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Approaches to Increase Water Use Efficiency in Horticultural and Grain Crops – An Overview

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ABSTRACT

Water use efficiency (WUE) is an important component of drought resistance and breeding. Horticultural crops consume more water than grain crops and hence are more susceptible to reduced water availability both in terms of yield and quality as well. Although management techniques are playing an important role in enhancing water use and WUE in horticultural crops, it is important to complement this by improving this characteristic at the whole plant level. Potato (*Solanum tuberosum* L.) and tomato (*Solanum lycopersicum* L.) are the ideal model horticultural crops important for studying water use and WUE. Knowledge of the traits contributing towards drought resistance of a crop need to be defined as these are often stage-dependent. Biotechnological approaches such as increasing the plant photosynthetic capacity and transferring important genes involved in metabolic pathways related to osmolyte synthesis, stress proteins and/or regulatory genes may also help to improve cellular stress tolerance. However, translating this advantage at the field level may require a coordinated approach involving other traits such as phenology, deep root system, optimum WUE, etc. Selecting genotypes having high WUE alone may not be rewarding, as it may be associated with low biomass. Hence, selecting genotypes with high biomass potential and having high WUE under a stress environment may be more appropriate. Understanding the mechanism enabling root growth in a water deficit environment and linking it with molecular markers may help to select this trait in segregating populations. Hence, a multidisciplinary approach is desired which helps in pyramiding traits imparting drought tolerance while retaining the productivity potential in an irrigated environment in a genotype.

Keywords: $\Delta^{13}C$, osmotic adjustment, partial root drying, potato, Pn/Tr, tomato

Abbreviations: ABA, abscisic acid; **AM**, arbuscular mycorrhiza; $\mathbf{\Delta^{13}C}$, carbon isotope discrimination; **DRE**, drought responsive elements; **ET**, evapotranspiration; **EUW**, effective use of water; **HI**, harvest index; **HSP**, heat shock protein; **LEA**, late embryogenesis-abundant; **MAS**, marker-assisted selection; **MDH**, NADP malate dehydrogenase; **MRI**, magnetic resonance imaging; **NADP-ME**, NADP-malic enzyme; **OA**, osmotic adjustment; **PEPC**, phosphoenolpyruvate carboxylase; **Pn/Tr**, photosynthesis per unit of water transpired; **PPDK**, pyruvate orthophosphate dikinase; **PRD**, partial root drying; **QTL**, quantitative trait locus; **RDI**, regulated deficit irrigation; **RUE**, radiation use efficiency; **SMD**, soil moisture deficit; $\mathbf{g_s}$, stomatal conductance; **TE**, transpiration efficiency; **VPD**, vapor pressure deficit; **WUE**, water use efficiency; $\mathbf{WUE_{DM}}$, water use efficiency for dry matter production

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INTRODUCTION

Agriculture accounts for at least 70% of the world's total water usage. Horticultural crops require significant amounts of water due to their perishable nature. With the increase in demand for water from the non-agricultural sectors such as urban and industrial usage as well, it becomes important to rationally utilize the available water resources for crop production. Environmental stresses compound this problem further and thus, are major factor in limiting stable food production. These stresses may further exacerbate in the near future due to climate variability induced by the projected climate change as a consequence of global warming. The most environmentally friendly and durable solution to

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Invited Review

the problem of water shortage is to complement more efficient irrigation approaches with crops with optimal water use efficiency (WUE), achieved either through genetic engineering and/or conventional breeding programmes, combined with high yields (Chaerle *et al*. 2005).

The water requirement of a crop is the quantity of water needed for normal growth, development and yield and may be supplied by precipitation or by irrigation or by both (Sinha *et al*. 1985). Water is needed mainly to meet the demands of evaporation (E), transpiration (T) and metabolic needs of the plants. The water requirement of any crop is dependent upon crop factors such as, variety, growth stage, duration, plant population and growing season besides soil texture, structure and depth and climatic factors like temperature, relative humidity and wind velocity. The amount of requirement of water and the critical stages at which water is required varies with different crops (Hukkeri and Pandey 1977). Some crops like cotton (*Gossypium hirsutum* L.), groundnut (*Arachis hypogaea* L.) and pulses prefer stress during early vegetative growth to suppress excessive vegetative growth (Sinha *et al*. 1985). In many crops the initial establishment and flowering stages are highly sensitive to conditions that results in the poor performance of the root system and also shedding of flowers, in addition to lodging at maturity in some crops. Hence, blind increase of irrigation inputs alone is not a viable option for high crop productivity and irrigation also depends on the availability of water. However, efficient use of water by the plants is desirable as the soil moisture is maintained for a longer period thus conferring drought tolerance to the plants.

In agriculture water use efficiency (WUE) has been slowly improving due to the use of genotypes with increased WUE (Condon *et al*. 2004) and due to the adoption of innovative irrigation practices such as drip irrigation and deficit irrigation (Pereira *et al*. 2006; Costa *et al*. 2007). Drip irrigation improves WUE by reducing run off and evapotranspiration losses. Deficit irrigation aims to optimize water productivity in crops by deliberately allowing crops to sustain some degree of water deficit and yield reduction to an extent with a significant reduction of irrigation water. Costa *et al*. (2007) have written an exhaustive review on deficit irrigation and its application in horticultural crops. The present review focuses on the various approaches to increase WUE in crops at the plant level with emphasis on potato and tomato. It is not a comprehensive review on WUE of horticultural crops.

WATER USE EFFICIENCY

Water use efficiency, defined as the amount of biomass produced per unit of water used in evapotranspiration (ET), is an important physiological trait for efficient use of water for crop production (de Wit 1958). There are considerable differences between plant species to produce a unit dry matter per unit amount of water used resulting in widely varying values of water use efficiency (Fischer and Turner 1978). Thus, genetic variation in WUE assumes considerable importance, especially in situations when water is limiting. Transpiration efficiency (TE) is a component of WUE, being aerial dry matter production per unit of water transpired by the crop. It is a direct measure of the crop species performance. A significant increase in WUE could be obtained by agronomic practices such as sub-surface irrigation and mulches and plant attributes like canopy structure, rate of canopy development, which would help in minimizing evaporative losses from soil (Rebetzke and Richards 1999; Richards and Luckacs 2002).

There are various morphological, anatomical, phenological, physiological and biochemical parameters, which enable crop plants to regulate water required for production of dry matter and yield. These include stomatal behaviour, canopy structure, leaf movements and surface reflectance, leaf area and root systems (Subbarao and Johansen 2002; Chaerle *et al*. 2005; Blum 2009). At present, only some of these traits have been studied at the molecular level.

At the leaf level, WUE is defined as the ratio of energy captured by photosynthesis per unit of water transpired (Pn/Tr). The differences in the leaf thickness also affect both Pn and WUE. Also, leaf movements and surface reflectance provide a means of optimizing the radiation load on the leaf for the maximization of TE. This can be of advantage in water deficit environments, to dissipate the energy as latent heat and minimize heat damage. Leaf movements allow maximum exposure of leaf area to direct radiation when evaporative demand is low and thus improve TE (Shackel and Hall 1979). At the canopy level, the boundary layer that forms over the crop canopies could cause gas exchange to be dependant less on stomatal conductance (g_s) and is thus one of the important factors affecting TE. Boundary layer resistance depends on the canopy architecture, which is determined by leaf size, leaf arrangement, growth habit and height of the canopy. With a low canopy conductance, leaf water equilibrates with an adjacent air space of higher humidity than the bulk atmosphere, thus having positive effects of a higher TE (Walker and Lance 1991).

Improvement in WUE can be exploited as a tool for crop improvement and for efficient use of limited water supply. Genetic improvement of WUE can be achieved through several ways such as improving Pn/Tr ratio, high harvest index, good drought resistance and selection of high WUE genotypes.

INCREASE IN Pn/Tr

Variation in TE in crop plants can result from changes in water vapour flux through stomata as in wheat (*Triticum aestivum* L.) (Condon *et al*. 1990) or changes in photosynthetic capacity as in groundnut (*Arachis hypogaea* L.) (Rao *et al*. 1995) A significant negative correlation has been shown between photosynthetic capacity and specific leaf area thus forming the basis of variations in TE. The C_4 plants exhibit certain advantages compared to C_3 plants such as reduced photorespiration, and increased nitrogen and WUE. So attempts were made to transfer the advantages of C_4 photosynthesis into C_3 plants by breeding. The C_3 and C_4 species of *Atriplex* were hybridized but the independent inheritance of the genes conferring C_4 characteristics such as Kranz anatomy, elevated phosphoenolpyruvate carboxylase (PEPC) and low CO_2 compensation point indicated that plant breeding was unlikely to be a successful route. However, the advent of genetic engineering in crop plants has resulted in attempts to introduce C_4 characteristics into C_3 plants such as tobacco (*Nicotiana tabacum* L.), potato (*Solanum tuberosum* L.) and rice (*Oryza sativa* L.) by genetic manipulation (Matsuoka *et al*. 2001). It is easier to over express enzymes of the C_4 pathway either individually or in concert than it is to introduce the complex Kranz leaf anatomy with its division of labour between thin-walled mesophyll cells surrounding the thick walled chlorenchymatous bundle sheath. Hence, considerable interest has focused on engineering single-celled C_4 type CO_2 concentrating mechanism such as that found in the aquatic plant *Hydrilla verticillata*. The *H. verticillata* system has PEPC in the mesophyll and the decarboxylation of C_4 acids occurs in the chloroplast. It simply refixes photorespired CO_2 rather than having an active CO_2 concentrating mechanism.

Attempts have been made to over express maize PEPC in tobacco, potato and rice. The transgenic plants exhibiting 2-3 fold higher PEPC activity did not show any effect on photosynthesis rate, CO_2 compensation point, etc. It is likely that PEPC introduced into rice is largely inactive *in vitro* because of down regulation by dephosphorylation (Ku *et al*. 1999). Similarly, transgenic potato over-expressing PEPC and NADP malic enzyme (NAPD-ME) either singly or in combination exhibited slight suppression of photorespiration. However, these are most likely a result of local changes in CO_2 concentration induced by increasing respiration (Häusler *et al*. 2002). Hence, it seems unlikely that attempts to introduce single cell CO_2 concentrating mechanisms will be successful without introducing some of the structural characteristics of C_4 photosynthesis. Engineering a single-celled C_4 system would require a reworking of C_3 leaf structure (Leegood 2002). Taniguchi *et al.* (2008) produced rice overproducing four C_4 enzymes, namely PEPC, pyruvate orthophosphate dikinase (PPDK), NADP malate dehydrogenase (MDH) and NADP-malic enzyme in the mesophyll cells independently or in combination. Overproduction of all four enzymes in combination slightly improved photosynthesis but at the same time caused slight but reproducible stunting of the transgenic plants. The study indicated that overproduction of the four enzymes did not act to concentrate CO_2 inside the chloroplast. It has been demonstrated that overproduction of PEPC almost void of activity regulation leads to serious stunting of transgenic *Arabidopsis* and potato plants (Rademacher *et al.* 2002; Chen *et al.* 2004). The metabolic response towards overexpression of C_4 cycle genes is species-specific and varies even among closely related species such as potato and tobacco, which may hinder the successful introduction of an operational C_4 cycle in all of the crop plants intended to be used (Häusler *et al.* 2001).

When stomata open and CO_2 is absorbed, water is inevitably lost. The balance between the two processes is maintained by the stomatal aperture (Woodward *et al.* 2002). Stomata respond directly to ambient humidity thereby strongly influencing plant TE. The efficiency and speed of stomatal movement to fluctuating water availability [influenced by the water vapor pressure deficit (VPD) between the leaf and surrounding air] are major factors in maximizing photosynthesis and WUE. Genotypic variations in TE can result from variation in g_s but with genotypes having the same level of photosynthetic capacity. The existence of genetic variation in stomatal characteristics suggests that it may be possible to develop cultivars that utilize water more efficiently, thus contributing to their adaptation under water limiting environments. In response to drought stress, plants minimize water loss by abscisic acid (ABA)-mediated-stomatal closure. In fact, it has been shown using tomato mutants that appropriate ABA perception and signalling is essential for osmotic tolerance (Borsani *et al.* 2002). Transgenic tomato lines overproducing ABA had lower stomatal conductance, increased TE and root hydraulic conductivity (Thompson *et al.* 2007). ABA may be synthesized in guard cells or transported to guard cells from other leaf cells or root under stress. The signaling events that bring about stomatal closure have been investigated at the molecular level (Schroeder *et al.* 2001). The ABA-insensitive 1/2 (ABI 1/2) protein serine/threonine phosphatase (PP2C) mutants are defective in drought-induced stomatal closure. Mutations in the farnesyl transferase gene (*ERA 1*) cause hypersensitivity to ABA-mediated stomatal closure. Under drought stress *era1* mutants showed enhanced stomatal closure and thus reduced wilting. Transgenic analysis of guard-cell signaling components will shed light on how these genes can be utilized to improve drought tolerance of crop plants. ABA-treated *Tradescantia virginiana* plants had significantly smaller stomata and higher stomatal density in their lower epidermis, compared with non-treated plants. These characteristics might enhance WUE in drought conditions. Small stomata can open and close more rapidly and thus provide the capacity for a rapid decrease in stomatal conductance of a leaf, minimizing water loss upon drought (Chaerle *et al.* 2005). However, screening for stomatal closure alone as a water conservation trait will probably be detrimental to crop yield.

HIGH HARVEST INDEX

Harvest index (HI) is defined as the ratio of economic yield to the total biological yield (Donald 1962). In grain crops, HI depends upon the distribution of dry matter between the grains and the remaining plant parts (Sinha and Khanna 1975). If post-anthesis water use as a proportion of total water use is large then HI will be large. Too little growth before anthesis may limit total dry matter production, maximizes HI, but leave water behind in the soil. On the other hand, too much growth before anthesis will ensure maximized dry matter production but will exhaust soil water, and consequently less grain yield and HI (Richards *et al.* 2001).

Genes contributing to height reduction and to early flowering are simple and effective ways to increase HI. Reduction in height results in less competition for assimilates between growing spike and the elongating stem. This is the main reason for the advantage of semi dwarf wheat varieties over standard wheat varieties. Since the upper limit of HI in wheat is considered to be 60%, it will therefore, be appropriate to focus on ways to increase biomass and HI. The opportunities lie in increasing the partitioning to reproductive organs such as manipulation of phasic development, grain set and abortion of sterile tillers (Richards *et al.* 2002). Plant phenology determines the amount of pre-anthesis and post-anthesis water use by crop. For instance, a few days earlier flowering may mean 10 to 15 mm of additional soil water for post-anthesis use and thereby a higher HI and may be greater yield (Angus and Herwarrden 2001). Combining early flowering with greater dry matter production may assist to increase HI.

GOOD DROUGHT RESISTANCE

Drought resistance is the generic term used to cover a range of mechanisms whereby plants withstands periods of dry weather. Breeding for drought resistance is an important objective in several national and international crop improvement programmes. Since drought resistance is a complex trait, breeding for such a trait could be simplified by identifying morphological or physiological characters that are closely linked to yield in water limited environments (Araus *et al.* 2008). It is now technically feasible to link phenotypic traits with molecular markers, and progeny carrying these markers can be selected at convenient stages in the form of pollen, seeds or young seedlings. The recently released Australian wheat variety 'Drysdale', developed by selection for low carbon isotope discrimination (higher water use efficiency) yields 10% better yield under dry conditions than other current varieties (Richards 2004; Passioura 2006).

Deep root system

WUE at the field level is determined to a great extent by the ability of the crop to capture water from the soil. Root traits are an important consideration in development of crop varieties suited to drier environments. The simplest way to increase rooting depth and distribution is to increase the duration of vegetative period in wheat (Richards *et al.* 2002). They also suggested that increased early vigour may also result in faster growth of roots enabling them to exploit deeper soil layers and production of more adventitious roots in the top soil. Maintenance of root growth during water deficits is an obvious benefit to maintain an adequate plant water supply and is under genetic control (O'Toole and Bland 1987). Primary root and nodal roots of maize (*Zea mays* L.) and many other species continue to elongate at water potential that completely inhibit shoot growth. This helps in seedling establishment under dry conditions by ensuring a supply of water before shoot emergence.

Sharp and co-workers worked on the physiological mechanisms underlying primary root growth maintenance under water deficit in maize and showed the involvement of turgor maintenance by osmotic adjustment and enhanced cell wall loosening (Sharp *et al.* 1990; van der Weele *et al.* 2000). It was observed that the apical 3mm region maintained the elongation rates while 3-7 mm region showed progressive inhibition under water deficit. The expansion activity and protein content was up regulated in the apical region and down regulated in the zone showing growth inhibition under water deficit. ABA accumulation was necessary for the maintenance of root growth under water deficit as it helped in enhancing antioxidant defense and thus prevent membrane damage. Differences in ABA have also been observed in plants which can establish a symbiotic association with arbuscular mycorrhiza (AM) fungi versus those which can not (Ruiz-Lozano *et al.* 2006). In lettuce (*Lactuca sativa*), AM plants regulated their ABA levels more efficiently than non-AM plants and had higher root hydraulic conductivity and reduced transpiration rates during drought stress (Aroca *et al.* 2008).

Identification of quantitative trait loci (QTLs) is the primary step for marker-assisted breeding and candidate gene cloning. Studies on QTLs have shown that Root ABA-1 is a major constitutive QTL which affects maize root architecture. There are ongoing projects on horticultural crops such as potato as well to define the genetic loci that control rooting depth and penetration (Thompson 2005) for improvement of rooting traits. In lettuce, 13 QTLs have been identified for phenotypic variation in roots and the wild variety was identified as a potential source of alleles to optimize water use (Johnson *et al.* 2000). Several chromosome regions affecting root traits in two or even three populations also affected grain yield under well-watered and/or drought-stressed conditions in maize (Tuberosa *et al.* 2003). In *Arabidopsis* too, 13 QTLs were identified for lateral root system and number and it was shown that variation depended on the growth of the existing lateral root system rather than the change in the number (Loudet *et al.* 2005). In rice, 36 QTLs were identified on all 12 chromosomes for root traits such as root penetration index, basal root thickness, penetrated root thickness, root pulling force, total root dry weight, penetrated root dry weight under drought stress (Zhang *et al.* 2001). The number for QTL for each trait varied from one to 11 and contribution of individual QTL varied from 8 to 38% of phenotypic variance. Gains in rice yields under water-limited conditions have been achieved by introgression of QTLs associated with deeper rooting into a high yielding cultivar (Babu *et al.* 2003). Although QTL analysis remains a resource-demanding undertaking, its integration with genomics and post-genomics approaches will play an increasingly important role for the identification of genes affecting root characteristics and grain yield and for harnessing the favourable allelic variation at such loci.

Osmotic adjustment

Osmotic adjustment (OA) refers to the active accumulation of solutes within plant cells during episodes of water stress leading to partial or complete maintenance of turgor. OA is recognized as one of the most important adaptive mechanism to water deficit in many crops and is considered as a major component of drought tolerance mechanisms (Zhang *et al.* 1999). Sharp *et al.* (1990) showed that substantial OA can be in the growing region of root at low water potential, and it is believed to be important for continued elongation of root at low water potential.

Genetic variation for OA has been recorded in wheat, sorghum, barley (*Hordeum vulgare* L.), pigeon pea (*Cajanus cajan* L.), and *Brassica* species (Khanna-Chopra and Sinha 1990; Khanna-Chopra 1999; Zhang *et al.* 1999). Morgan and Condon (1986) extended the relationship of OA with water use, WUE in addition to HI, biomass and yield in selected F6 lines under high water deficit conditions. They observed a significant positive correlation between OA and all these parameters and concluded that, in case of limited water, plants with higher OA extract more water from the soil, because of the connection between water supply and growth, and produce more dry matter and grain. In chickpea (*Cicer arietinum* L.) also OA helped in maintaining better plant water status and seed yield at moderate and severe moisture stress levels (Moinuddin and Khanna-Chopra 2004). Hence OA is a major cellular stress adaptive response in certain crop plants that enhances dehydration avoidance and supports yield under stress. There is no proof that OA entails a cost in terms of reduced yield potential (Blum 2005).

Analysis of a single chromosome substitution series of Chinese Spring/Red Egyptian wheat indicated that genes responsible for OA are located on chromosome 7A. The analysis of linkage with RFLP studies reconfirmed the probable position of OA on short arm of chromosome 7A of wheat. QTLs for OA have already been identified in wheat, rice and barley. Surprisingly, the region of rice chromosome 8 containing OA QTL is homeologous with a segment of wheat chromosome 7. Rather, it appears that some genomic regions for drought tolerance are conserved among cereals (Zhang *et al.* 2001), which can thus be used for marker-assisted selection breeding to improve stress tolerance.

There is need for studies on osmotic adjustment in horticultural crops under water deficit environment. In potato, osmotic adjustment was very limited and did not correlate with the ability to maintain leaf expansion with increasing soil moisture deficit (Jefferies and Mackerron 1993). Accumulation of proline, generally associated with drought tolerance mechanisms, did not correlate with the yield or biomass production of drought stressed potato plants indicating that proline measurement may not be a suitable screening method for drought tolerance in potato (Schafleitner *et al.* 2007). Osmotic adjustment was observed in tomato and *Lycopersicon pennellii* L. subjected to NaCl and Polyethylene glycol-6000 induced osmotic stresses. Organic solutes i.e. sugars, amino acids and organic acids markedly increased in both tomato species while ion content decreased under osmotic stress. OA did not correlate with the tolerance of the two species to osmotic stress. Free proline also increased under both stresses in the tomato species but did not contribute to OA significantly (Pérez-Alfocea *et al.* 1993). However transgenic approaches to increase the biosynthesis of compatible compounds such as trehalose have been attempted in tomato with favorable results (Cortina and Cullianez-Macia 2005).

Transgenic approaches

Plants react to drought stress by adjusting themselves at various levels of morphology, phenology, physiology, and biochemistry. Since, these responses are regulated by genes, efforts in recent years have focused on the isolation and characterization of genes induced by stresses. Among stress-induced genes isolated to date, several major groups have been targeted for improving drought stress resistance in plants. These include genes encoding enzymes for the biosynthesis of compatible compounds, enzymes for scavenging active oxygen species, heat shock proteins (HSPs), late embryogenesis-abundant (LEA) proteins, enzymes modifying membrane-lipid saturation, transcription factors, and proteins required for ion homeostasis.

While introduction of the key abiotic stress resistance/tolerance genes into plants increased their stress resistance in some recent experiments, simultaneous transfer of several genes should be carried out to achieve practical levels of plant resistance/tolerance. To achieve this, genetic engineering through manipulation of signaling molecules and transcription factors are important as they regulate a group of genes involved in stress response (Chinnusamy *et al.* 2005). Thus, knowledge of regulatory genes that coordinate the complex gene expression cascade during abiotic stress in the plant is important (Sreenivasulu *et al.* 2007). To overcome the problems of energy loss and growth related problems which occur during such manipulations a stress-inducible promoter should be considered. Study of the *cis*-elements responsible for stress induction is necessary to temporally and spatially target the transgenes.

In general, gene products induced during drought stress are classified into two groups. The first group includes proteins that probably have a direct function in stress tolerance (functional proteins). Other proteins are involved indirectly and show regulatory functions in signal transduction and gene expression (regulatory proteins) (Yamaguchi-Shinozaki *et al.* 2002). Some results of recent research findings

Table 1 Genes encoding various enzymes and proteins involved in drought stress resistance in transgenic plants.

Gene	Gene action	Species	Phenotype	Reference
BADH-1	Betaine aldehyde dehydrogenase	Tomato	Maintenance of osmotic potential	Moghaieb *et al*. 2000
CBF3	Transcription factor	Rice	Drought and salt resistance	Oh *et al*. 2005
CYP707A3	Regulate ABA levels	*Arabidopsis*	Dehydration and rehydration responses	Umezawa *et al*. 2006
DREB1 or *OsDREB1*	Transcription factor	Rice	Drought, salt and cold tolerance with reduced growth under non-stress	Ito *et al*. 2006
ERA1	Farnesyl transferase	Canola	Antisense promote drought resistance in the field	Wang *et al*. 2005
FAD3, *FAD8*	Increased fatty acid desaturation	Tobacco	Drought resistance	Zhang *et al*. 2005
HARDY	Transcription factor	Rice	Improved WUE by reducing transpiration and enhancing photosynthesis	Karaba *et al*. 2007
HVA1	Group 3 LEA protein gene	Wheat	Increased biomass and WUE under field drought conditions	Bahieldin *et al*. 2005
ME-leaN4	LEA protein	Chinese cabbage	Drought and salt resistance	Park *et al*. 2005a
		Lettuce	-do-	Park *et al*. 2005b
P5CS	Pyrroline carboxylate synthase (proline synthesis) (tomato)	Citrus	Osmotic adjustment and drought resistance	Molinari *et al*. 2004
PPO	Polyphenol oxidases suppression	Tomato	Drought resistance	Thipyapong *et al*. 2004
RGS1	Regulation of G-protein signalling	*Arabidopsis*	ABA mediated root elongation and drought resistance	Chen *et al*. 2006
RWC3	Aquaporin overexpression	Rice	Maintenance of leaf water potential and transpiration under 10 h PEG stress	Lian *et al*. 2004
TPS1	Trehalose synthesis	Tomato	Drought, salt and oxidative stress tolerance	Cortina and Culiáñez-Macià 2005
ZPT2-3	Encodes a Cys2/His2-type zinc finger protein	Petunia	Dehydration tolerance	Sugano *et al*. 2003
SKIPa	Transcriptional regulator	Rice	Grain yield, spikelet fertility Oxidative stress tolerance	Hou *et al*. 2009
Ap37	Transcriptional regulator	Rice	Grain yield	Oh *et al*. 2009

Table 2 A partial list of QTLs associated with WUE in various crops.

Crop	No. of QTLs	References
Wheat	10 QTLs affecting per plant WUE (Total dry matter/ amount of water used by per plant)	Zhang *et al*. 2002
	2 QTLs controlling leaf WUE	
	6 QTL significantly controlling leaves and stems WUE (Dry weight of stem and leaves/amount of water used per plant).	
	4 QTL influencing leaves and stems WUE	
	5 QTL significantly controlling roots WUE (Dry weight of roots /amount of water used per plant)	
	QTLs for WUE	
	QTL on chromosome 6A associated with longer coleoptile, greater seedling vigour	Spielmeyer *et al*. 2007
Soybean	4 QTLs accounting for 16% variation in Δ.	Mian *et al*. 1996
	2 QTLs one on LG C1, one on LG L	Mian *et al*. 1998
	2 QTLs on the linkage group U22 for WUE	Tischner *et al*. 2003
Tomato	22 QTLs located on 11 chromosomes	Lin *et al*. 1998
	3 QTLs accounting for 70% variation in Δ.	Martin *et al*. 1989
	1 OTL for Δ accounting for 25.6% phenotypic variation.	Xu *et al*. 2008
Brassica	QTLs for plant WUE accounting for 3.4% to 36.6% genetic variance	Hall *et al*. 2005
Rice	QTLs for WUE	Laza *et al*. 2006
	7 QTLs for WUE located in 5 chromosomal regions.	Xu *et al*. 2009
	Improvement of WUE in rice by expression of *HARDY* gene(drought and salt tolerant gene) from *Arabidopsis*	Karaba *et al*. 2007
Barley	10 QTLs for grain WUE,8 QTLs co-located with QTLs for physiological traits under water stress conditions	Teulat *et al*. 2002
	One major QTL linked to the *ERECTA* gene	
Arabidopsis	5 QTLs for plant WUE	Masle *et al*. 2005
	QTLs for WUE as an integrated measure of drought avoidance and plant water relations	Juenger *et al*. 2004

have been summed up (**Table 1**). Overexpression of stress-related genes has afforded some stress protection in transgenic plants. However, comprehensive physiological and biochemical testing of transgenic plants under drought stress conditions must be conducted in the field for true assessment of drought resistance. There are few field evaluation studies of this nature, with some exceptions such as in (i) transgenic wheat expressing the barley *HVA 1* gene showed drought tolerance and increased WUE in the field (Bahieldin *et al*. 2005) and in (ii) transgenic rice overexpressing transcriptional regulators AP37 and SKIPa showing enhanced spikelet fertility, grain number and grain yield under drought stress in the field (Hou *et al*. 2009; Oh *et al*. 2009).

Drought tolerance being a complex quantitative trait, an increasing number of studies has strived to map QTLs affecting drought-related traits and yield in major crops exposed to water deficit. In a limited number of cases, marker-assisted selection (MAS) has been used as an integral component of breeding strategies to incorporate target traits and to increase tolerance to drought (Nguyen *et al*. 2004). However, since WUE is a complex trait and determination of WUE under field condition needs accurate measurement of water and carbon budget of plants, identification of QTLs directly associated with WUE have been few (**Table 2**). Rather, QTLs for physiological traits such as deep roots etc which indirectly improve WUE have been identified (MacMillan *et al*. 2006).

SELECTION OF HIGH WUE GENOTYPES

Numerous studies have noted considerable variation in WUE among crop plants. However, breeding for high WUE was not an attractive goal for breeders for a long time until carbon isotope discrimination was shown to be related to WUE.

Carbon isotope discrimination

Farquhar *et al.* (1982) suggested that carbon isotope discrimination might serve as a mechanistic means of evaluating genotypes within a given C_3 species for differences in TE. Plants discriminate against the naturally occurring and heavier isotope of carbon (^{13}C) during the diffusion and fixation of CO_2 in photosynthesis, resulting in depletion of the ^{13}C content in the biomass. This deviation of carbon isotopic ratio ($^{13}C/^{12}C$) of biomass from that of air is called discrimination ($\Delta^{13}C$). WUE at the level of instantaneous leaf gas exchange (photosynthesis rate/transpiration rate) and stable carbon isotope discrimination ($\Delta^{13}C$) are independently linked to the partial pressure of CO_2 inside leaf to that in ambient air (p_i/p_a). Based on theory, they suggested that Δ measured in plant dry matter should be positively correlated with the ratio of p_i/p_a and negatively associated with WUE. This negative relation of Δ with WUE is robust for plants of many C_3 species such as rice, cowpea and chickpea. The theory proposed by Farquhar *et al.* (1982) relating Δ and WUE is therefore well established at both the leaf and whole-plant levels.

Till date numerous field studies have critically examined the relevance of WUE through Δ in breeding to achieve higher productivity. It is clear that low Δ types have distinct advantages under water-limited environments. Genotypes with low Δ should have greater biomass and hence potential for higher yields, assuming that all genotypes use the same amount of water for transpiration. However, these advantages significantly decreased when rainfall and soil water availability increased (Richards *et al.* 2002). Such genotypes use significantly less amount of water because of a strong stomatal control of transpiration. In the conditions of intermittent water stress and/or irrigated environments, selection for low Δ would be counterproductive as it may be coupled with low biomass (Richards *et al.* 2002).

One approach to identify relatively high water use coupled with high WUE is by identifying genotypes with low Δ and high oxygen isotope ($\Delta^{18}O$). The oxygen isotope enrichment is a good time averaged surrogate for transpiration rate and stomatal conductance at a given VPD. Hence, dual isotope ratio (($\Delta^{13}C/(\Delta^{18}O$) should strongly augment the selection of parents from germplasm and segregating progenies for crop improvement (Sheshshayee *et al.* 2003).

INCREASING WUE IN HORTICULTURAL CROPS

Horticultural crops demand significant amounts of water due to their perishable nature. Tree fruit and nut crops are not only comprised of large amount of water, but the trees are perennial plants. Hence, stress event not only affects the current season's crop, but may also influence the future productivity as well. In the case of apple (*Pyrus malus* L.), for instance, it is reported that 38.1 to 50.8 mm of precipitation per week are needed in an orchard because an individual tree may lose between 30 to 45 litres of water in a day. At this transpiration rate, it does not take very long to deplete the soil available water and for the trees to begin to show drought stress symptoms. Vegetables are also quite perishable but they represent annual crops and thus only one year of production is affected.

Since water is such a critical component for the growth and development of horticultural crops, it is recommended that none of these crops be established without full irrigation capabilities. Normally, 30.24-37.8 liters of water per min per acre well capacity for each acre planted are required for horticultural crops. These are several management decisions, which must be undertaken to reduce the risk of overextending an irrigation system.

Generally, drought management plans are same for vegetable and orchard crops, orchardists are looking at such steps to ease their water shortage immediately. One such approach is application of regulated deficit irrigation (RDI) during phenological stages which are less sensitive to water deficit. The effects of two different levels of RDI ranging from 100% ET to 25% ET were studied in a peach (*Prunus persica* L.) orchard (cv. 'Springcrest'). No significant reductions in yield or quality were found for the 50% ET treatment where as about 1000, 1800 and 2400 m^3ha^{-1} of water were saved in the first, the second and the third year. Thus, a clear benefit can be obtained through the use of 50% RDI during the post harvest period, especially for early harvest peaches under scarce water supply conditions (Dichio *et al.* 2004). In case of premium wine grapes (*Vitis vinifera* L.), it was observed that a longer deficit applied between fruit set and variation reduced seasonal irrigation by 38% with no apparent negative impact on fruit quality, suggesting that reduction in irrigation below current levels of RDI can be accomplished without negative impact on fruit (Tarara *et al.* 2005).

Vegetable producers have the ability to evaluate all aspects of their water situation prior to planting and thus have the opportunity to reduce the size of planting etc. For orchard crops many aspects need to be considered like: current irrigation method inefficiencies, irrigation scheduling techniques, less than optimum irrigation, block productivity and more efficient irrigation systems. In the US, especially the South-Western part many vegetable production regions are strictly regulated on water use and in addition demand high quality and nutritious vegetables. In this regard studies have been performed to explore the effects of deficit irrigation on yield, fruit quality and lycopene content of red-fleshed diploid and triploid watermelon (*Citrullus lanatus*) cultivars. Irrigation treatments were 1.0, 0.75 and 0.5 ET rates and 395, 298 and 173 mm, total water was applied through a subsurface drip system, respectively (Leskovar *et al.* 2004). Lycopene content increased slightly with maturity and was significantly higher at 0.75 ET than 1.0 ET in melons at ripe and overripe maturity stages.

Although management techniques are playing an important role in enhancing water use and WUE in horticultural crops, it is important to complement this by improving this characteristic at the plant level. Water stress anytime during the growth and development of citrus fruit can reduce yield and fruit quality compared to that of well-watered trees. Such losses cannot be completely recovered through proper irrigation during the rest of the season. Water stress may result in smaller, lighter fruit with thicker peel and reduced juice content. Excessive rainfall/or irrigation immediately after harvest results in a dilution of soluble solids whereas drought conditions concentrate soluble solids. Even though fruit from water-stressed trees may have higher total soluble solids and acids per fruit, solids per acre may be reduced because of lower total yield per acre. Water stress also affects the fruit at harvest, with soft or dehydrated fruit experiencing more plugging. Conversely, irrigation or rainfalls near harvest or harvesting with dew on the plant often results in fruit with very turgid rind, susceptible to olecellosis (rupture of oil cells upon impact). Heavy rains after drought can result in "Zebra skin" (areas of necrotic peel over the raised sequerts) (Ritenour *et al.* 2003).

PARTIAL ROOT DRYING

An important contribution of plant physiology in improving water use efficiency in horticultural crops such as vines, raspberry etc is the introduction of "Partial Root Drying (PRD)" technique (Loveys *et al.* 2000). This was derived from the "split-plot principle" which was used to demonstrate the non-hydraulic regulation of growth. In the field the split-plot principle is applied by supplying irrigation to a proportion of the roots while a proportion of the roots are allowed to dry the soil in which they are rooted. Regular alternation of the proportion of the roots that are allowed to dry the soil enables the entire root system to be maintained in a viable state, PRD offers the ability to better control the water relations of the crop when compared to conventional deficit irrigation, practices, which have negative impact on yield quantity and quality. The early experiments at CSIRO demonstrated that by using PRD the total amount of water applied to the crop during the season could be significantly reduced, the amount of water lost through transpiration decreased as a result of partial stomatal closure and vegetative vigor was reduced. The crop yield was not affected and the quality characteristics of the berries and subsequently wine were significantly enhanced.

PRD was extended to other crops such as cotton, tomato and raspberry (*Rubus idaeus* L.) wherein marginal decline in yield was associated with significant increases in water use efficiency and positive effects on yield quality. Maize plants subjected to PRD exhibited significant increase in biomass allocation to the roots and increased nutrient use efficiency which might help in improving WUE (Kirda *et al*. 2005). In potato, PRD treatment saved 30% water and increased WUE by 59% and contributed to lowered stomatal conductance and leaf area expansion by ABA-signaling (Liu *et al*. 2006). There is need for more research to unravel the physiological mechanisms underlying the benefits of PRD vs traditional deficit irrigation (Davies *et al*. 2000).

A key issue limiting both evolution of and breeding for WUE is an inescapable trade-off between traits enhancing total water use versus efficient water use. Biotechnological approaches such as increasing the photosynthetic capacity and by transferring genes involved in metabolic pathways related to osmolyte synthesis, stress proteins and regulatory genes may also help to improve cellular stress tolerance. A coordinated approach involving other traits such as phenology, deep root system, optimum water use efficiency etc is also needed. Potato and tomato are the ideal model horticultural crops where wide information is available. Hence, some of the above features are discussed taking these two crops as examples.

POTATO

Potato is an important crop grown throughout the world and is of particular importance in the temperate climates. It is the fourth most important food crop after maize, rice and wheat. Present world production is some 308 million tons fresh tubers from 19 million ha. Potato varieties can be grouped into early (90 to 120 days), medium (120 to 150 days) and late varieties (150 to 180 days). For high yields, the crop water requirements (ETm) for a 120 to 150 day crop are 500 to 700 mm, depending on climate. Potato is relatively sensitive to soil water deficits (Iwama and Yamaguchi 2006). Depletion of the total available soil water during the growing period of more than 50% results in lower yields. Water deficit during the period of stolonization and tuber initiation and yield formation have the greatest adverse effect on yield, whereas ripening and the early vegetative periods are less sensitive (Lynch and Tai 1989; van Loon 1981; Minhas and Bansal 1991).

Water deficit in the early part of the yield formation period increases the occurrence of spindled tubers, and if water deficit is followed by irrigation tuber cracking or tubers with black hearts may result (Robins and Domingo 1956). Dry matter content may increase slightly with limited water supply during the ripening period (MacKerron and Jefferies 1985). Frequent irrigation does reduce occurrence of tuber malformation (Shock *et al*. 1992). In drought stressed crops, leaf growth rate was reduced in comparison with that in irrigated crops when leaf water potential (Ψ_l) and turgor (*P*) were less than –0.28 and 0.5 MPa respectively (Jefferies 1989). Leaf growth was highly correlated to soil moisture deficit (*SMD*) and declined rapidly when the *SMD* was greater than 16 mm. Reduction in dry matter accumulation in drought affected potato is attributed primarily to lower interception of radiation as a result of reduced size of canopy especially in cool climate regions. But in hot, dry regions, where the transpiration demand is severe, a decrease of radiation use efficiency (RUE) also affects dry matter production (Jefferies and MacKerron 1989; Trebejo and Midmore 1990). Therefore, the reduction of intercepted radiation in potato, even under mild water-stress condition, may be a reflection of higher sensitivity of its leaf growth to water-stress.

The primary effect of a period of drought on potato plants was to reduce the rate of photosynthesis. Levels of sugars and of starch-synthesizing enzymes in extracts from tubers of drought stressed and control plants were similar, and it is likely therefore that the effect of drought on tuber growth is exerted mainly via a reduction in the supply of assimilate (Basu *et al*. 1999).

Good yields under irrigation of a crop of about 120 days in the temperate and subtropical climates are 25 to 35 ton/ha fresh tubers and in tropical climates yields are 15 to 25 ton/ha. The water utilization efficiency for harvested yield (Ey) for tubers containing 70 to 75% moisture is 4 to 7 kg/m^3 Rational use of natural resources, especially water, is one of the most important objectives in the sustainable management of cropping systems. WUE was calculated on the basis of the results of an experiment in which a ETc 100% drip irrigation control was compared with treatments providing for early or late water stress or over-watered conditions (ETc 120%) (Battilani *et al*. 2004). In a field experiment the effects of three treatments (Rainfed, ETc 100%, ETc 100% followed by ETc 70%, with a change of irrigation regime at an average tuber diameter of 35 mm) on four varieties (one determinate and three indeterminate) were compared. In the field the WUE of the irrigated plots was 38% lower than under rainfed conditions, depending on rain distribution during the growth cycle and whether calculated on fresh matter or on dry matter. Water supplies of approximately 300 mm y^{-1} resulted in highest yields both of fresh tubers and tuber dry matter. In terms of profitability WUE$_{DM}$ was optimal when the total available water was approximately 250 mm y^{-1}.

Both soil matric potential and drip irrigation frequency influence potato ET, Y and WUE. In an experiment in North China, potato ET increased as irrigation frequency and soil matric potential increased (Kang *et al*. 2004). The highest ET was 63.4 mm more than the lowest value, and the difference was 32.1% of the highest ET under the various soil water potential treatments. Based on irrigation frequency treatments, the highest ET was 36.7 mm more than the lowest value, and the difference was 19.2% of the highest ET. Potato Y and WUE were also found to increase as irrigation frequency increased. Potato Y increased with increase in soil water potential and then decreased. The highest Y and WUE values were found for soil matric potential -25 kPa and irrigation once every day.

Potato production under deficit irrigation is not economically justifiable under normal conditions. However, under regional drought conditions, it may sometimes be unavoidable. Timing of water stress is important in order to maximize yield and quality under restricted water availability. Spreading water deficits over the latter part of the season will result in the least reduction in tuber yield and quality. If possible, irrigation deficits should be avoided during tuber initiation and mid-bulking. Modification of irrigation, variety selection, fertility, and cultural management practices according to anticipated water availability can partially mitigate tuber yield and quality reductions. In fact, it has been observed that water stress effects on canopy growth and development of potato plants can be mediated somewhat under CO_2 enrichment (Fleisher *et al*. 2008).

Abiotic stresses such as drought, cold and salinity have not been concerns in potato production because of intensive management of the crop. As a consequence, potato production is a heavy user of groundwater for irrigation during the summer months. Use of water by the industry is beginning to come under greater scrutiny by the public and legislature. Addressing drought stress facing potato through breeding and biotechnological approaches will position the industry with tools to manage sub-optimal water management. Drought tolerance of Andean potato genotypes was evaluated using microarray analysis and metabolite profiling. The tolerant genotype, SUL, had a greater induction of chloroplast-localized antioxidant and chaperone genes (Vasquez-

Robinet *et al.* 2008). Most of the antioxidant genes induced were glutathione related. Expressed sequence tags have also been generated in potato in response to various abiotic stresses such as heat-, cold-, salt- and drought stress and analyzing this transciptome revealed sequences known to respond to abiotic stresses as well as several which were unique to potato (Rensink *et al.* 2005). Research has also started on transgenic approaches to improve abiotic stress tolerance. *CBF1* (C repeat/ dehydration-responsive element binding factor 1) genes are referred to as master switches that activate expression of *COR* genes, increasing stress tolerance (Thomashow 1999). *CBF1*-expressing transgenic potato lines have been created that can be further characterized under field and growth chamber conditions for their tolerance to water deficit conditions (Douches, unpublished data, http://fieldcrop.msu.edu/documents/GR03-011.pdf). The clones with greater tolerance to drought stress conditions will be studied to understand the contributing physiological mechanisms (i.e. increased root growth, stomatal closure, osmotic adjustment and synthesis of stress proteins). Concurrently, this more drought tolerant germplasm can be introgressed into the potato breeding variety development program. A few of the more drought tolerant lines identified are being considered for release by the breeders of these lines.

DREB/CBF genes are a small family of transcription factors that bind to the drought responsive elements (DRE) found in the promoters of many drought-responsive genes of *Arabidopsis* (Liu *et al.* 1998), and other plants. Enhanced tolerance of drought, cold and salt was observed in *Arabidopsis* plants in which the AtDREB1A gene was over-expressed. Constructs containing the AtDREB1A gene under the control of various promoters have been introduced into several CGIAR mandated crops such as groundnut, potato, wheat and rice in the expectation that AtDREB1A would recognize the DREs of endogenous genes and enhance stress responsiveness (Jackson 2004). In potato, the existing DREB+ transformants are being assessed and are at the field trials stage (Watanabe *et al.* unpublished data). Transgenic potato accumulating fructans have been shown to have high amounts of proline under drought stress (Knipp and Honermeier 2006)

In plants, oxidative stress is one of the major causes of damage as a result of various environmental stresses. In order to develop plants with enhanced tolerance to environmental stress, transgenic potato plants were generated (cv. 'Atlantic') expressing both Cu/Zn superoxide dismutase (SOD) and ascorbate peroxidase (APX) in chloroplasts under the control of an oxidative stress-inducible *SWPA2* promoter. These plants showed enhanced tolerance to oxidative stress and high temperature (42°C). On exposure to high temperature stress at 42°C for 20 hr, the photosynthetic activity of transgenic plants only decreased 6%, compared with that of plants grown at 25°C (Tang *et al.* 2006). Thus, alterations in the expression of one or more antioxidative enzymes in chloroplasts can be used to develop crop plants with increased tolerance to diverse environmental stresses including drought stress.

TOMATO

Tomato is a warm season crop, which cannot withstand adverse climatic conditions such as frost and high humidity. It requires different climatic range for seed germination, seedling growth, flower and fruit set, and fruit quality. Global tomato production (processing and fresh) has increased reaching 108 million metric tons in 2002 (FAO 2003). Most of the production growth occurred in Asia, which accounted for 50 percent of global production in 2002. Temperatures below 10°C and above 38°C adversely affects plant tissues thereby slowing down physiological activities. Water stress and long dry periods causes cracking of fruits (Guichard *et al.* 2001).

It has been reported that transgenic tomato containing *Arabidopsis* CBF1 were more resistant to water deficit stress than wild type plants (Hsieh *et al.* 2002). CBF1 (DREB1B), a homolog of DREB1A, is a transcriptional activator that binds to the CRT/DRE element, in the promoter region of cold-regulated (*COR*) genes that respond to both low temperature and water deficit. However, the transgenic plants exhibited growth retardation by showing dwarf phenotype, and the fruit and seed numbers and fresh weight of the transgenic tomato plants were less than those of the wild-type plants. The stomata of the transgenic *CBF1* tomato plants closed more rapidly than the wild type after water deficit treatment. It has also been reported that transgenic tomato plants over-expressing the gene *AVP1* led to upregulation of H^+-pyrophosphatase (H^+-PPase), found in all plants, which resulted in stronger, larger root systems which made better use of limited water (Park *et al.* 2005). Control tomatoes used in the experiment suffered irreversible damage after five days without water, as opposed to the transgenic tomatoes, which began to show signs of damage after 13 days but rebounded completely as soon as they were watered. This study was described for the first time in a commercially viable crop.

As part of multi-institutional collaborations, researchers are investigating the genetic basis for naturally-occurring variation in WUE of tomato and rice. Martin *et al.* (1989) were the first to report potential genes associated with WUE in tomato by combining molecular markers and $\Delta^{13}C$ technologies. In order to improve crop productivity in water limited environments, the scarce water resource must be used more efficiently. Since then several such studies have been reported for tomato. A data base that enables use of RFLP markers as selection tools to breed for improved crop WUE was developed (Lin *et al.* 1998). In this study, 22 genomic regions distributed on 11 chromosomes associated with WUE in tomato were identified. The QTLs (identifiable by RFLP markers) that govern each trait were different and unique in well-watered and water-stressed environments. It was confirmed that WUE and Δ are polygenically controlled in various environments.

In another study, it was observed that WUE can be increased by selecting for low Δ, but selecting for low Δ alone may identify a subpopulation of small plants. It was suggested that dry weight could probably be increased by traditional breeding techniques (Martin *et al.* 1999). In yet another study in tomato, QTLs associated with high WUE were consistently associated with low specific leaf area (m^2 leaf area/g dry weight) and high nitrogen content per unit leaf area (Comstock *et al.* 2005). More scientists are giving attention to studying the genetic background and genetic manipulation for improving crop WUE. The water use efficiency genomic project was also set up by UAS national science fund in 2001 in China, with the aim to use stable isotope technique to screen genotypes of modern crops, as represented by tomatoes, rice and their wild relatives, for differences in WUE.

CONCLUSIONS AND FUTURE PROSPECTS

High WUE in crops as a selection criterion has gained considerable interest especially since the finding that it is negatively related to ^{13}C discrimination (Δ). This is so, since genetic variation in WUE exists for many crop species in both well-watered and moisture deficit environments. The high levels of heritability for Δ have further strengthened the argument that Δ is amenable to genetic improvement (Subbarao and Johansen 2002). Different WUE genes have been located in different crops, for instance in chromosome 4 in barley (Handley *et al.* 1994) and chromosome 7D in wheat (Gorny 1999). However, selection for high WUE alone can result in selection for low total biomass. This negative trade off can be reduced by taking into account the intrinsic mesophyll carbon assimilating capacity of the plants. Thus, not only is it useful for developing crop varieties that require less water to produce the same amount of yield but also for much more rational usage of irrigation water.

In water-limited environments selection for high WUE tends to result in a genetic shift towards plant traits that limit crop water use, such as early flowering and smaller leaf area (Martin *et al.* 1999). This is also exemplified by the improvement of dryland wheat grain yield based on selection for high WUE in NSW Australia (Condon *et al.* 2002) where wheat is grown mainly on stored soil moisture. A major avenue for yield improvement is the control of water used during the early part of the growing season in order to avoid lack of soil moisture during reproduction. Blum (2009) is of the view that effective use of water (EUW) is a major target for yield improvement under water limited environment. EUW implies maximum soil moisture capture for transpiration, which is tightly linked with biomass production, reduced non-stomatal transpiration and minimal water loss by soil evaporation. Even osmotic adjustment which is a major stress adaptive trait in crop plants is recognized as enhancing soil moisture capture and transpiration (Chimenti *et al.* 2006). High harvest index (HI) expresses successful plant reproduction and yield and assimilate partitioning towards grains. In most rainfed environments crop water deficit develops during the reproductive growth stage thus reducing HI. EUW by improving plant water status helps sustain, assimilate partitioning and grain yield (Merah 2001; Kirkegaard *et al.* 2007).

Since, WUE is a trait under multigenic control, it can be improved directly or indirectly by multiple transfer of genes involved in drought resistance, increasing photosynthesis etc. For example, in maize a C_4-PEPC overexpressing line, incorporated with the *Asr1* (a transcription factor linked to drought tolerant QTLs) gene showed a 30% increase in WUE (Jeanneau *et al.* 2002). Genes, *ERECTA* and *HARDY*, influencing WUE have been reported from *Arabidopsis* by Masle *et al.* (2005) and Karaba *et al.* (2007). The gene *ERECTA*, a putative leucine-rich repeat receptor like kinase is a major contributor to a locus for $\Delta^{13}C$ on *Arabidopsis* chromosome 2. It affects several processes including stomatal density, epidermal cell expansion, mesophyll cell proliferation which may explain its effect on transpiration efficiency. Karaba *et al.* (2007) showed that expression of Arabidopsis *HARDY* (*HRD*) gene, an AP2/ERF-like transcription factor, improved WUE by enhancing photosynthetic assimilation and reducing transpiration in rice. *HRD* overexpression in *Arabidopsis* and rice produced thicker leaves with more chloroplast bearing mesophyll cells, which probably contributed to enhanced photosynthesis assimilation and efficiency. However, the effect of these genes on grain yield needs to be examined as increased WUE is associated with thicker leaves and hence leaf expansion is influenced (Araus *et al.* 2008).

A promising approach is to integrate useful drought tolerance traits through genetic engineering into lines or varieties at the advanced breeding level for drought tolerance. Genes carrying these traits along with conservation agriculture techniques may be useful under water-limited conditions. Use of transgenics is both a challenge and an opportunity for public breeding programs. Over expression of stress-related genes has afforded some stress protection in transgenic plants. However, the results are not always consistent. Comprehensive physiological and biochemical testing of transgenic plants under stress conditions must be conducted, which requires a careful evaluation of the methods for assessing stress tolerance (Araus *et al.* 2008; Salekdeh *et al.* 2009). Desiccation and salt stresses applied by most researchers are ‘shock’ treatments. For most crops, drought tends to develop slowly as the soil dries. Plants that are subjected to drought conditions in this gradual manner accumulate solutes that maintain cell hydration and undergo complex adjustments in their morphology and photosynthetic characteristics (Khanna-Chopra and Sinha 1990; Blum 2005). Thus, to ensure that the responses of the transformed plants to water stress treatments are agronomically relevant, plants must be subjected to the same drought regime that crops experience in the field (Salekdeh *et al.* 2009). Molecular markers (such as restriction fragment length polymorphisms, RFLPs) could be linked to water use efficiency and other physiological traits such as osmotic adjustment (Jamaux *et al.* 1997).

The above approaches can be supplemented by newer techniques such as thermal imaging which permits automated, non invasive monitoring of evaporation at the leaf surface (Chaerle *et al.* 2005). Thermography reveals the temperature distribution of objects by visualizing their emitted long-wave infrared radiation. As water transport in plants is driven by leaf transpiration, thermal imaging can quantitatively estimate plant water usage. Thermography can also help to characterize transpiration kinetics in mutants isolated by alternative screening approaches. Thus, this technique provides new leads to the characterization of the signaling cascade for stomatal control. However, the quantitative yield parameters should be followed up simultaneously instead of solely relying on thermal imaging. In this respect, screening for alterations in chlorophyll fluorescence emission could help to highlight photosynthetic efficiency. Hence, plants can be subjected to multiple screening procedures where each screening can lead to a short listing of crops showing drought resistance. Recently, Henk van As and his team at Wageningen University in the Netherlands have developed a portable MRI for studying living plants, from tobacco to maize. So far his team has found that drought-susceptible and drought-resistant plants store water and move it around in different ways. This has immediate applications in agriculture for optimal growth of plants (van As 2006). Thus, multiple approaches can lead to a better integration of physiological traits into crop breeding programs for the development of cultivars that are better adapted to moisture deficit environments without a loss in yield potential.

REFERENCES

Angus JF, van Herwaarden AF (2001) Increasing water use and water use efficiency in dryland wheat. *Agronomy Journal* **93**, 290-298

Araus JL, Slafer GA, Royo C, Serret MD (2008) Breeding for yield potential and stress adaptation in cereals. *Critical Reviews in Plant Science* **27**, 377-412

Aroca R, Vernieri P, Ruiz-Lozano JM (2008) Mycorrhizal and non-mycorrhizal *Lactuca sativa* plants exhibit contrasting responses to exogenous ABA during drought stress and recovery. *Journal of Experimental Botany* **59**, 2029-2041

Babu RC, Nguyen BD, Chamarerk V, Shanmugasundaram P, Chezhian P, Jeyaprakash P, Ganesh SK, Palchamy A, Sadasivam S, Sarkarung S, Wade LJ, Nguyen HT (2003) Genetic analysis of drought resistance in rice by molecular markers: Association between secondary traits and field performance. *Crop Science* **43**, 1457-1469

Bahieldin A, Mahfouz HT, Eissa HF, Saleh OM, Ramadan AM, Ahmed IA, Dyer WE, El-Itriby HA, Madkour MA (2005) Field evaluation of transgenic wheat plants stably expressing the HVA1 gene for drought tolerance. *Physiologia Plantarum* **123**, 421-427

Basu PS, Sharma A, Garg ID, Sukumaran NP (1999) Tuber sink modifies photosynthetic response in potato under water stress. *Environmental and Experimental Botany* **42**, 25-39

Battilani A, Dalla Costa L, Lovatti L (2004) Water and nitrogen use efficiency of potato grown in a sub-humid area. *Acta Horticulturae* **664**, 63-70

Blum A (2005) Drought resistance, water-use efficiency, and yield potential – are they compatible, dissonant, or mutually exclusive? *Australian Journal of Agricultural Research* **56**, 1159-1168

Blum A (2009) Effective use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress. *Field Crop Research* **112**, 119-123

Borsani O, Cuartero J, Valpuesta V, Botella MA (2002) Tomato *tos1* mutation identifies a gene essential for osmotic tolerance and abscisic acid sensitivity. *The Plant Journal* **32**, 905-914

Chaerle L, Saibo N, Van Der Straeten D (2005) Tuning the pores: Towards engineering plants for improved water use efficiency. *Trends in Biotechnology* **23**, 308-315

Chen L-M, Li K-Z, Miwa T, Izui K (2004) Overexpression of a cyanobacterial phosphoenolpyruvate carboxylase with diminished sensitivity to feedback inhibition in *Arabidopsis* changes amino acid metabolism. *Planta* **219**, 440-449

Chen Y, Ji F, Xie H, Liang J (2006) Overexpression of the regulator of G-protein signalling protein enhances ABA-mediated inhibition of root elongation and drought tolerance in *Arabidopsis. Journal of Experimental Botany* **57**, 2101-2110

Chinnusamy V, Xiong L, Zhu JK (2005) Use of genetic engineering and molecular biology approaches for crop improvement for stress environments. In: Ashraf M, Harris PJC (Eds) *Abiotic Stresses: Plant Resistance through Breeding and Molecular Approaches*, The Haworth Press Inc., New York, pp 47-107

Chimenti CA, Marcantonio M, Hall AJ (2006) Divergent selection for osmotic adjustment results in improved drought tolerance in maize (*Zea mays* L.) in both early growth and flowering phases. *Field Crops Research* **95**, 305-315

Comstock JP, McCouch S, Martin BC, Tauer CG, Vision TJ, Xu Y, Pausch RC (2005) The effects of resource availability and environmental conditions on genetic rankings for carbon isotope discrimination during growth in tomato and rice. *Functional Plant Biology* **32**, 1089-1105

Condon AG, Farquhar GD, Richards RA (1990) Genotypic variation in carbon isotope discrimination and transpiration efficiency in wheat. Leaf gas exchange and whole plant studies. *Australian Journal of Plant Physiology* **17**, 9-22

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

Condon AG, Richards RA, Rebetzke GJ, Farquar GD (2004) Breeding for high water-use efficiency. *Journal of Experimental Botany* **55**, 2447-2460

Cortina C, Culiáñez-Macià FA (2005) Tomato abiotic stress enhanced tolerance by trehalose biosynthesis. *Plant Science* **169**, 75-82

Costa JM, Ortuño MF, Chaves MM (2007) Deficit irrigation as a strategy to save water: Physiology and potential application to horticulture. *Journal of Integrative Plant Biology* **49 (10)**, 1421-1434

Davies WJ, Bacon MA, Thompson DS, Sobeih W, Gonzalez RL (2000) Regulation of leaf and fruit growth on plants in drying soil: Exploitation of the plants, chemical signalling system and hydraulic architecture to increase the efficiency of water use in agriculture. *Journal of Experimental Botany* **51**, 1617-1637

de Wit CT (1958) Transpiration and crop yields. Institute of Biological and Chemical Research on Field Crops and Herbage. Wageningen, The Netherlands, *Verslag Landbouwkundig Onderzoek* **64 (6)**, 24 pp

Dichio B, Xiloyannis C, Nuzzo V, Montanaro G, Palese AM (2004) Postharvest regulated deficit irrigation of peach tree in a mediterranean environment: Effects on vegetative growth and yield. *Acta Horticulturae* **664**, 169-174

Donald CM (1962) In search of high yield. *Journal of Australian institute of Agricultural Science* **28**, 171-178

FAO (2003) Horticulture crops. In: *Selected Indicators of Food and Agriculture Development in Asia-Pacific Region 1999-2002*, RAP Publication - 2003/10, FAO Corporate Document Repository, pp 1-55

Farquhar GD, O'Leary MH, Berry JA (1982) On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Australian Journal of Plant Physiology* **9**, 121-137

Fischer RA, Turner NC (1978). Plant productivity in the arid and semiarid zones. *Annual Review on Plant Physiology* **29**, 277-317

Fleisher DH, Timlin DJ, Reddy VR (2008) Interactive effects of carbon dioxide and water stress on potato canopy growth and development. *Agronomy Journal* **100**, 711-719

Górny AG (1999) Effects of D-genome substitutions on the water use efficiency and response of the 'Langdon' durum wheat (*Triticum turgidum* L. var. durum) to reduced nitrogen nutrition. *Cereal Research Communications* **27**, 83-90

Guichard S, Bertin N, Leonardi C, Gary C (2001) Tomato fruit quality in relation to water and carbon fluxes. *Agronomie* **21**, 385-392

Hall NM, Griffiths H, Corlett JA, Jones HG, Lynn J, King GJ (2005) Relationships between water-use traits and photosynthesis in *Brassica oleracea* resolved by quantitative genetic analysis. *Plant Breeding* **124**, 557-564

Handley LL, Nevo E, Raven JA, MartInez-Carrasco R, Scrimgeour CM, Pakniyat H, Forster BP (1994) Chromosome 4 controls potential water use efficiency (δ^{13}C) in barley. *Journal of Experimental Botany* **45**, 1661-1663

Häusler RE, Hirsch H-J, Kreuzaler F, Peterhänsel C (2002) Overexpression of C_4-cycle enzymes in transgenic C_3 plants: A biotechnological approach to improve C_3-photosynthesis. *Journal of Experimental Botany* **53**, 591-607

Häusler RE, Rademacher T, Li J, Lipka V, Fischer KL, Schubert S, Kreuzaler F, Hirsch H-J (2001) Single and double overexpression of C_4-cycle genes had differential effects on the pattern of endogenous enzymes, attenuation of photorespiration and on contents of UV protectants in transgenic potato and tobacco plants. *Journal of Experimental Botany* **52**, 1785-1803

Hou X, Xie K, Yao J, Qi Z, Xiong L (2009) A homolog of human ski-interacting protein in rice positively regulates cell viability and stress tolerance. *Proceedings of the National Academy of Sciences USA* **106**, 6410-6415

Hsieh T-H, Lee J-T, Charang Y-Y, Chan M-T (2002) Tomato plants ecotopically expressing *Arabidopsis CBF1* show enhanced resistance to water deficit stress. *Plant Physiology* **130**, 618-626

Hukkeri SB, Pandey SL (1977) Water requirement and irrigation of crops. In: *Water Requirement and Irrigation Management of Crops in India*, Monograph No. 4 (new series). Water technology center, Indian Agricultural Research Institute, New Delhi, pp 163-273

Ito Y, Katsura K, Maruyama K, Taji T, Kobayashi M, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2006) Functional analysis of rice DREB1/CBF-type transcription factors involved in cold-responsive gene expression in transgenic rice. *Plant and Cell Physiology* **47**, 141-153

Iwama K, Yamaguchi J (2006) Abiotic stresses. In: Gopal J, Paul Khurana SM (Eds) *Handbook of Potato Production, Improvement and Post Harvest Management*, The Haworth Press. Inc, New York, pp 231-273

Jackson MT (2004) Evaluation and deployment of transgenic drought-tolerant varieties. IRRI Ref. No. DPPC 2004-73

Jamaux I, Steinmetz A, Belhassen E (1997) Looking for molecular and physiological markers of osmotic adjustment in sunflower. *New Phytologist* **137**, 117-127

Jeanneau M, Gerentes D, Foueillassar X, Zivy M, Vidal J, Toppan A, Perez P (2002) Improvement of drought tolerance in maize: Towards the functional validation of the *Zm-Asr1* gene and increase of water use efficiency by over-expressing C_4–PEPC. *Biochimie* **8**, 1127-1135

Jefferies RA (1989) Water-stress and leaf growth in field-grown crops of potato (*Solanum tuberosum* L.). *Journal of Experimental Botany* **40**, 1375-1381

Jefferies RA, MacKerron DKL (1989) Radiation interception and growth of irrigated and droughted potato (*Solanum tuberosum*). *Field Crops Research* **22**, 101-112

Jefferies RA, Mackerron DKL (1993) Responses of potato genotypes to drought. II. Leaf area index, growth and yield. *Annals of Applied Biology* **122**, 105-112

Johnson WC, Jackson LE, Ochoa O, Wijk RV, Peleman J, St. Clair DA, Michelmore RW (2000) Lettuce, a shallow-rooted crop, and *Lactuca serriola*, its wild progenitor, differ at QTL determining root architecture and deep soil water exploitation. *Theoretical and Applied Genetics* **101**, 1066-1073

Juenger TE, McKay JK, Hausmann N, Keurentjes JJB, Sen S, Stowe KA, Dawson TE, Simms EL, Richards JH (2004) Identification and characterization of the QTL underlying whole-plant physiology in *Arabidopsis thaliana*: Delta C^{13}, stomatal conductance and transpiration efficiency. *Plant Cell and Environment* **28**, 697-708

Kang Y, Wang FX, Liu HJ, Yuan BZ (2004) Potato evapotranspiration and yield under different drip irrigation regimes. *Irrigation Science* **23**, 133-143

Karaba A, Dixit S, Greco R, Aharoni A, Trijatmiko KR, Marsch-Martinez N, Krishnan A, Nataraja KN, Udayakumar Makarla, Pereira A (2007) Improvement of water use efficiency in rice by expression of *HARDY*, an *Arabidopsis* drought and salt tolerance gene. *Proceedings of the National Academy of Sciences USA* **104**, 15270-15275

Khanna-Chopra R (1999) Osmotic adjustment and yield stability in wheat genotypes and species grown in water limited environments. *Journal of Plant Biology* **26**, 173-178

Khanna-Chopra R, Sinha SK (1990) Genetic aspects of water relations and drought resistance in crops. In: Khanna KR (Ed) *Advances in Biochemical Genetics*, CRC Press, San Francisco, pp 153-178

Kirda C, Topcu S, Kaman H, Ulger AC, Yazici A, Cetin M, Derici MR (2005) Grain yield and N-fertilizer recovery of maize under deficit irrigation. *Field Crops Research* **93**, 132-141

Kirkegaard JA, Lilley JM, Howe GN, Graham JM (2007) Impact of subsoil water use on wheat yield. *Australian Journal of Agricultural Research* **58**, 303-315

Knipp G, Honermeier B (2006) Effect of water stress on proline accumulation of genetically modified potatoes (*Solanum tuberosum* L.) generating fructans. *Journal of Plant Physiology* **163**, 392-397

Ku MSB, Agarie S, Nomura M, Fukayama H, Tsuchida H, Ono K, Hirose S, Toki S, Miyao M, Matsuoka M (1999) High-level expression of maize phosphoenolpyruvate carboxylase in transgenic rice plants. *Nature Biotechnology* **17**, 76-80

Laza MR, Kondo M, Ideta O, Barlaan E, Imbe T (2006) Identification of quantitative trait loci for δ C-13 and productivity in irrigated lowland rice. *Crop Science* **46**, 763-773

Leegood RC (2002) C_4 photosynthesis – principles of CO_2 concentration and prospects for its introduction into C_3 plants. *Journal of Experimental Botany* **53**, 581-590

Lian H-L, Yu X, Ye Q, Ding X-S, Kitagawa Y, Kwak S-S, Su W-A, Tang Z-C (2004) The role of aquaporin RWC3 in drought avoidance in rice. *Plant and Cell Physiology* **45**, 481-489

Leskovar DI, Bang HJ, Franco JA, Perkins-Veazie P (2004) Deficit irrigation influences yield and lycopene content of diploid and triploid watermelon. *Acta Horticulturae* **628**, 147-151

Lin RK, Albert TL, Zhu Y, Tauer CG, Martin BC (1998) Identification of quantitative trait loci associated with water-use efficiency in tomato. In: *Plant and Animal Genome VI Conference*, San Diego, CA, January 18-22, Poster P255

Liu F, Shahnazari A, Andersen MN, Jacobsen SE, Jensen CR (2006) Physiological responses of potato (*Solanum tuberosum* L.) to partial root-zone drying: ABA signalling, leaf gas exchange, and water use efficiency. *Journal of Experimental Botany* **57**, 3727-3735

Liu Q, Kasuga M, Sakuma Y, Abe H, Miura S, Yamaguchi-Shinozaki K, Shinozaki K (1998) Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low-temperature-responsive gene expression, respectively, in *Arabidopsis*. *The Plant Cell* **10**, 1391-1406

Loudet O, Gaudon V, Trubuil A, Daniel-Vedele F (2005) Quantitative trait loci controlling root growth and architecture in *Arabidopsis thaliana* confirmed by heterogeneous inbred family. *Theoretical and Applied Genetics* **110**, 742-753

Loveys BR, Dry PR, Stoll M, Mc Carthy MG (2000) Using plant physiology to improve the water use efficiency of horticultural crops. *Acta Horticulturae* **537**, 187-197

Lynch DR, Tai GCC (1989) Yield and yield component response of eight potato genotypes to water stress. *Crop Science* **29**, 1207-1211

MacKerron DKL, Jefferies RA (1985) Observations on the effects of relief of late water stress in potato. *Potato Research* **28**, 349-359

MacMillan K, Emrich K, Piepho HP, Mullins CE, Price AH (2006) Assessing the importance of genotype × environment interaction for root traits in rice using a mapping population II: Conventional QTL analysis. *Theoretical and Applied Genetics* **113**, 953-964

Martin B, Nienhuis J, King G, Schaefer A (1989) Restriction fragment length polymorphisms associated with water use efficiency in tomato. *Science* **243**, 1725-1728

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

Masle J, Scott R, Gilmore, Graham FD (2005) The *ERECTA* gene regulates plant transpiration efficiency in *Arabidopsis. Nature* **436**, 866-870

Matsuoka M, Furbank RT, Fukayama H, Miyao M (2001) Molecular engineering of C_4 photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* **52**, 297-314

Merah O (2001) Potential importance of water status traits for durum wheat improvement under Mediterranean conditions. *Journal of Agricultural Sciences* **137**, 139-145

Mian MAR, Bailey MA, Ashley DA, Wells R, Carter Jr. TE, Parrott WA, Boerma HR (1996) Molecular markers associated with water use efficiency and leaf ash in soybean. *Crop Science* **36**, 1252-1257

Mian MAR, Ashley DA, Bailey MA (1998) An additional QTL for water use efficiency in soybean. *Crop Science* **38**, 390-393

Minhas JS, Bansal KC (1991) Tuber yield in relation to water stress at different stages of growth in potato (*Solanum tuberosum* L.). *Journal of the Indian Potato Association* **18**, 1-8

Moghaieb REA, Tanaka N, Saneoka H, Hussein HA, Yousef SS, Ewada MAF, Aly MAM, Fujita K (2000) Expression of betaine aldehyde dehydrogenase gene in transgenic tomato hairy roots leads to the accumulation of glycine betaine and contributes to the maintenance of the osmotic potential under salt stress. *Soil Science and Plant Nutrition* **46**, 873-883

Moinuddin, Khanna-chopra R (2004) Osmotic adjustment in chickpea in relation to seed yield parameters. *Crop Science* **44**, 449-455

Molinari HCB, Marur CJ, Bepalhok-Filho JC, Kobayashi AK, Pileggi M, Leite Júnior RP, Pereira LFP, Vieira LGE (2004) Osmotic adjustment in transgenic citrus rootstock Carrizo citrange (*Citrus sinensis* OSB. X *Poncirus trifoliate* L. Raf.) overproducing proline. *Plant Science* **167**, 1375-1381

Morgan JM, Condon AG (1986) Water use, grain yield and osmoregulation in wheat. *Australian Journal of Plant Physiology* **13**, 523-532

Nguyen TT, Klueva N, Chamareck V, Aarti A, Magpantay G, Millena AC, Pathan MS, Nguyen HT (2004) Saturation mapping of QTL regions and identification of putative candidate genes for drought tolerance in rice. *Molecular Genetics and Genomics* **272**, 35-46

O'Toole JC, Bland WL (1987) Genotypic variation in crop plant root systems. *Advances in Agronomy* **41**, 91-145

Oh S-J, Kim YS, Kwon C-W, Park HK, Jeang JS, Kim J-K (2009) Overexpression of the transcription factor AP37 in rice improves grain yield under drought conditions. *Plant Physiology* **150**, 1368-1379

Oh S-J, Song S I, Kim Y S, Jang H-J, Kim S Y, Kim M, Kim Y-K, Nahm BH, Kim J-K (2005) *Arabidopsis* CBF3/DREB1A and ABF3 in transgenic rice increased tolerance to abiotic stress without stunting growth. *Plant Physiology* **138**, 341-351

Park B-J, Liu Z, Kanno A, Kameya T (2005a) Genetic improvement of Chinese cabbage for salt and drought tolerance by constitutive expression of a *B. napus* LEA gene. *Plant Science* **169**, 553-558

Park B-J, Liu Z, Kanno A, Kameya T (2005b) Increased tolerance to salt- and water-deficit stress in transgenic lettuce (*Lactuca sativa* L.) by constitutive expression of LEA. *Plant Growth Regulation* **45**, 465-171

Park S, Li J, Pittman JK, Berkowitz GA, Yang H, Undurraga S, Morris J, Hirschi KD, Gaxiola RA (2005). Up-regulation of a H^+-pyrophosphatase (H^+-PPase) as a strategy to engineer drought-resistant crop plants. *Proceedings of the National Academy of Sciences USA* **102**, 18830-18835

Pereira JS, Chaves MM, Cladeira MC, Correia AV (2006) Water availability and productivity. In: Morison J, Croft M (Eds) *Plant Growth and Climate Change*, Blackwell Publishers, London, pp 118-145

Pérez-Alfocea F, Estañ MT, Caro M, Guerrier G (1993) Osmotic adjustment in *Lycopersicon esculentum* and *L. Pennellii* under NaCl and polyethylene glycol 6000 iso–osmotic stresses. *Physiologia Plantarum* **87**, 493-498

Passioura JB (2006) Increasing crop productivity when water is scarce – from breeding to field management. *Agricultural Water Management* **80**, 176-196

Rademacher T, Häusler RE, Hirsch H-J, Zhang L, Lipka V, Weier D, Kreuzaler F, Peterhänsel C (2002) An engineered phosphoenolpyruvate carboxylase redirects carbon and nitrogen flow in transgenic potato plants. *The Plant Journal* **32**, 25-39

Rao RCN, Udaykumar M, Farquar GD, Talwar HS, Prasad TG (1995) Variation in carbon isotope discrimination and its relationship to specific leaf area and ribulose-1,s-bisphosphate carboxylase content in groundnut genotypes. *Australian Journal of Plant Physiology* **22**, 545-551

Rebetzke GJ, Richards RA (1999) Genetic improvement of early vigour in wheat. *Australian Journal of Agricultural Research* **50**, 291-301

Rensink W, Hart A, Liu J, Ouyang S, Zismann V, Buell CR (2005) Analyzing the potato abiotic stress transcriptome using expressed sequence tags. *Genome* **48**, 598-605

Richards RA (2004) Physiological traits used in breeding of new cultivars for water-scarce environments. In: *New Directions for a Diverse Planet, Proceedings of the 4th International Crop Science Congress*, Brisbane, Australia, pp 1-12

Richards RA, Condon AG, Rebertzke (2001) Traits to improve yield in dry environment. In: Reynolds MP, Oritz-Monasterio JI, McNab A (Eds) *Application of Physiology in Wheat Breeding*, CIMMYT, Mexico, pp 88-100

Richards RA, Luckacs Z (2002) Seedling vigour in wheat-sources of variation for genetic and agronomic improvement. *Australian Journal of Agricultural Research* **42**, 111-121

Richards RA, Rebetzke GJ, Condon AG, van Herwaarden AF (2002) Breeding opportunities for increasing the efficiency of water use and crop yield in temperate cereals. *Crop Science* **42**, 111-121

Ritenour MA, Wardowski WF, Tucker DP (2003) Effects of water and nutrients on the postharvest quality and shelf life of *Citrus*. Horticultural Sciences Department, Florida Cooperative Extension Service, Institute of Food and Agricultural sciences, University of Florida. Doc No. HS942. Available online: http://edis.ifas.ufl.edu/CH158

Robins JS, Domingo CE (1956) Potato yield and tuber shape as affected by severe soil-moisture deficits and plant spacing. *Agronomy Journal* **48**, 488-492

Ruiz-Lozano JM, Porcel R, Aroca R (2006) Does the enhanced tolerance of arbuscular mycorrhizal plants to water deficit involve modulation of drought-induced plant genes? *New Phytologist* **171**, 693-698

Salekdah GH, Reynolds M, Bennett J, Boyer J (2009) Conceptual framework for drought phenotyping during molecular breeding. *Trends in Plant Science* **14**, 488-496

Schafleitner R, Gaudin A, Rosales ROG, Aliaga CAA, Bonierbale M (2007) Proline accumulation and real time PCR expression analysis of genes encoding enzymes of proline metabolism in relation to drought tolerance in Andean potato. *Acta Physiologiae Plantarum* **29**, 19-26

Schakel KA, Hall AE (1979) Reversible leaf movements in relation to drought adaptation of cow peas (*Vigna ungiculata* (L.) Walp). *Australian Journal of Plant Physiology* **6**, 265-276

Schroeder JI, Allen GJ, Hugouvieux V, Kwak JM, Waner D (2001) Guard cell signal transduction. *Annual Review of Plant Physiology and Plant Molecular Biology* **52**, 627-658

Sharp RE, Hsiao TC, Kuhn SW (1990) Growth of the maize primary root at low water potentials: II. Role of growth and deposition of hexose and potassium in osmotic adjustment. *Plant Physiology* **93**, 1337-1346

Sheshshayee MS, Bindumadhava H, Shankar AG, Prasad TG, Udayakumar M (2003) Breeding strategies to exploit water use efficiency for crop improvement. *Journal of Plant Biology* **30**, 253-268

Shock CC, Zalewski JD, Stieber TD, Burnett DS (1992) Early season water deficits on Russet Burbank plant development, yield, and quality. *American Potato Journal* **69**, 793-804

Sinha SK, Agarwal PK, Khanna-Chopra R (1985) Irrigation in India: A physiological and phenological approach to water management in grain crops. In: Hillel D (Ed) *Advances in Irrigation Science*, Academic Press, New York, pp 130-212

Sinha SK, Khanna R (1975) Physiological, biochemical and genetic basis of hetrosis. In: Brady NC (Ed) *Advances in Agronomy*, Academic Press, New York, pp 123-174

Spielmeyer W, Hyles J, Joaquim P, Azanza F, Bonnett D, Ellis ME, Moore C, Richards RA (2007) A QTL on chromosome 6A in bread wheat (*Triticum aestivum*) is associated with longer coleoptiles, greater seedling vigour and final plant height. *Theoretical and Applied Genetics* **115**, 59-66 **S**

Sreenivasulu N, Sopory SK, Kavi KIshore PB (2007) Deciphering the regulatory mechanisms of abiotic stress tolerance in plants by genomic approaches. *Gene* **388**, 1-13

Subbarao GV, Johansen C (2002) Transpiration efficiency: Avenues for genetic improvement. In: Pessarakli M (Ed) *Handbook of Plant and Crop Physiology*, Marcel Dekker Inc., New York, pp 835-855

Sugano S, Kaminaka H, Rybka Z, Catala R, Salinas J, Matsui K, Ohme-Takagi M, Takatsuji H (2004) Stress-responsive zinc finger gene ZPT2-3 plays a role in drought tolerance in petunia. *The Plant Journal* **36**, 830-841

Taniguchi Y, Ohkawa H, Masumoto C, Fukuda T, Tamai T, Lee K, Sudoh S, Tsuchida H, Sasaki H, Fukayama H, Miyao M (2008) Overproduction of C_4 photosynthetic enzymes in transgenic rice plants: An approach to introduce the C_4-like photosynthetic pathway into rice. *Journal of Experimental Botany* **59**, 1799-1809

Tang L , Kwon S-Y , Kim S-H , Kim J-S, Choi JS, Cho KY, Sung CK, Kwak S-S, Lee H-S (2006) Enhanced tolerance of transgenic potato plants expressing both superoxide dismutase and ascorbate peroxidase in chloroplasts against oxidative stress and high temperature. *Plant Cell Reports* **25**, 1380-1386

Tarara JM, Perez Pen JE, Keller M (2005) Using whole-vine photosynthesis to understand the effects of water deficit on premium wine grapes. *Acta Horticulturae* **689**, 301-307

Teulat B, Merah O, Sirault X, Borries C, Waugh R, This D (2002) QTLs for grain carbon isotope discrimination in field-grown barley. *Theoretical and Applied Genetics* **106**, 118-126

Tischner T, Allphin L, Chase K, Orf JH, Lark KG (2003) Genetics of seed abortion and reproductive traits in soybean. *Crop Science* **43**, 464-473

Trebejo I, Midmore DJ (1990) Effect of water stress on potato growth, yield and water use in a hot and a cool tropical climate. *Journal of Agricultural Sciences* **114**, 321-334

Thipyapong P, Melkonian J, Wolfe DW, Steffens JC (2004) Supression of polyphenol oxidase increases stress tolerance in tomato. *Plant Science* **167**, 693-703

Thomashow MF (1999) Plant cold acclimation: Freezing tolerance genes and regulatory mechanisms. *Annual Review of Plant Physiology and Plant Molecular Biology* **50**, 571-599

Thompson AJ (2005) Genetic control of root traits important for water use in horticultural crops. *Department of Environment, Food and Rural Affairs* 2005-2010

Thompson AJ, Andrews J, Mulholland BJ, McKee JMT, Hilton HW, Horridge JS, Farquhar GD, Smeeton RC, Smillie IRA, Black CR, Taylor IB (2007) Overproduction of abscisic acid in tomato increases transpiration efficiency and root hydraulic conductivity and influences leaf expansion. *Plant Physiology* **143**, 1905-1917

Tuberosa R, SalviI S, Sanguineti MC, Maccaferri M, Giuliani S, Landi P (2003) Searching for QTLs controlling root traits in maize: A critical appraisal. *Plant and Soil* **255**, 35-54

Umezawa T, Fujita M, Fujita Y, Yamaguchi-Shinozaki K, Shinozaki K (2006) Engineering drought tolerance in plants: Discovering and tailoring genes to unlock the future. *Current Opinion in Biotechnology* **17**, 113-122

van As H (2006) Portable scanner designed for plants. *New Scientist* **2546**, 20

van der Weele CM, Spollen WG, Sharp RE, Baskin TI (2000) Growth of *Arabidopsis thaliana* seedlings under water deficit studied by control of water potential in nutrient-agar media. *Journal of Experimental Botany* **51**, 1555-1562

van Loon CD (1981) The effect of water stress on potato growth, development and yield. *American Potato Journal* **58**, 51-69

Vasquez-Robinet C, Mane SP, Ulanov AV, Watkinson JI, Stromberg VK, Koeyer DD, Schafleitner Roland, Willmot DB, Bonierbale M, Bohnert HJ, Grene R (2008) Physiological and molecular adaptations to drought in Andean potato genotypes. *Journal of Experimental Botany* **59**, 2109-2123

Walker CD, Lance RCN (1991) The fractionation of ^{2}H and ^{18}O in leaf water of barley. *Australian Journal of Plant Physiology* **18**, 411-425

Wang Y, Ying J, Kuzma M, Chalifoux M, Sample A, McArthur C, Uchacz T, Sarvas C, Wan J, Dennis DT, McCourt P, Huang Y (2005) Molecular tailoring of farnesylation for plant drought tolerance and yield protection. *The Plant Journal* **43**, 413-424

Woodward FI, Lake JA, Quick WP (2002) Stomatal development and CO_2: Ecological consequences. *New Phytologist* **153**, 477-484

Xu X, Martin B, Comstock J, Vision T, Tauer C, Zhao B, Pausch R, Knapp S (2008) Fine mapping a QTL for carbon isotope composition in tomato. *Theoretical and Applied Genetics* **117**, 221-233

Xu Y, This D, Pausch R, Vonhof W, Coburn J Comstock J, McCouch S (2009) Leaf-level water use efficiency determined by carbon discrimination in rice seedlings: Genetic variation associated with population structure and QTL mapping. *Theoretical and Applied Genetics* **118**, 1065-1081

Yamaguchi-Shinozaki K, Kasuga M, Liu Q, Nakashima K, Sakuma Y, Abe H, Shinwari ZK, Seki M, Shinozaki K (2002) Biological mechanisms of drought stress response. *Japan International Research Center for Agricultural Sciences Working Report* **23**, 1-8

Zhang J, Nguyen HT, Blum A (1999) Genetic analysis of osmotic adjustment in crop plants. *Journal of Experimental Botany* **50**, 291-302

Zhang J, Zheng HG, Aarti A, Pantuwan G, Nguyen TT, Tripathy JN, Sarial AK, Robin S, Babu RC, Nguyen BD, Sakarung S, Blum A, Nguyen HT (2001) Locating genomic regions associated with components of drought resistance in rice: Comparative mapping within and across species. *Theoretical and Applied Genetics* **103**, 19-29

Zhang M, Barg R, Yin M, Gueta-Dahan Y, Leikin-Frenkel A, Salts Y, Shabtai S, Ben-Hayyim G (2005) Modulated fatty acid desaturation via overexpression of two distinct ω-3 desaturases differentially alters tolerance to various abiotic stresses in transgenic tobacco cells and plants. *The Plant Journal* **44**, 361-371

Zhang ZB, Xu P (2002) Review on QTL study of wheat traits. *Development of Research and Technology in the World* **1**, 52-58