BREEDING FOR DROUGHT TOLERANCE IN CEREALS: AN OVERVIEW

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ABSTRACT

Drought is among the most serious abiotic stresses in many parts of the world where cereal crops are essential human food source. Breeding cereals for such stress tolerance is strongly challenging the breeders' efforts where difficulties are compounded by the complexity of crop yield on the genetic and physiological bases. For cereal plants, drought tends to develop slowly under field conditions as the soil dries. Plants that are subjected to drought stress in such gradual manner accumulate solutes that maintain cell hydration and undergo complex adjustments in their morphology and photosynthetic characteristics. Many investigators explained the plant response to drought through escape, avoidance, and/or tolerance mechanisms. Substantial cooperative efforts among physiologists and breeders have been devoted toward understanding and manipulating such complex of morpho-physiological traits for better sustainable crop performance under stress.

Such cooperative efforts, beside advances in the field of stress physiology, resulted in many techniques of testing and selecting drought-tolerant plants. An effective screening tool in the hand of a plant breeder should be relatively simple, accurate, inexpensive, and dependable on physiological traits that are highly inherited and well correlated with crop performance under actual field-stressful conditions. Selection using such screening tools in many cases of well planned breeding programs resulted in sustainable cereals' yield under actual shifting dry field conditions.

In recent years, more positive role of genomic-based approaches is expected toward improving drought tolerance of cereal crops. Several studies have been able to map

quantitative trait loci (QTL) for traits associated with drought tolerance in major cereal crops. Marker-assisted selection (MAS) has been employed as an integral component of breeding strategies to improve stress tolerance. Despite impressive technological breakthroughs, the results of such efforts are not always consistent due to the multi-genic nature of drought tolerance. Selection based on a comprehensive approach of testing might be more effective in breeding better drought-tolerant cultivars.

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1- Importance of breeding cereals for drought tolerance

A shortage of human food production has been very early predicted. In 1798, the English economist, Thomas Malthus, argued that the growth of the human population occurs geometrically while the growth of food production occurs arithmetically and a human famine is a final destiny (Encyclopedia Britannica, 1983). Some changes have occurred since that early date, like new methods of birth control and the outstanding role of technology of enlarging food production. However, the Malthus's argument is still controversial and many scientists still warning from a human food shortage, especially in some parts of the world, as long as people have children and eat food.

Cereals are the most widely grown crops around the world. It is used as a direct source of human food and feed for domestic animals. The total world production of the different cereals reached more than 2373 million tons in 2004 (FAO, 2006). The serious efforts toward producing enough food will push farming to new areas of marginal nature and will present new stress problems. Breeding new stress-tolerant cereal cultivars would enhance food production in such environments.

In the recent years, there has been wide consensus about fears of global climatic shift (Weiss, 2007). Human population and food supply in many parts of the world are in a delicate balance. A climatic shift, even of a local short duration, can cause crop losses and a serious imbalance in cereal production. There have been outstanding achievements in breeding for high potential yields of cereals. However, stabilizing yield performance of cereals in face of changing environment would be very rewarding.

Organization of the United Nations has predicted a 14% increase in water use for irrigation in developing countries between 2002 and 2030 that will lead to additional water shortages in one out of five developing countries (FAO, 2002). Furthermore, as water shortages recently approach critical levels around the world, particularly in developing countries where more than 60% of the inhabitants already live under precarious conditions with no access to safe sources of fresh water, there is a clear need to quick solutions to the multi-faceted dilemma of irrigation water shortages. One promising approach is to integrate useful drought tolerance traits through genetic improvement into the cultivated varieties.

Another argument was made by Jensen (1978) about the importance of breeding cereals for stress tolerance. Figure 1 shows the grain yields of the released and expected wheat cultivars over 11 decades in New York State and other U.S states. Jensen (a wheat breeder at Cornell Univ.) argued that the increase rate in yield average of the new cultivars is decreasing in the later decades and we are approaching a theoretical yield ceiling using the traditional methods of plant breeding. Furthermore, as the genetic yield potential of improved cultivars is raised, the problem of environmental stresses, causing crop loss becomes more acute. Therefore, breeding for specific stressful environments and a new concept for obtaining more profitable yield under such dry environments became a necessity.

2- Traits associated with drought tolerance in cereals

Drought tolerance is among the most challenging goals to breeders due to its quantitative genetic basis and poor understanding of yield physiological basis in water-limited conditions (Passioura, 2007; McWilliams, 1989). Studying the plant physiological responses to drought can provide a rational basis for evaluating drought-induced yield losses in dry environments. Three major components that contribute to crop-plant response to drought stress have been stated as: 1) Stress escape, in which a plant fastens its phonological development to escape drought during its critical stages of development, 2) Stress avoidance, in which tissues of a plant subjected to drought have lower constrain than other control plants, and 3) Actual stress tolerance, where essential plant functions are

maintained when plant tissues become under actual constrain due to ambient water deficit. The different mechanisms of drought resistance differ in different plant species (Foulkes *et. al*., 2007). From crop breeding point of view, tolerance is considered to be prior and more beneficial than drought escaping or avoidance. This review article will emphasize essentially on aspects of drought tolerance.
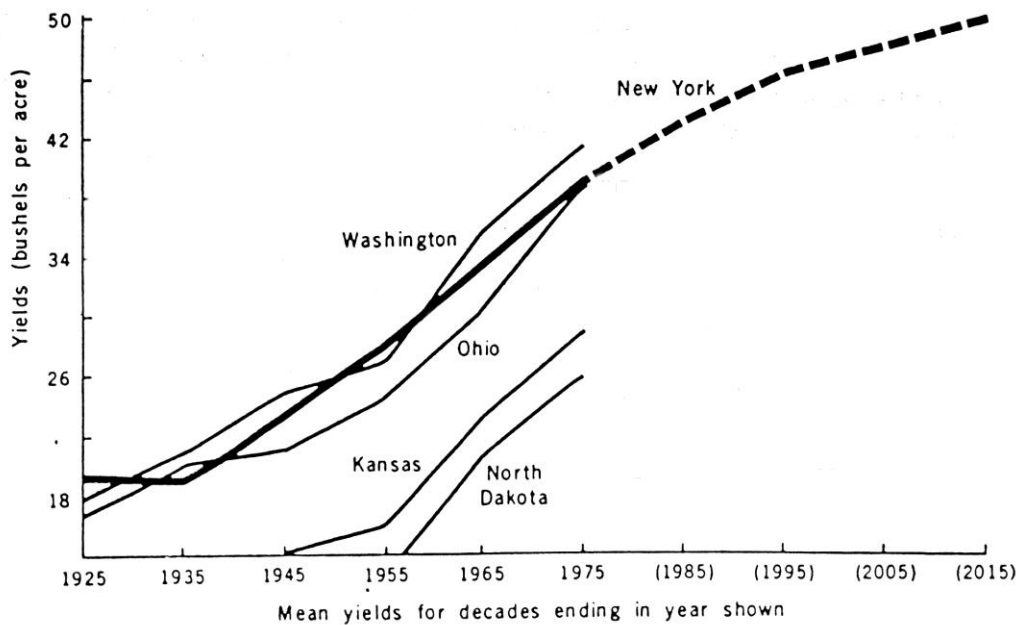
Figure 1: Mean yields of wheat cultivars for New York and other U.S. States for the 110-years period from 1866 to 1975 (Jensen, 1978).

In General, the effects of drought on a cereal crop depends on different factors as; severity of stress, duration of stress, the developmental stage of plant growth, the genotypic ability of crop plant to tolerate the stress, and the genotypic x environmental interaction. However, from an agronomic point of view, our review article will concentrate on the affected traits that have been demonstrated to have direct relationship to productivity of stressed field-grown cereals.

2.1. Morpho-phenological traits

Many morpho-phenological traits could be noticed with water deficit or could be beneficial to field-grown cereals under drought as:

2.1.1. Leaf waxes

A thick or waxy cuticle would be advantageous in plant adaptation to drought where it reduces plant water loss. Borrell *et al*. (2006) pointed out that the sorghum genotypes possessing heavy epicuticular layer were more drought tolerant. The improved drought resistance came from increased reflection of solar radiation and reduced cuticular permeability to water loss and consequently maintaining higher leaf water potential.

2.1.2. Awns characteristics

Long awns have been demonstrated to be a useful selection indicator in wheat for improved production in hot, dry environments where impaired function due to abiotic stress is common. Potential advantages of awns under drought; i.e. high water use efficiency (Blum, 1986) and high sensible heat transfer responsible for a cooler canopy (Weyhrich *et al*., 1994) were confirmed by several researchers. However, Foulkes *et al*. (2007) reported less significance of awns role under moderate droughts in the UK than under more severe droughts in other regions worldwide.

2.1.3. Leaf angle and leaf rolling

The sharp leaf angle is a common characteristic in some cereal cultivars as in maize and sorghum. Some crop plants have the ability of active leaf movement under stress. Changing the leaf angle leads to parallel orientation of the leaf to the incident radiation. In cereal grasses, leaf rolling is a common response to drought stress and results in a 50 to 70% reduction in transpiration (Gusta and Chen, 1987). The reduced leaf angle along with rolling the leaf blade under stress effectively reduces the radiation load on leaves (Boyer, 1996).

2.1.4. Sustainable green leaf area

Producing less leaf area is a common response of the cereal plants under drought. It has been considered one of the most plant adaptive traits to reduce water loss and to increase plant survival under stress (Boyer, 1996). Loss and dryness of lower leaves is common. However, reduced leaf area through the early leaf senescence profoundly reduces the photosynthetic activity of the plant. Drought-tolerant cultivars maintain reasonable photosynthetic leaf area under stress comparing to drought-avoidant cultivars (Baker, 1989). Foulkes *et al*. (2007) reported that the persistence of flag leaf showed the clearest correlation with maintenance of wheat yield under drought. Genetic differences in leaf area duration was also reported in sorghum (Borrell *et al*. 2000) where in 'stay-green' lines the persistence of green leaf area was associated with greater leaf N concentration at anthesis and greater N uptake during grain filling.

2.1.5. Reduced tillering

Tillering in some cereals, *i.e.* wheat and barley, is extremely sensitive to drought. In wheat, Keim and Kronstand (1981) reported a 55% reduction in tiller number in water-stressed plants compared with well-watered control. Although some researchers reported mobilization of stem reserves from late proliferated tillers to the developing grains of the main stem, these late tillers don't contribute significantly to the grain yield. Reducing the number of late-proliferated tillers relatively conserve soil moisture for later grain development periods (Quarrie, *et al*., 1999).

2.1.6. High root/shoot ratio

The root system in cereals has an essential role in the response to drought through its capacity to absorb the depleting soil water under stress. The higher root/shoot ratio could be due to a decline in shoot growth or an increase in root growth or both. Narayan (1991) studied the rooting behavior under water-deficit in comparison to well-watered conditions and reported that roots reached lower depth under drought condition. Also he found that cultivars with lower root depth yielded better than cultivars with shallow root system under drought conditions. When plants relay entirely on stored soil water, water conservation during the vegetative stage would be advantageous so

some water would be available for late use during the critical grain filling period. However, for plants growing in sandy soils, with low water storage capacity, this criterion would not be advantageous (Baker, 1989).

2.2. Physiological traits

Many physiological response-related traits have been reported on plant response to drought in numerous review articles (*i.e*.; Blum, 1996; Boyer, 1996; Foulkes *et al*., 2007). Some of these traits are most common among cereals as:

2.2.1. Proline accumulation

Total free amino acids increase in water-stressed cereal leaves, with proline being the most pronounced (Baker, 1989). Hale and Orcutt (1987) stated that proline accumulation in stressed plants could be as a result of stress damage in the metabolic plant functions while its protective role is not understood. Some researchers suggested its role in protecting membrane systems of plant cell. Boyer (1996) concluded that proline contribution to plant adaptation to water stress is probably not significant. Chandrasekar *et al*. (2000) suggested a protective role of proline as being more synthesized in the most drought-tolerant genotypes.

2.2.2. Osmotic adjustment

Recent interest has been focused on osmotic adjustment, turgor maintenance, and growth responses in cereals. These subjects are discussed in detail by some authors (Yoshiba *et al*., 1997; El-Hafid *et al*. 1998). Turgor is generally thought to be essential for cell enlargement and, therefore, maintaining turgor is essential for maintaining growth. Turgor can be maintained by increasing osmotic concentration, increasing elasticity or decreasing cell size. Li *et al*. (1992) demonstrated that differences in the osmoregulation ability of stressed wheat cultivars are due to their ability to concentrate some different electrolytes, namely in descending order according their importance, potassium, soluble sugars, amino acids, calcium, magnesium, and proline.

2.2.3. Stomatal behavior

In cereal plants drought stress results in a decrease in transpiration, due to stomatal closure (Baker, 1989). Water loss through transpiration is, in general, correlated with size of stomatal aperture and is therefore used as an indicator of stomatal closure. There is a threshold level of leaf water potential before stomatal closure is occurred causing higher leaf resistance to water loss. El-Hafid *et al*. (1998) reported that wheat cultivars with a wide stomatal aperture produced higher yields without consuming more water. Shimshi *et al*. (1982) compared stomatal response to water stress in several Triticum species and they reported that not all species closed their stomata at the same water potential. They also reported that the stomata in *T. aestivum* did not start to open until 2 days after re-watering, and even after 5 days the leaf permeability was only 70% of those non-stressed plants.

2.2.4. Photosynthesis

Water stress severely reduces net photosynthesis in cereal species (Tiesson *et al*., 2006). The reduction in CO_2 assimilation could be due to stomatal closure, which restricts the inward diffusion of CO_2 into the leaf. Also, there are many evidences of non-stomatal effects on net assimilation through the deleterious effects of water deficit on thylakoid membranes, electron transport systems, and enzymes activities. Another indirect effect of drought on photosynthesis would be through reducing the photosynthetic leaf area by reducing leaf elongation and early leaf senescence. Many authors reviewed the deleterious effects of water-deficit stress on cereals photosynthesis (Boote *et al*., 1994; Shah and Paulsen, 2003).

2.2.5. Translocation and allocation of assimilates

Translocation is less sensitive to drought stress than photosynthesis (Gusta and Chen. 1987). In general, drought reduces total assimilates allocated to the developing grain in cereals (Boote *et al*., 1994). There has been a general increase in yield of modern cereal cultivars with little change in the total aboveground biomass, and this increase is attributable to an increase in the allocated assimilate to grain development. This increase has come without much change in the amount of water consumed by plants (Richards *et al.*, 1993). In

recent years, the isotope ^{14}C has been widely used to determine assimilate allocation under stresses in cereals. Many studies indicated negative effects of drought on phloem loading and unloading and consequently less mobilized assimilate to developing grains under stress (Arraudeau, 1989; Wang *et al.*, 2001; Inoue *et al.*, 2004).

2.2.6. Stem carbohydrate reserves

By flowering, reserves of water soluble carbohydrates accumulate in the stems and leaf sheaths of the cereal plant. A significant proportion of these reserves can be subsequently re-translocated to grains under water-deficit conditions (Palta *et al.* 1994). Many researchers reported their evidence that the utilization efficiency (the proportion of the maximal reserves accumulated subsequently re-translocated to the grain) is increased under drought. Palta *et al.* (1994) found post-anthesis assimilation was reduced by 57% by drought, while remobilization of reserves was increased by 36% in Australian spring wheat. Yang *et al.* (2000) in northern China reported that senescence of winter wheat induced by drought during grain filling increased the remobilization of pre-stored carbohydrate assimilates to the grains from 57 to 79%. Foulkes *et al.* (2007) concluded that stem reserves is a trait which favors yield in all situations in the UK rather than specifically maintenance of yield under late-season drought stress.

3. Selection techniques for drought tolerance

Genetic improvement of drought tolerance in crop plants requires identification of relevant drought tolerance mechanisms and the development of a suitable methodology for their measurement in well-planned breeding programs. Many advances in the field of stress physiology resulted in new techniques of breeding for dry environments. Relatively simple, accurate, and inexpensive methodology for screening and selecting tolerant crop plants has become an effective tool in the hands of a plant breeder as a result of merging plant breeding and plant stress physiology. These screening tools depend on different physiological traits that are mostly highly inherited and well correlated with performance under field stress conditions. Selection on the basis of these traits, in most cases, results

in improved yield under recognized stresses and sustained yield under shifting environmental factors.

Blum (1988) stated some criteria that an ideal tolerance test should have as:

1) Rapid to enable screening large number of entries.
2) Sensitive enough to detect differences of segregates within crosses.
3) Non-damaging for the tested plots or material.
4) Inexpensive.
5) Relates to crop performance under field stressful conditions.

Additionally, the choice of selection environment mostly influences the ability to detect morphological and/or physiological traits conferring stress tolerance. In many cases selection under actual stressful environment or preconditioning of the tested materials is critical, especially for drought, heat, salinity and some other stresses. Recently, some techniques and plant criteria have been widely used in screening for drought tolerance in plant breeding programs as:

3.1. Drought susceptibility index:

This index is based on the field performance and is used to compare genotypes in terms of their ability to minimize the reduction in yield under drought-stressful compared with non-stressful environments. The index was initially proposed by Fischer and Maurer (1978) and was widely used by latter investigators (*i.e*., Bruckner and Frohberg, 1987; Clark *et al*. 1992; Saadalla, 1994). It is calculated independently for each environment from a genotype mean as:

$$Si = (1 - Yd / Yp) / D$$

where Yd is mean yield in stress environment, *Yp* is the potential yield in non-stressful environment, and *D* is the environmental stress intensity = 1- (mean *Yd* of all genotypes / mean *Yp* of all genotypes).
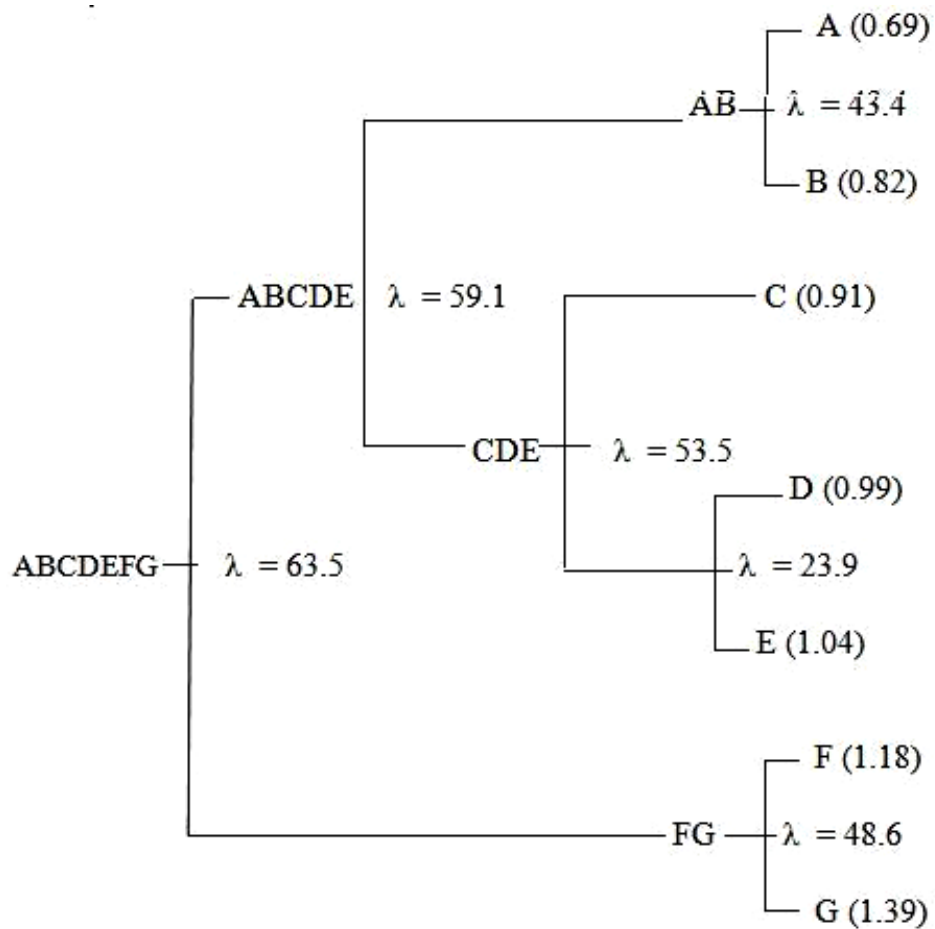
λ is the χ^2 approximation value at 0.05 probability level.

Cluster Key:
A = Waverly + Pitic 62,
B = Sakha 8 + Giza 160,
C = WB926 + Yecora Rojo + Giza 155 + Ast11 + Sakha 69,
D = ID303 +Sakha 61 + Pejamo + Ast12,
E = Probread + Mexipack + Naamhia + Blanca,
F = Shenap 70 + Inia 66 + Ast10 + UT209 + Sonnora,
G = Norin 28 + Kloka + SD956

Figure (2) Cluster analysis and clusters mean (in parentheses) of 25 wheat entries for yield-based susceptibility index (Sy), (Saadalla, 1994).

Saadalla (1994) used the susceptibility index to quantify drought tolerance in 25 wheat entries based on losses in grain yield (Sy) and in grain weight (Sk) by exposing the genotypes to water-deficit treatment for three consecutive seasons in field experiments. A year-to-year comparison of the genotypic response showed a reasonable degree of consistency for both indices; Sy and Sk. Using cluster analysis to separate the entries into distinct groups, entries were divided into significantly different seven clusters based on Sy (Fig. 2). The effectiveness of clustering was tested by portioning mean squares of both genotypes and genotypes x environment into among and within-cluster components (Table 1). Homogeneity of response to drought was verified within the clusters for yield and grain weight, while among clusters variation was highly significant. Additionally, there were significant correlations between the two indices and each of grain yield and grain weight under stress.

3.2.   Chemical desiccation:

Sizable amount of nonstructural carbohydrates accumulate in stems of cereals immediately prior to and after anthesis and although some of these carbohydrates are consumed in plant respiration, some are remobilized to the developing grains (Davidson and Chevalier, 1992; and Schnyder, 1993). When cereal crops are subjected to severe post-anthesis drought, grain growth is increasingly supported by the mobilization of stem reserves relative to transient photosynthesis. These reserves have been estimated to contribute from 10-12% of the final grain yield in wheat under normal conditions comparing to more than 40% under drought or heat stress (Nicolas and Turner, 1993; Blum *et al*., 1991).

Chemical desiccants have been suggested as an indirect simple method to simulate post-anthesis drought for small-grain cereals. Spraying plants (grown in the field under well-watered conditions) at a precise time after anthesis causes total destruction of the plant's photosynthetic activities and consequently allows assessment of genotypic ability for translocation-based grain growth (Haley and Quick, 1993; Royo and Blanco, 1998; Saadalla, 2001a).

Many Chemicals were used as desiccants; Magnesium Chlorate $MgClO_3$ (Blum *et al*. 1983), Sodium Chlorate $NaClO_3$ (Nicolas and Turner, 1993), and Potassium Iodide KI (Saadalla, 2001a). However,

Nicolas and Turner (1993) proved that Potassium Iodide *KI* was the most suitable since it rapidly stops photosynthesis without any direct toxic effects on grain filling from the translocated carbohydrates.

Nicolas and Turner (1993) reported a strong linear relationship between the reduction in kernel weight and the level of stem reserves of carbohydrates where a low reduction in kernel weight was associated with high levels of stem reserves (Fig. 3).

Table 1. Among and within cluster variances for grain yield-based susceptibility index (Sy) and grain weight-based susceptibility index (Sk) for genotypes and genotypes x years interaction (Saadalla, 1994).

Source	Sy		Sk	
	df	Variance	df	Variance
Genotypes	24	934**	24	16**
Among Clusters	6	3593**	5	3252**
Among genotypes / Clusters	18	48	19	49
Genotypes x Years	48	56	48	50
Clusters x Years	12	88	10	40
Genotypes / Clusters x Years	36	46	38	53

** Significant at 0.01 probability level.

Selection for post-anthesis drought tolerance under field conditions is mostly impractical due to uncontrolled field environment and the complicating effects of the genotypic variation in plant phenology. In contrast, post anthesis chemical desiccation was found to be potentially useful technique for differentiation among genotypes in terms of their ability to utilize stem reserves in addition to overcoming the changing precipitation and other interfering environmental factors.

3.3. Canopy temperature:

The remote-sensing infrared thermometry (IR) was proposed to meet the criteria of a rapid, instantaneous, nondestructive, and monitoring whole-plant response to drought in the field (Amani *et al*.,

1996; Saadalla and Alderfasi, 2000). The technique is an indirect method for assessing stomatal conductance of canopy leaves where canopies tend to warm up with stomatal closure. Two strategies explain how canopy temperature (Tc) might best be used for evaluating plant adaptability to drought. The first clarify the ability of a plant canopy to continue extracting and transpiring water from drying soil and consequently cooler Tc, higher yield, and better water use efficiency under drought conditions (Blum *et al*. 1989; Amani *et al*., 1996). The second strategy suggests that genotypes with warmer Tc during vegetative growth stages under well-watered conditions may save more soil water to avoid water deficit which can develop latter during the critical stages of anthesis and grain development (Hatfield *et al*., 1987). Recently, the battery-operated, hand-held infrared thermometer let the field use of Tc as plant-water status monitor more feasible (Fig. 5).
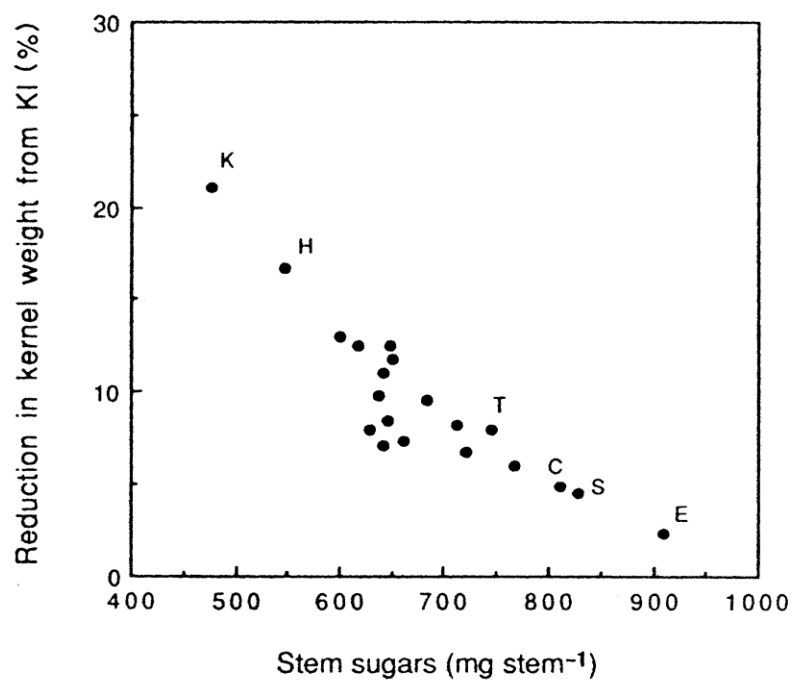

Figure 3. Reduction in kernel weight due to KI treatment as a function of the sugar content of stems at the time when KI was applied (Nicolas and Turner, 1993).

Saadalla (2001a) demonstrated a strong linear relationship between kernel-weight susceptibility indices calculated under both drought and desiccation treatments for 18 spring wheat genotypes (Fig. 4). Similar relationship (with lower R^2 value) existed between yield susceptibility indices calculated under both conditions.
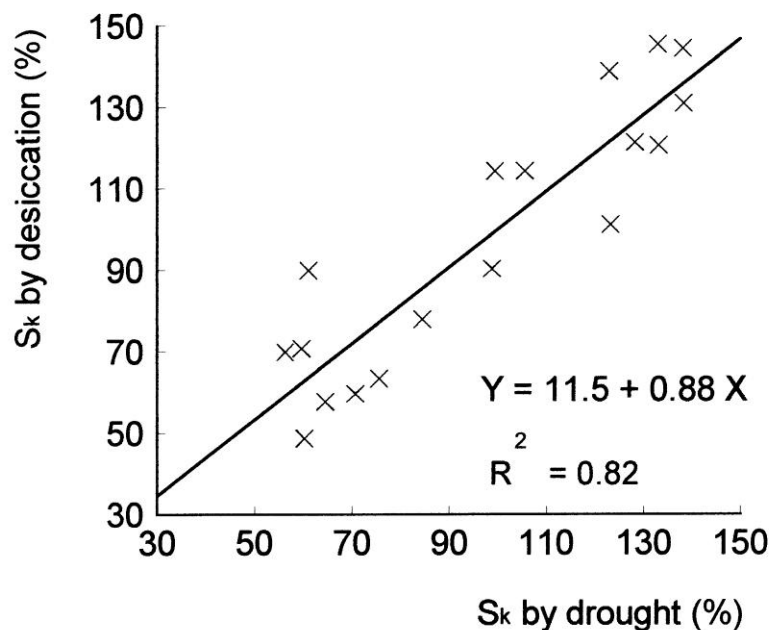

Figure 4. The relationship between grain weight susceptibility indices calculated under desiccation treatment and under soil water deficit (Saadalla, 2001a).

The measurements of Tc have been frequently expressed as air minus canopy temperature and referred to as canopy temperature depression (TD) that is usually a positive number and getting increased with well-watered conditions (Pinter *et al*. 1990; Fischer *et al*., 1998).

Among other weather factors, the atmospheric vapor pressure deficit was reported to have a major effect on Tc, while net radiation, air temperature, and wind speed have minor effects on Tc (Smith *et*

al., 1986). However, the technique relies on the speed of IR measurements and considering TD instead of Tc to avoid complications due to changing weather.

Figure (5) The battery-operated, hand-held infrared thermometer let the use of *Tc* more feasible, easy to use, rapid, instantaneous, nondestructive, and monitoring whole-plant response in the field.

3.4.   Excised-leaf water loss:

Plants extract water from their environment and keep dynamic water balance between their evapotranspiration and water uptake by the root system. Many studies showed that the rate of water loss from excised plant leaves is related to drought resistance in cereals (Winter *et al*., 1988; McCaig and Romagosa, 1989). Hale and Orcutt (1987) stated that excised leaf water loss seems to be a tool for screening for low cuticular transpiration rate. Water retention by leaves of durum

wheat (McCaig and Romagosa, 1989) and hexaploid wheat (Sharid, 1999; Cseuz *et al.*, 2002) was positively correlated with yield.

Clark and Townley-Smith (1986) showed that the excised-leaf water retention trait seems to be somewhat heritable, and is positively related to durum wheat yield under drought stress

The use of modern sophisticated scaling machines reduces to much extent the labor input for this method. Large number of entries can be processed in less time and in an accurate and easy way.

3.5.   Cell membrane stability:

For drought tolerance, the cell membrane stability method is based on dehydration *in vitro* of leaf tissue by a solution of an osmotic (Polyethylene Glycol, PEG) and a subsequent measurement of electrolyte leakage in an aqueous medium. Brief description of the method (Blum and Ebercon, 1981) includes washing the leaf diskettes several times with de-ionized water, placing the treated samples in PEG (6000) and the control samples in the same size of de-ionized water, incubation for 24 h, draining the media, equilibrating the samples in a thermo-stated water bath at 25 ℃ and conductivity of the incubation medium is measured after a vigorous mixing of the vial content. After reading, samples are autoclaved for 15 minutes to kill leaf tissue causing a full release of electrolytes. A second conductivity measurement is taken upon equilibration at 25 ℃. Calculation of percent injury due to desiccation is calculated as:

%   Injury $= 1$- [1-($T1/T2$) / 1-($C1/C2$)] x 100

where T and C refer to mean of treatment and control, respectively, and 1 and 2 refer to initial and final conductivities, respectively.

Blum and Ebercon (1981) tested 77 wheat cultivars in two seasons. The percent injury ranged from 0 to 87% indicating a wide range of genotypic variability in the membrane stability. However, the cultivars that tested both years showed significant year effect and a significant year x cultivar interaction. The difference in results between years was attributed to more soil moisture in the first year. The strong drought stress in the second year resulted in more differentiation among cultivars indicating the importance of testing under actual drought stressful environment.

3.6.   Leaf water potential:

The pressure chamber was used to measure the osmotic potential of plant leaf. However, many obstacles hindered using it on a large scale for evaluating plants in the field. Some of these obstacles are the difficulty of handling it in the field due to its heavy weight, the high vibration in its measurements, the influence of the environmental factors on plant osmotic potential value, and the relatively long time required for its measurements (Wall *et al*. 2006).

Some relatively recent instruments have been used for estimating the water potential of plant leaves; *i.e.* psychrometers. A reasonable usage of these instruments has enabled evaluation of relatively large number of plant entries. However, the fluctuating measurements of genotypic water potential under field conditions necessitate using such instruments under controlled environments. Leaf water potential was reported to be highly related to the water status of the stressed cereal plants. Li *et al*. (1992) demonstrated that leaf water potential of stressed four wheat cultivars was a very reasonable evaluation of the cultivars ability of osmoregulation and consequently their drought tolerance level.

3.7.   Relative leaf water content:

Relative water content (RWC) of the plant leaf was proposed as a better indicator of plant water status than leaf water potential (Sinclair and Ludlow, 1985), because RWC, through its relation to cell volume, may more closely reflect the balance between water supply to the leaf and transpiration rate. Schonfeld *et al*. (1988) studied the RWC in relation to the drought reaction of six populations of wheat. The RWC was measured at 7-days intervals on single leaves until flag leaf senescence. RWC declined with increasing drought stress, but no significant differences among populations were found in water potential, solute potential, or turgor potential. Furthermore, RWC differed significantly among populations under increasing drought stress. The parent tolerant cultivar, TAM105, maintained higher RWC under stress than the sensitive parent, Strudy, and had longer grain filling period. The study indicated that differences were controlled predominantly by genes with additive effects. Relatively high narrow sense heritability (0.64) was estimated for RWC

indicating it as promising selection criterion for drought tolerance. Siddique *et al*. (2000) reported that higher leaf water potential and relative water content were associated with higher photosynthetic rate in four drought-tolerant wheat cultivars.

3.8.   Water use efficiency:

Water use efficiency is generally defined as dry mater produced from consumed water units. For a cereal breeder, the more critical criterion is grain yield produced from the consumed water. Blum (1988) summarized this relation as:

$$Y = ET \times WUE \times HI$$

where Y is the grain yield, ET is the evapotranspiration, WUE is water use efficiency on the dry matter basis, and HI is the harvest index.

WUE is an important criterion in cereals production especially in areas where water resources are limited. Nevertheless, reducing irrigation frequency in the arid regions may have another benefit of reducing soil salinization (Turner, 1993). Some various phenological, morphological, and physiological traits may improve the WUE of the cereal crops as: 1) the development of deep root system (Asseng *et al*., 1998), 2) shoot physiological traits, *i.e.* reduced leaf expansion and stomatal conductance, which help plants to establish a better canopy structure with reduced water consumption (Martin and Ruiz-Torres, 1992), 3) an improved harvest index (Richards, 2006).

Saadalla (2001b) studied the components of water use efficiency on grain yield basis (WUEg); evapotranspiration efficiency (ETE) and harvest index (HI), in different wheat cultivars under contrasting water regimes; stressful vs. non-stressful. He reported significant effects of water regimes on WUEg and significant variation in WUEg and its components among the genotypes under both environments. The studied short cultivars, on average, were less susceptible to drought and they had higher WUEg than the tall cultivars under both non-stressful and drought-stressful regimes. Water deficit negatively affected WUEg mainly through its adverse effect on HI, especially for tall cultivars. The short cultivars had higher HI and less affected WUEg under drought. The path analysis showed that HI accounted for

more variation in WUEg under both stressful and non-stressful regimes with higher relative contribution of HI to WUEg under stress. The relatively high ETE mean for the tall cultivars did not compensate for their low HI and consequently they kept lower WUEg under both non-stressful and stressful conditions.

3.9.   Root/shoot dry weight ratio

Many researchers reported significant genotypic variation in the root characteristics among cereal cultivars (*i.e*., O'Toole and Bland, 1987; Narayan, 1991). Several traits have been taken in root system evaluation as the root depth, root system density, the growth rate of the root system, and the root/shoot ratio. Plant breeders used to give less attention to improve the allocation of plant dry matter between roots and shoots, whereas their attention was essentially to improve the allocation of dry matter between the vegetative growth and grain yield of the cereals. Huge efforts in improving dry matter allocation to grain development resulted in prominent improvement in harvest index of the dwarf and semidwarf cultivars of cereal species. However, in breeding for drought-tolerant cultivars more attention should be directed to improve the root system development (Blum, 1996).

3.10.   *In-vitro* cell lines selection

Plant cell culture offers breeders an alternative strategy to conventional methodology for plant improvement. Since plant cells in culture may be genetically variable, *in vitro*-induced, spontaneous mutations can be selected for a specific tolerance-related trait (Smith *et al*., 1993). Factors affecting somaclonal variation in cultured cells include time in culture, explant source, pathway of regeneration, genotype of the donor plant, environmental conditions during culture, concentration and type of plant growth regulators in the culture media and presence or absence of *in-vitro* selective agents (Bhaskaran and Smith 1990).

The first step after the establishment of a cell culture from a suitable source material is the induction and isolation of drought-tolerant cells and cell lines in the culture. Drought simulation is commonly applied by gradually increasing concentration of some

osmotic agents to the nutrient medium; *i.e.* polyethylene glycol (PEG) [Abdel-Hady and Hoda El-Naggar, 2007], mannitol (AbdElGhany *et al*. 2004,).

The osmotic pressure has to be high enough to kill more than 95% of the cells. The surviving cells are re-cultured and reselected until certain resistant cell lines are recovered. Cell lines may be reselected under higher osmotic pressure levels. The stability of the drought-tolerant cell line has to be proved by passing the culture through non-stressful media back to stressful media (Bajji *et al*., 2000).

The second stage after cell lines selection involves the regeneration of tolerant plants from tolerant cell lines. Irrespective of stress tolerance, plant regeneration from cell lines is an established routine for some plants (*e.g.*, tobacco) and more difficult problem in others (*e.g.* some cereals). It is very common to find varietal differences in the capacity for plantlet or plant regeneration from cultured cells or calluses among cereals (Bhaskaran and Smith, 1990). It is highly advisable to choose cultivars with high regeneration capacity before work in cell culture is initiated, rather than to find out that one is faced with a non-regenerating cultivar after selection in cell culture has been performed.

The maintenance of drought tolerance through plant regeneration is another matter. Regenerated plantlets may or may not carry tolerance. Furthermore, the drought tolerance of the regenerated plants must be retained through plant reproduction. Drought tolerance in cell lines is often ascribed to epigenetic changes and gene expression, which could not be transmitted through sexual reproduction (Tuberosa, and Salvi. 2006). Stable drought-tolerant variants were reported for rice (Blum, 1988), maize (Bruce *et al*. 2002), barley (Teulat *et al*. 2003), and wheat (Barakat and Abdel-latif, 1995).

Undoubtedly, much progress has been made in the last few decades in the attempt to use cell lines and tissue culture for improvement of drought tolerance in cereals. Many of technical problems were being solved. However, the contribution of this method in the form of commercial cereal cultivars is still behind.

4.   Molecular aspects of breeding for drought tolerance

Recent genetic studies and efforts to understand/improve drought tolerance of crop plants using traditional protocols and transgenic approaches have largely determined that plant drought tolerance is a multigenic trait. Different components of tolerance, controlled by different sets of genes, are critical for drought tolerance at different stages of plant development or in different tissues (Boyer and Westgate, 2004). Thus, the use of genetic stocks with different degrees of drought tolerance, correlation and co-segregation analyses, molecular biology techniques and molecular markers to identify tolerance QTLs (quantitative trait loci) are promising approaches to genetic basis of improvement (Collard *et al*., 2004).

Compared to conventional approaches, genomics offers unprecedented opportunities for dissecting quantitative traits into their single genetic determinants, the so-called QTLs (Diab, 2003), thus paving the way to marker-assisted selection (MAS) [Morgante and Salamini, 2003; Nguyen *et al*., 2004] and eventually, cloning of QTLs (Salvi and Tuberosa, 2005) and their direct manipulation via genetic engineering. The increasing number of studies reporting QTLs for drought-related traits and yield in drought-stressed crops indicates growing interest in this approach (Sanchez, *et al*. 2002). However, despite all the recent technological breakthroughs, the overall contribution of genomics-assisted breeding to the release of drought-tolerant cultivars has so far been marginal. How genomics can accelerate the release of improved drought-tolerant cultivars is out scope of this review article. For further readings about genomics-based approaches of breeding, see Collard *et al*. (2004), Tuberosa and Salvi (2006), Somers (2004), Gupta and Varshney (2004).

REFERENCES

Abdel-Hady, M.S., and Hoda M.H. El-Naggar. 2007. Wheat genotypic variation and protein markers in relation with *in vitro* selection for drought tolerance. J. Appl. Sci. Res. 3(10):926-934.

AbdElGhany, H.M., A.A. Nawar, M.E. Ibrahim, Sh.A. El-Shamarka, M.M. Selim, and A.I. Fahmy. 2004. Using tissue culture to select for drought tolerance in bread wheat. Proceedings of the 4th international crop science congress, Brisbane, Australia, 26 Sep. - 1 Oct. 2004. [on line]. Available at:
http://www.cropscience.org.au/ics2004/poster/3/4/563_hatem.htm

Amani, I., R.A. Fischer, and M.P. Reynolds. 1996. Canopy temperature depression association with yield of irrigated spring wheat cultivars in a hot climate. J. Agron. & Crop Sci. 176:119-126.

Arraudeau, M.A. 1989. Breeding strategies for drought resistance. p.107-116. In F.W.G. Baker (ed.). Drought resistance in cereals. CAB International, UK.

Asseng, S., J.T. Ritchie, A.J.M. Smucker, M.J. Robertson. 1998. Root growth and water uptake during water deficit and recovering in wheat. Plant & Soil 201(2):265-273.

Austin, R.B. 1994. Plant breeding opportunities. p.567-585. *In* K. Boote (ed.) Physiology and Determination of Crop Yield. ASA, CSSA, and SSSA, Madison. WI.

Bajji, M., S. Lutts and J.M. Kinet. 2000. Physiological changes after exposure to and recovery from polyethylene glycol induced water deficit in callus cultures issued from durum wheat (*Triticum durum*) cultivars differing in drought resistance. J. Plant Physiol., 156:75-83.

Baker, F.G. 1989. Drought Resistance in Cereals. CAB International UK.

Barakat, M.N., and T.H. Abdel-latif. 1995. *In vitro* selection for salt-tolerant lines in wheat. II. *In vitro* characterization of cell lines and plant regeneration. Alex. J. Agric. Res. 40(3):139-165.

Bhaskaran, S. and R.H. Smith. 1990. Regeneration in cereal tissue culture: A Review. Crop Sci., 30:1328-1336.

Blum, A. 1986. The effect of heat stress on wheat leaf and ear photosynthesis. J. Exp. Bot. 37:111-118.

Blum, A. 1988. Plant Breeding For Stress Environments. CRC Press. Boca Raton. Fl.

Blum, A. 1996. Crop responses to drought and the interpretation of adaptation. Plant Growth Regulation. 20:135-148.

Blum, A., and A. Ebercon. 1981. Cell membrane stability as a measure of drought and heat tolerance in wheat. Crop Sci. 21:43-47.

Blum, A., L. Shpiler, G. Golan, and J. Mayer. 1989. Yield stability and canopy temperature of wheat genotypes under drought stress. Field Crop Res. 5:137-146.

Blum, A., L. Shpiler, G. Gozlan, J. Mayer, and B. Sinmena. 1991. Mass selection of wheat for grain filling without transient photosynthesis. Euphytica. 54:111-116.

Blum, A., Poiarkova, H. Gozlan, and J. Mayer. 1983. Chemical desiccation of wheat plants as a simulator of post-anthesis stress. I. Effects on translocation and kernel growth. Field Crop Res. 6:51-58.

Boote, K.J., J.M. Bennett, T.R. Sinclair, and G.M. Paulsen. 1994. Physiology and determination of crop yield. CSSA, and SSSA, Madison, WI.

Borrell G.K, G.L. Hammer and R.G. Henzell. 2000. Does maintaining green leaf area in sorghum improve yield under drought? II. Dry matter production and yield. Crop Sci. 40:1037-1048.

Borrell, A., D. Jordan, J. Mullet, B. Henzell, and G. Hammer. 2006. Drought adaptation in sorghum. *In* Jean-Marcel Ribaut (ed.) Drought Adaptation in Cereals. Haworth Press Inc.

Boyer, S.J. 1996. Advances in drought tolerance in plants. Advances in Agron. 56:187-218.

Boyer, J.S. and M.E. Westgate. 2004. Grain yields with limited water. J. Exp. Bot. 55:2385-2394.

Bruce, W.B., G.O. Edmeades, and T.C. Baker. 2002. Molecular and physiological approaches to maize improvement for drought tolerance. J. Exp. Bot. 53:13-25.

Bruckner, P.L., and R.C. Frohberg. 1987. Stress tolerance and adaptation in spring wheat. Crop Sci. 27:731-736.

Chandrasekar, V., R.K. Sairam, and G.C. Srivastava. 2000. Physiological and biochemical responses of hexaploid and tetraploid wheat to drought stress. J. Agron. Crop Sci. 185(4):219-227.

Clark, J.M., R.M. DePauw, and T.F. Townley-Smith. 1992. Evaluation of methods for quantification of drought tolerance in wheat. Crop Sci. 32:723-728.

Clark, J.M. and T.F. Townley-Smith. 1986. Heritability and relationship to yield of excised-leaf water retention in durum wheat. Crop Sci. 26:289-292.

Collard, B.C., M.Z. Jahufer, J.B. Brouwer, and E.C.K. Pang. 2004. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts.

Cseuz, L., J. Pauk, Z. Kertesz, J. Matuz, P. Fonad, I. Tari, and L. Erdei. 2002. Wheat breeding for tolerance to drought stress at the cereal research non-profit company. Acta Biologica Szegediensis 46(3-4):25-26.

Davidson, D.J., and P.M. Chevalier. 1992. Storage and remobilization of water-soluble carbohydrates in stem of spring wheat. Crop Sci. 32:186-190.

Diab, A.A. 2003. Genomic analysis of drought tolerance in wheat and barley. Ph.D. diss. Cornell University. Ithaca. NY.

El-Hafid, R., D.E. Smith, M. Karrou, and K. Samir. 1998. Physiological responses of spring durum wheat cultivars to early-season drought in a Mediterranean environment. Ann. Bot. 81(2):363-370.

Encylopedia Britannica. 1983. Malthus, Thomas Robert. p.394-395. H.H. Benton Publ., Chicago.

Fischer, R.A., and R. Maurer. 1978. Drought resistance in spring wheat cultivars: I. Grain yield responses. Aust. J. Agric. Res. 29:897-912.

Fischer, R.A., D. Rees, K.D. Sayre, Z.M. Lu. A.G. Condon, and A. L. Saavedra. 1998. Wheat yield progress associated with higher stomatal conductance and photosynthetic rate, and cooler canopies. Crop Sci. 38:1467-1475.

Food and Agriculture Organization of the United Nations (FAO). 2002. World Agriculture 2030: Main Findings [on line]. Available at
http://www.fao.org/english/newsroom/news/2002/7833-en.html
(verified 13 Apr 2007).

Food and Agriculture Organization of the United Nations (FAO). 2006. FAO Statistical Yearbook 2005-2006 [on line]. Available at
http://www.fao.org/statistics/yearbook/vol_1_1/index_en.asp
(verified 12 June 2007).

Foulkes M. J., R. Sylvester-Bradley, R. Weightman, and J. W. Snape. 2007. Identifying physiological traits associated with improved drought resistance in winter wheat. Field Crops Research 103(1):11-24.

Gupta, P.K., and R.K. Varshney. 2004. Cereal genomics: An overview. p. 1-19 *In* Gupta, P.K., and R.K. Varshney (ed.) Cereal Genomics. Kluwer Academic Publishers. Netherlands.

Gusta, L.V., and T.H. Chen. 1987. The physiology of water and temperature stress. P.115-150. In E.G Heyne (ed.) Wheat and wheat improvement. 2nd ed. American Society of Agronomy, Madison, WI

Hale, M.G., and D.M. Orcutt. 1987. The Physiology of Plant Under Stress. John Wiley & Sons. New York.

Haley, S.D., and J.S. Quick. 1993. Early generation selection for chemical desiccation tolerance in winter wheat. Crop Sci. 33:1217-1223.

Hatfield, J.L., J.E. Quisenberry, and R.E. Dilbeck. 1987. Use of canopy temperature to identify water conservation in cotton germplasm. Crop Sci. 27:269-273.

Inoue T., S. Inanaga, Y. Sugimoto, K. El Siddig. 2004. Contribution of pre-anthesis assimilates and current photosynthesis to grain yield, and their relationships to drought resistance in wheat cultivars grown under different soil moisture. Photosynthetica 42:99-104.

Jensen, N.F. 1978. Limits to growth in world food production. Ceilings for wheat yields are coming in developed countries. Science. 28:317-320.

Keim, D.L., and W.E. Kronstand. 1981. Drought response of winter wheat cultivars grown under field stress conditions. Crop Sci. 21:11-15.

Li, D.Q., O. Zou, and B.S. Chong. 1992. Osmotic adjustment of wheat cultivars with different drought resistance. Acta, Potophysiologica, Sinica. 18:(1):37-44.

Martin, B., and N.A. Ruiz-Torres. 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(2):733-739.

McCaig, T.N., and I. Romagosa. 1989. Measurement and use of excised-leaf water status in wheat. Crop Sci. 29:1140-1145.

McWilliams, J.R. 1989. The dimensions of drought. p. 1-11. *In* Baker F.W.G. (ed.) Drought Resistance in Cereals. CAB International, UK.

Morgante, M., and F. Salamini, 2003; From plant genomics to breeding practice. Curr. Opin. Biotechnol 14:214-219.

Narayan, D. 1991. Root growth and productivity of wheat cultivars under different soil moisture conditions. International J. Ecology and Environmental Sci. 17:19-26

Nicolas, M.E., and N.C. Turner. 1993. Use of chemical desiccants and senescing agents to select wheat lines maintaining stable grain size during post-anthesis drought. Field Crops Res. 31:155-171.

Nguyen, T.T., N. Klueva, V. Chamareck, A. Avrti, G. Magpantay, A.C. Millena, M.S. Pathan, and H.T. Nguyen. 2004. Saturation mapping of QTL regions and identification of putative candidate genes for drought tolerance in rice. Molecular and General Genomics. 272:35-46.

O'Toole, J.C., and W.L. Bland. 1987. Genotypic variation in crop plant root systems. Adv. in Agron. 41:91-145.

Palta, J.A., T. Kobata, N.C. Tumer, and I.R. Fillery. 1994. Remobilization of carbon and nitrogen in wheat as influenced by post-anthesis water deficit. Crop Sci. 34:118-124.

Passioura, J. 2007. The drought environment: physical, biological, and agricultural prospective. J. Exp. Bot. 58(2):113-117.

Pinter, J.P. Jr., G. Zipoli, R.J. Reginato, R.D. Jackson, S.B. Idso, and J.P. Hohman. 1990. Canopy temperature as an indicator of

differential water use and yield performance among wheat cultivars. Agricultural Water Management, 18:35-48.

Quarrie, S.A., J. Stojanovie, and S. Pekie. 1999. Improving drought resistance in small-grained cereals: a case study, progress and prospects. Plant Growth Regul. 29:1-21.

Richards, R. 2006. Physiological traits used in breeding of new cultivars for water scarce environments. Agric. Water Manage. 80:197-211.

Richards, R.A., C. Lopez-Castaneda, H. Gomez-Macpherson, and A.G. Condon. 1993. Improving the efficiency of water use by plant breeding and molecular biology. Irrig. Sci. 14:93-104.

Royo, C., and R. Blanco. 1998. Use of potassium iodide to mimic drought stress in triticale. Field Crop Res. 59:201-212.

Saadalla, M.M. 1994. Quantification of drought tolerance in spring wheat. Alex. J. Agric. Res. 39(1):177-195.

Saadalla, M.M., and A.A. Alderfasi. 2000. Infrared-Thermal Sensing as a Screening Criterion for Drought Tolerance in Wheat. Annals of Agric. Sci., Ain Shams Univ., Cairo, 45(2): 421-437.

Saadalla, M.M. 2001a. Chemical desiccation as a screening method for post-anthesis drought tolerance in wheat. Bull. Fac. Agric., Cairo Univ. 52:385-400.

Saadalla, M.M. 2001b. Water use efficiency and its components of wheat genotypes of varying drought tolerance. Annals Agric. Res., Ain Shams Univ., 46(1):85-102.

Salvi, S. and R. Tuberosa. 2005. To clone or not to clone plant QTLs: present and future challenges. Trends Plant Sci. 10:297-304.

Sanchez, A.C., P.K. Subudhi, D.T. Rosenow, and H.T. Nguyen. 2002. Mapping QTLs associated with drought resistance in sorghum (*Sorghum bicolor* L. Moench). Plant Molecular Biology 48(5-6):713-726.

Schnyder, H. 1993. The role of carbohydrate storage and redistribution in the source-sink relations of wheat and barley during grain filling. New Physiol. 123:233-245.

Schonfeld, M.A., R.C. Johnson, B.F. Carver, and D.W. Mornhinweg. 1988. Water relations in winter wheat as drought resistance indicators. Crop Sci. 28:526-530.

Shah, N.H., and G. M. Paulsen. 2003. Interaction of drought and high temperature on photosynthesis and grain-filling of wheat. Plant and Soil 257(1):219-226.

Sharid, A.A. 1999. Comparison of evaluation methods for drought tolerance in wheat. Ph.D. Dissertation, King Saud University, Riyadh, Saudi Arabia.

Shimshi, M.L., M.L. Mayoral, and D. Atsmon. 1982. Responses to water stress in wheat and related wild species. Crop Sci. 22:123-128.

Siddique M.R.B., A. Hamid, M.S. Islam. 2000. Drought stress effects on water relations. Bot. Bull. Acad. Sin. 41:35-39.

Sinclair, T.R., and M.M. Ludlow. 1985. Who taught plants thermodynamics? The unfulfilled potential of plant water potential. Aust. J. Plant Physiol. 12:213-217.

Somers, D.J. 2004. Molecular marker systems and their evaluation for cereal genetics. p. 19-34. *In* Gupta, P.K., and R.K. Varshney (ed.) Cereal Genomics. Kluwer Academic Publishers. Netherlands.

Smith, R.C.G., H.D. Barrs, and J.L. Steiner, 1986. Alternative models for predicting the foliage-air temperature difference of well irrigated wheat under variable meteorological conditions. Irrig. Sci. 7:225-236.

Smith, R.H., R.R. Duncan, and S. Bhaskaran. 1993. *In vitro* selection and somaclonal variation for crop improvement. In D.W. Buxton (ed.) Proc. 1st Int'l Crop Sci. Congress. pp. 629-632, July 1992. Ames, IA. Crop Sci. Soc. America, Madison, WI.

Teulat, B., N. Zoumarou-Wallia, B. Rotter, M. Ben Salem, H. Bahri and D. This. 2003. QRL fro relative water content in field-grown barley and their stability across Mediterranean environments. Thor. Appl. Genet. 108:181-188.

Tiesson A., J. Lunn, P. Geigenberger. 2006. Carbohydrate metabolism under water limited conditions. P 449-489. *In* J.M. Ribaut (ed). Drought Adaptation in Cereals. Haworth Press Inc.

Tuberosa R., and S. Salvi. 2006. Genomics-based approaches to improve drought tolerance of crops. Trends Plant Sci. 11(8):405-412.

Turner, N.C. 1993. Water use efficiency of crop plants: potential for improvement. p. 75-82. *In* D.R. Buxton (ed.) International Crop Science I. CSSA, Madison, WI, U.S.A.

Wall, G.W., R.L. Garcia, B.A. Kimball, D.J. Hunsaker, P.J. Pinter, S.P. Long, C.P. Osborne, D.L. Hendrix, F. Wechsung, G. Wechsung, S.W. Leavitt, R.L. LaMorte, and S.B. Idso. 2006. Interactive effects of elevated carbon dioxide and drought on wheat. Agron. J. 98:354-381.

Wang, Z.M., A.L. Wei, and D.M. Zheng. 2001. Photosynthetic characteristics of non-leaf organs of winter wheat cultivars differing in ear type and their relationship with grain mass per ear, Photosynthetica 39:239-244.

Weiss, R. 2007. Facing a threat to farming and food supply [on line]. Available at http://www.washingtonpost.com (posted November 19, 2007).

Weyhrich, R.A., B.F. Carver, and E.L. Smith. 1994. Effects of awns suppression on grain yield and agronomic traits in hard red winter wheat. Crop Sci. 34:965-969

Winter, S.R., J.T. Musick, and K.B. Porter. 1988. Evaluation of screening techniques for breeding drought-resistant winter wheat. Crop Sci. 28:512-516.

Yang, J., J. Zhang, Z. Huang, Q. Zhu, and L. Wang. 2000. Remobilization of carbon reserves is improved by controlled soil-drying during grain filling of wheat. Crop Sci. 40:1645-1655.

Yoshiba, Y., T. Kiyosue, K. Nakashima, K. Yamaguchi-Shinozaki. 1997. Regulation of levels of proline as an osmolyte in plants under water stress. Plant Cell Physiol. 38(10):1095-1102.