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: Δ13C, osmotic adjustment, partial root drying, potato, Pn/Tr, tomato

Abbreviations: ABA, abscisic acid; AM, arbuscular mycorrhiza; Δ13C, 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; gs, stomatal conductance; TE, transpiration efficiency; VPD, vapor pressure deficit; WUE, water use efficiency; WUEDM, water use efficiency for dry matter production

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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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 (Santos et al. 2000). Water is used 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 (Hakkeri and Pandey 1977). Some crops like cotton (Gossypium hirsutum L.), groundnut (Arachis hypogaea L.) and mulches 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 evapotranspiration from soil (Rebetzke and Richards 1999; Richards and Luckacs 2002).

There are various morphological, anatomical, phenological, physiological and biochemical parameters, which can help crops 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 the plant to optimize exposure of leaf area to the incoming solar 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 dependent less on stomatal conductance (gsp) 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 Lahn 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 in many ways such as improving Pn/Tr ratio, higher 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 C₄ plants exhibit certain advantages compared to C₃ plants such as reduced photorespiration, and increased nitrogen and WUE. So attempts were made to transfer the advantages of C₄ photosynthesis into C₃ plants by breeding. The C₄ and C₃ species of Atriplex were hybridized but the independent inheritance of the genes conferring C₄ characteristics such as Kranz anatomy, elevated phosphoenolpyruvate carboxylase (PEPC) and low CO₂ 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 C₄ characteristics into C₃ 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 C₄ 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 C₄ type CO₂ concentrating mechanism in C₃ plant Hydrilla verticillata. The H. verticillata system has PEPC in the mesophyll and the decarboxylation of C₄ acids occurs in the chloroplast. It simply refixes photorespired CO₂ rather than having an active CO₂ concentrating mechanism.

Attempts have been made to over express maize PEPC in tobacco, potato and rice. The transgenic plants exhibiting in 5-fold higher PEPC activity, as well as those found in the aquatic plant Hydrilla verticillata. 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 (NADP-ME) either singly or in combination exhibited slight suppression of photorespiration. However, these are most likely a result of local changes in CO₂ concentration induced by increasing respiration (Häuśler et al. 2002). Hence, it seems unlikely that
attempts to introduce single cell C2O2 concentrating mechanisms will be successful without introducing some of the structural characteristics of C4 photosynthesis. Engineering a single-celled C4 system would require a reworking of C3 leaf structure (Leegood 2002). Taniguchi et al. (2008) produced rice overproducing four C4 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 reproducible stunting of the transgenic plants. The study indicated that overproduction of the four enzymes did not act to concentrate CO2 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 C4 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 C4 cycle in all of the crop plants intended to be used (Häusler et al. 2001).

When stomata open and CO2 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, 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 signaling 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 (ABI1/2) and probably also PPF2C mutants are defective in drought-induced stomatal closure. Mutations in the farnesyl transferase gene (ERA1) 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 (Cao 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 (Singh 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 have an important role in such crop water use efficiency. Deep root systems are 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’Toloe 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 the advanced benefit to maintain an adequate plant water supply 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 neces-
sary for the maintenance of root growth under water deficit as it helps in enhancing antioxidant defense and thus prevent membrane damage. Differences in ABA in OAs 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 conductance, root growth, and 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 chromosomes 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, root length, and root volume. 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 (Baba 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 mechanisms 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 (Sorghum vulgare L.), pigeon pea (Cajanus cajan L.), and Brassica species (Khana-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 QTLs is also conserved in the short arm 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 (Schalleitner et al. 2007). Osmotic adjustment was observed in tomato (Lycopersicon esculentum 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 (Perez-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 Culllianez-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...
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 HV A 1 gene showed drought tolerance and increased WUE in the field (Bahieldin et al. 2005) and in (ii) transgenic rice over-expressing transcriptional regulators AP37 and SKI Pi a 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 C₃ species for differences in TE. Plants discriminate against the naturally occurring and heavier isotope of carbon (¹³C) during the diffusion and fixation of CO₂ in photosynthesis, resulting in depletion of the ¹³C isotope of biomass. This deviation of carbon isotopic ratio (¹³C/¹²C) of biomass from that of air is called discrimination (Δ¹³C). WUE at the level of instantaneous leaf gas exchange (photosynthesis rate/transpiration rate) and stable carbon isotope discrimination (Δ¹³C) are independently linked to the partial pressure of CO₂ inside leaf to that in ambient air (p₁/pₐ). Based on theory, they suggested that Δ measured in plant dry matter should be positively correlated with the ratio of p₁/pₐ and negatively associated with WUE. This negative relation of Δ with WUE is robust for plants of many C₃ 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 (¹⁸O). The oxygen isotope enrichment is a good time averaged surrogate for transpiration rate and stomatal conductance at a given VPD. Hence, dual isotope ratio (Δ¹³C/Δ¹⁸O) 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 litres 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 m³/ha 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 the earlier harvested crops such as peaches (Dickio 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 (Citrus 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 sequets) (Ritoune 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 by transferring genes (*Solanum lycopersicum* 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 Jeffries 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 (ΨL) and turgor (ΨP) were less than –0.28 and 0.5 MPa respectively (Jeffries 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 (Jeffeiries and Mackerron 1989; Trebajo 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 assimilates (Bassu et al. 1997).

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/m³. 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 result 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 variables (determinant 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 WUEcm 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 that are sensitive 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 CO2 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 transcriptome 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 (for cbf response/dehydration responsive 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 SWP12 promoter. The 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 tomato 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 Δ13C 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 (m² 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 (WUE) was also selected for in a 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 Δ13C 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 use by early maturation (Mefford 1991). Karkegarian et al. (2006) have recognized as enhancing soil moisture capture and transpiration (Chimienti 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 (Mefford 1991).

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 C4-PEPC overexpressing line, incorporating with the Asr1 (a transpiration 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 Δ°C on Arabidopsis chromosome 2. It affects several processes including stomatal density, epidermal cell expansion, mesophyl 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 the transgene has affected reproduction 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 automatic, 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 (Borsani et al. 2005). 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.

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