealed the highest yield. It was observed that drought stress had a negative effect on yields of different genotypes but the genotypes that were able to maintain their chlorophyll content were also able to maintain its yield potential (Khayatnezhad and Gholamin, 2012). Drought stress imposed during vegetative growth or anthesis significantly decreased chlorophyll a, chlorophyll b and total chlorophyll content. The results showed that mesophyll resistance is the basic determinate of rate of photosynthesis under drought stress conditions. Under drought conditions, the drought tolerant variety gave the highest yield whereas the drought sensitive variety gave the lowest yield (Mafakeri et al., 2010). Based on the above findings, the measurement of chlorophyll fluorescent was used as new tool to investigate effects of different stresses such as drought, salinity and heat on crop yield (Ort, 2002). Nowadays, handheld instruments such CCI-200 device and chlorophyll meter SPAD 502 Plus that measure the leaf chlorophyll content are

available. These instruments determine the relative chlorophyll present in leaf by measuring the absorbance of leaf.

BIOTECHNOLOGICAL APPROACHES

Many biotechnological studies based on molecular biology have the aim to show the gene function and gene expression profile. To achieve this goal, different molecular approaches such as analysis of genome, transcriptome, metablome and proteome were developed (Carpentier, 2007). The analysis of the genome is a complex study of an organism's genome. This discipline known as genomics, is based on a complete genome analysis and involves DNA sequencing, assembly of sequences, annotation and mapping of genes (Arabidopsis, 2000).

The study of gene expression and its regulation is another approach for understanding the gene function. This approach known as transcriptomics uses efficient tools such as microarray analysis, cDNA fragment fingerprinting and serial analysis of gene expression (SAGE) to carry out the transcriptome analysis (Brown and Botstein, 1999; Schena et al., 1998). Metabolome represents a collection of all metabolites in a biological organism at a specific time and under specific conditions. These metabolites are the end products of genes expression of the organism.

The study of metablome (metabolomics) is a comprehensive, qualitative and quantitative study of all small molecules (less than or equal to 1500 daltons) participating in important metabolic functions and fulfilling critical roles such as drought tolerance, signaling molecules or secondary metabolites in an organism (Oliver et al., 1998). The main methods for metablome analysis are metabolite profiling and metabolite fingerprinting (Hall, 2006). Proteomics is another approach to understand the gene function and expression profile. It focuses on the characterisation of the cellular proteome which is defined as a set of protein species present in a biological unit at a specific developmental stage and under determined external biotic and abiotic conditions (Jorin et al., 2006; Klug et al., 2000; Prescott et al., 2005). Proteomics involves protein biochemistry and bioinformatics to determine the spatial and temporal expression of proteins in cells and tissues of a living thing (Karr, 2007). The main tool of proteome analysis is a two dimensional gel electrophoresis (2-DE).

All these approaches (genomics, transcriptomics, metabolomics and proteomics) are powerful tools for massive screening of several genes and aim to reveal the changes of what might be happening in a cell. They can also serve as tools to identify candidates with desired traits such as drought tolerance in sweet potato germplasm.

CONCLUSION

The effect of drought stress on yield is highly complex and involves diverse processes in plants. Tolerance to a combination of different stress conditions, particularly those that mimic the field environment, should be the focus of future research programmes aimed at developing transgenic crops with an enhanced tolerance to naturally occurring environmental conditions. Modern genetic and molecular tools will significantly contribute to understanding physiological mechanisms related to drought and forge the way forward to develop new crop varieties with drought tolerance. The advent of biotechnology will contribute significantly to survey drought tolerance in various crop species, including sweet potato, cassava and potato. Further, advancements in marker technology and genetic transformation are expected to contribute significantly to the development of plants with various tolerances in the future. Integration of both agricultural practices, conventional and contemporary breeding strategies are necessary in developing crop varieties tolerant to abiotic and biotic stresses to insure food security of the worldwide population.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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