

REVIEW ARTICLE

Advances and prospects: Biotechnologically improving crop water use efficiency

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Abstract

Bio-water saving can be defined as the reduction of crop water consumption employing biological measures. This is the focus of efforts to save water in agriculture. Different levels of water-use efficiency (WUE) have been developed. The genetic diversity of WUE has been confirmed in several crops. WUE is the basis of bio-watering and physiological WUE is the key. The degree to develop physiological WUE potential decides the performance of bio-watering in the field. During this process, fine management is important. Thus bio-watering is closely related to WUE. Crop WUE has improved and evolved as a result of breeding programs. Many WUE genes have been located in different genomic and aneuploid materials and have been mapped by various molecular markers in a number of crops. Two genes, (*Erecta* and *alx8*), which control water use efficiency; have been cloned in *Arabidopsis thaliana*. Eleven WUE genes have been identified by microarray analysis. Six genes associated with drought resistance and photosynthesis have been transferred into crops which have resulted in improving WUE and drought resistance. WUE is important on the basis of functional identification of more drought resistant gene resources. The popularity on the industrial-scale of transgenic plants is still in its infancy and one of the reasons for this is the lack of knowledge regarding molecular mechanisms and it is a very immature technology. Enhanced agricultural practices and the theoretical aspects of improving crop WUE have been developed and are discussed in this review paper. Rapid progress will be made in bio-water savings and that crop WUE can be substantially improved under both favorable and unfavorable water-limited environments. This will be achieved by a combination of traditional breeding techniques and the introduction of modern biotechnology

Keywords: Breeding, crops, drought tolerance, genetics, quantitative trait location (QTL), transgenic technology, water use efficiency

Introduction

The current reduction in the potential crop yield due to water stress is estimated to be 65% globally and in excess of 80% in many developing countries (Gleick, 1998). N. E. Borlaug (2000), a Nobel Prize winner for peace, said: "How can we continue to expand food production for a growing world population within the parameters of limited water availability? The answer is that humankind will need to bring about a 'Blue Revolution -more crops for every drop' in the 21st century to complement the Green

Revolution in the 20th century. Water use productivity must be wedded to land use productivity. Science and technology will be called upon to show the way."

There are many techniques for saving water, but their application faces many problems with respect to, for example, different soil and geographical conditions, and only a limited potential for improvement has been exploited (Zhang et al., 2007). Many research programs have been set up for improving water-use efficiency (WUE=productivity/water used, which is a

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general description) by the use of biological technology (Comstock, 2001; Ribaut, 2003; Tuberosa, 2003, 2005; Zhang et al., 2007). There are reports of work with *Triticum aestivum* L. (Zhang, 1998; Richards et al., 2002; Sheshshayee et al., 2003; Tuberosa, 2003, 2005), *Triticum vulgare* L. (Handley et al., 1994; Teulat et al., 2002; Ellis et al., 2002; Molnar et al., 2007), *Oryza sativa* L. (Fischer et al., 2003), *Zea mays* L. (Banziger et al., 2000), *Gossypium spp.* (Warwick et al., 2005; Saranga et al., 2001), *Glycine max* L. (Mian et al., 1996, 1998), *Lycopersicon esculentum* Mill (Martin et al., 1989; Lin et al., 2002), and *Brassica oleracea* (Hall et al., 2005). Compared with other methods for saving water, breeding new crop varieties with a high yield and WUE as well as drought-resistance has the following merits: less investment by the grower, greater uptake through low costs of technology transfer, sustainable efficiency; and more potential exploitation. Research on the fundamental aspects of biological water-saving, physiological and genetic improvement of crop WUE has a great promise for the future (Stanhill, 1992; Richards, 1987; Zhang et al., 1992, 2001, 2003, 2007; Bray, 1997; Araus, 2002; Shan et al., 2006). WUE is proportional to yield and drought resistance in most cases, which is also related to soil water status, and natural rainfall. The physiological-centered management in the field will play an active role in increasing yield and drought resistance. There are papers that review the physiology and basic breeding of plant WUE (Richards et al., 2002; Condon et al., 2002, 2004; Blum, 2005; Tambussi et al., 2007; Tuberosa et al., 2007) and genomics on drought resistance (Cattivelli et al., 2008), but no related paper reviews on improving crop WUE from genetics, biotechnology, and physiology together.

Our current paper will concentrate on crop genetics and improving crop WUE by potential biotechnological applications.

The definition and concept of water use efficiency (WUE) at different scales

WUE is a complex trait that is controlled by many genes that are related to physiological drought-resistance traits. In agronomic terms, WUE is equal to biomass yield, or economic yield, or economic value/amount of water used (Condon et al., 2002, 2004). There are three requirements: the first is that WUE is related to drought resistance and drought tolerance, and utilization of water with high efficiency but little biomass and yield under serious water stress; the second is that WUE is related to water-saving and the highly efficient use of water, medium drought resistance (tolerance), and medium or medium-high yield under moderate water stress. The third is that WUE is related to the highly efficient use of water and maximum potential yield. These three kinds of description for WUE basically reflect the nature of traditional crop WUE although they emphasize correspondingly different aspects (Zhang et al., 1992, 2001, 2003, 2007; Shao et al., 2010). Blum (2009) recently established the concept of

effective use of water (EUW), which is the most important determinant of plant production under most conditions of limited water supply. This originated when considering practical crop yield (Passioura, 2006). But in practice, this EUW is difficult to detect. For practical purposes, plant breeders targeting water-limited environments should consider the use and give reference to WUE and consider plant constitutive and adaptive traits that drive the EUW and the resultant dehydration avoidance as major traits for yield improvement in drought prone environments (Blum, 2009; Shao et al., 2010). From the viewpoint of agriculture management and physiological manipulation, we should pay more attention to physiological WUE, which is our focus of the current report (Zhang et al., 1992, 2001, 2003, 2007; Blum, 2009; Shao et al., 2010).

In terms of physiology, WUE is equal to the accumulation of assimilation products/amount of water used (transpiration, T), which reflects the energy conversion efficiency per unit of water used in the plant. WUE could be defined as encompassing three concepts: one is that leaf WUE (WUE_l) or transpiration efficiency (TE_l) is photosynthesis rate (P_n)/transpiration rate (T), where P_n and T are measured with suitable apparatus. The second is that WUE for the whole plant (WUE_p) is the weight of biomass or economic weight / amount of water used (ET , T). Under an evaporation controlled condition, WUE_p is the weight of biomass or economic weight / amount of water used (T), which can be considered as the whole-plant transpiration efficiency (TE_p). WUE_p or TE_p can be divided into above-ground WUE (WUE_{ls} , TE_{ls}), the biomass of leaves and stems or economic weight/amount of water used, and the below-ground WUE (WUE_r , TE_r), root weight/amount of water used, which is a useful parameter for tuberous root crops such as potato. WUE_p can be measured for both aquaculture and pot culture. The third is population WUE in the field (WUE_f), where WUE_f is the economic harvest or grain yield/ amount of water used (ET) (Zhang, 2003; Bacon, 2004; Toldi et al., 2009).

WUE is an important factor in the evaluation of drought resistance and drought tolerance of plant crops, and water-saving in a variety of water stress conditions. In general, however, drought resistance and drought tolerance are difficult to quantify in terms of efficiency of water use and yield (Moore et al., 2009; Nicotra and Davidson, 2010).

Genetic diversity and evolution of crop WUE

It has been known for some time that crop WUE is a multi-gene trait. Understanding the genetic determinants of crop WUE is very important for genetic improvement of crop WUE. Before the 1900s, few agronomists showed interest in understanding water use in different crops, and only 14 researchers had carried out studies related to water requirement (WR) or ET efficiency. King from the University of Wisconsin was the first in the United States to study the water required to grow field crops. His work, using small lysimeters in glasshouses and fields, was

done between 1890 and 1902. From 1911 to 1917, Briggs and Shantz studied WR of different plants throughout growth, and they studied corn, sorghum, millet, wheat, oat, barley, potato, alfalfa, and soybean (Briggs and Shantz, 1913, 1914; Shantz and Piemeisel, 1927; Tanner and Sinclair, 1983). These early studies revealed that there was genetic diversity in WUE between C3 and C4 plants, and the C4 plants had higher WUE than the C3 plants. Jones (1992) later attributed a WUE to photosynthetic species of C4 plants of about twice that of C3 species. Briggs and Shantz (1913) stated: "Measurable differences in the water requirement also exist between different varieties of the same crop, and this suggests the possibility of developing strains through selection, which are still more efficient in the use of water".

Single-leaf WUE ($\text{g CO}_2/\text{g H}_2\text{O}$) of several plants was studied by Fischer et al., (1978). In the early stages, many plant physiologists showed more attention to the photosynthetic rate, which is closely related to WUE_1 and high yield. But most studies showed that the photosynthetic rate had tended to decrease in wheat evolution. There were significant differences among wheat species with different chromosome ploidy. In general, the photosynthetic rate was reportedly highest in diploid species, intermediate in tetraploids, and lowest in hexaploid wheat (Khan and Tsunoda, 1970; Dunstone et al., 1973; Austin et al., 1982). Leaf area had tended to increase with chromosome ploidy levels, and modern hexaploid wheat lines generally have larger leaves and a lower photosynthetic rate than their progenitor species (Bhagsari and Brown, 1986). Johnson et al., (1987) studied the relationship among photosynthesis, stomatal conductance, and WUE_1 in various *Triticum* accessions at the tillering stage under growth-chamber conditions. The results show the average WUE_1 ($4.91 \mu\text{mol}/\text{mmol}$) of modern hexaploid varieties (TMA-1Sturdy; AABBDD) was greater than that of the diploid ($4.16 \mu\text{mol}/\text{mmol}$) (*T. tauschyi*, DD; *T. speltoides*, BB; two *T. monsoecum* accession, AA), which in turn was greater than that of the tetraploid ($4.14 \mu\text{mol}/\text{mmol}$) (five *T. dicoccoides* accession, AABB; *T. kotschyi*, VVS^v).

In studies with 15 genotypes of winter wheat (13 were planted in the central and southern Great Plains of the USA, and two were planted in the USSR) growing in well-irrigated fields there were significant genotype-related effects for photosynthetic rate per leaf area (CER), leaf conductance (g_s), leaf WUE_g ($= \text{CER}/g_s$), the ratio of intercellular $[\text{CO}_2]/\text{ambient } [\text{CO}_2]$, specific leaf weight, and concentration of chlorophyll (Morgan and Lecain, 1991). Among the genotypes with the lowest WUE_g were the two old, large-leaved Soviet genotypes, and Vona and Sturdy, the two oldest Great Plains genotypes were included in the study. The negative correlation of leaf size with CER and WUE_g was mostly due to the differences in leaf size and CER between the relatively small-leaved Great Plains genotypes and the large-leaved Soviet genotypes. Significant variations in WUE_g from 34 to $46.9 \mu\text{mol}/\text{mmol}$ among winter wheat genotypes were

attributed to genotype-related differences in both photosynthetic capacity and g_s (Morgan and Lecain, 1991).

The flag leaf WUE_1 (or TE_1) of 44 genotypes including wild species and cultivars, comprising five diploids (AA, DD, RR), seven tetraploids (AABB, AAGG), 13 hexaploids (AABBDD, AAAAGG, AABBRR), and one octaploid (AABBDDRR), seven varieties from irrigated land and 11 varieties from dryland, were studied under dryland field conditions. The result showed that the flag leaf WUE_1 of diploids and tetraploids increased as the wild species became domesticated. The flag leaf WUE_1 increased as the chromosome ploidy levels increased ($2x \rightarrow 4x \rightarrow 6x$) in wheat evolution. Among modern cultivars, the flag leaf WUE_1 of varieties for irrigated land are higher than for those for dryland (Zhang and Shan 1998). In the physiological changes of wheat WUE_1 ($2x \rightarrow 4x \rightarrow 6x$), the flag leaf area and stomata size have increased, but the frequency of stomata has decreased. This could explain the trend for decreases of stomata conductance (G), photosynthetic rate (P) and transpiration rate (T) and intercellular concentration of carbon dioxide (C_i) in wheat evolution. Although the photosynthetic rate and transpiration rate have decreased, the photosynthetic rate has decreased less than the transpiration rate through the optimum control of stomata. Therefore, the $\text{WUE}_1 (= P/T)$ increased during the evolution of wheat (Zhang and Shan, 1998; Zhang et al., 2000; Zhang, 2003).

The leaves of wild diploid wheat are small and grow slowly in the early stages of growth. The stoma frequency per unit area is high, which results in high rates of photosynthesis and transpiration, but whole-leaf photosynthetic productivity ($= \text{photosynthetic rate} \times \text{leaf area} \times \text{time stay green}$) and TE_1 are not high, thus biomass and grain weight, as well as WUE_p , are much lower. Modern hexaploid wheat has early vigor and larger leaves. The photosynthetic rate and transpiration rate are lower, but WUE_1 is high. Whole-leaf photosynthetic productivity is high, because the large, wide leaf area contributes greatly to photosynthetic productivity, and yields more biomass and transfer into grain, leading to a high harvest index (HI) and yield.

Since 1980 there have been many studies of the genetic diversity in leaf WUE, per plant WUE and field WUE in pots or rows and field experiments. Richards (1987) reported that there was no consistent difference of WUE (dry weight of aboveground biomass/amount of water used) between old and new varieties of wheat planted in large soil containers in the glasshouse. However, in those glasshouse experiments, domesticated wheat varieties had a higher WUE than their diploid and tetraploid ancestors. Siddique et al., (1990) reported that WUE_t of modern cultivars was higher than those of old varieties, by comparing nine varieties planted for 100 years in Australia. Improved WUE_t for grains of modern wheat cultivars is related to higher HI, which is realized mainly through reduction of plant height by the introduction of dwarfing genes.

Nienhuis et al., (1994) studied WUE (moles of carbon accumulated per mole of water used) of ecotypes and

recombinant inbred lines (RILs) of *Arabidopsis thaliana*, WUE of *Arabidopsis* ecotypes varied from 1.86 mg/g to 2.40 mg/g. Genetic variance for WUE of RILs was significant.

Zhang et al., (2002) reported that during the evolution of wheat from diploid to hexaploid, WUE_p increased in ten species and cultivars planted in soil rows. Zhang (2003) reported that because wild wheat diploid species had small leaves and grew slowly in the early stage of growth, stems elongated, the ear emerged, and they matured later than modern hexaploid cultivars, they produced much biomass but little grain yield post-anthesis, because their small plant size cannot cover the row space as early in the early growth stage, and thus evaporation of water from the soil was greater but the grain yield was less than that of modern hexaploid cultivars. Modern hexaploid dwarf cultivars have large leaves and produce more biomass pre-anthesis, and produce a large grain yield in the late stage from grain-filling to maturity. The roots of diploid species are thin and grow slowly; most roots do not penetrate deeply into the soil, thus these wild wheat species cannot obtain much water from deep soil. In contrast, modern hexaploid cultivars have big tillers; the roots are big, and grow deeply into the soil, thus the modern hexaploid cultivars can absorb more water from deep soil and use the water efficiently. Because modern cultivars have earlier vigor, higher yield, and HI than ancient species, the WUE_t of wheat species of different ploidy have increased during evolution from diploid to hexaploid modern dryland cultivars and from these on to modern irrigated land cultivars. Huang et al., (2007) reported that water consumption for transpiration decreased remarkably, correlated with the decline of the growth period, while grain yield, HI, WUE_p, N, P and K uptake efficiency, and N, P and K utilization efficiency increased significantly. Grain yield, HI and WUE_t decreased in the same order: *T. vulgare* (AABBDD) > *T. dicoccon* (AABB) > *T. dicoccoides* (AABB) > *Ae. tauschii* (DD) > *Ae. speltoides* (BB) > *T. boeoticum* (AA).

During the evolution of wheat from a diploid to a tetraploid species, the leaf size, stem diameter, stem biomass, plant height, seed size, spike weight, and whole plant biomass have increased, but the tiller number has decreased. Grain yield has increased markedly, mainly due to the increased biomass and spike weight. During evolution from a wheat hexaploid species to the modern cultivars, the plants have become significantly shorter, the leaf shape has become wider, shorter, and erect, the plant density has increased strikingly, and thus HI and grain yield as well as WUE_t have increased greatly (Zhang and Wan, 1992; Zhang, 1998, 2003, 2006).

There are four basic types of winter wheat cultivars in North China: The first type has a lower yield but uses less water, thus its WUE_t also is lower, most wild wheat species and old landraces are of this type. The second type has an intermediate to high yield and uses less water, thus its WUE_t is moderate, most tall and semi-dwarf cultivars that are usually planted in poor-quality, low-fertility dryland

are of this type. The third type has a high yield but uses intermediate amounts of water, thus its WUE_t is usually the highest, some dwarf and limited-irrigated-land varieties are of this type. The fourth type has the highest yield and uses the most water, but its WUE_t is only average, some fully irrigated-land varieties are of this type (Zhang and Wan, 1992; Zhang, 1998, 2003; Dong et al., 2003, 2005; Huang et al., 2003; Zhang et al., 2005; Zhao et al., 2006; Hu et al., 2006; Shao et al., 2007a,b; Khazaei et al., 2010).

There is a great deal of evidence that crop WUE has been improved by breeding and agronomic culture methods (Richards et al., 1987, 2002; Zhang, 1998, 2006; Sheshshayee et al., 2003; Zhang et al., 2003; 2007; Shan et al., 2006; Tuberosa et al., 2007; 2005). For example, in most winter wheat areas of Northwest China, annual precipitation has been about 500 mm for many years, but wheat yield has increased gradually from 750 kg/ha before 1950 to more than 3750 kg/ha. In China, WUE is often measured as WUE_{pr}, which is grain yield (kg)/ha/seasonal or annual precipitation (mm) in rainfed land. Calculation of WUE_{pr} for different crop cultivars under different conditions of rainfall is simple and rapid, but not very accurate. Wheat WUE_{pr} has increased from 0.1 kg/ha/mm before the 1950s to 0.5 kg/mm or even more in the dryland areas (Zhang, 2003).

In the North China Plain, winter wheat and maize are the two main staple crops in annual rotation. While annual ET has increased slightly, crop yield has improved by 50% and resulted in significant increases of WUE_t from 1982 to 2002. WUE_t has improved from 10 kg/ha/mm to 15 kg/ha/mm for winter wheat and from 14 kg/ha/mm to 20 kg/ha/mm for maize. This increased yield was associated with increased kernel numbers per unit area without alteration of the weight of the kernels for both winter wheat and maize. The number of kernels per spike of winter wheat was increased from ~22 for the cultivars used in the 1980s to ~28 for the cultivars in current use. The number of kernels per cob of maize was increased from ~300 for the cultivars used in the 1980s to ~450 at present (Zhang et al., 2005a,b,c). From this report, we can see the considerable impact of plant breeding on crop yield and WUE, especially in increasing the number of kernels per spike in wheat and maize. Crop breeders have improved crop yield and WUE more quickly than crop WUE has been increased in genetic studies (Zhang et al., 2007).

It is known that there is a large genetic diversity of drought resistance in rice. There are many lowland rice and upland rice landraces and cultivars in south China and south-eastern Asia. A famous upland rice variety was introduced from Brazil in 1990s and it is widely planted in China. There is a large genetic diversity of WUE in rice (Flowers et al., 1988;). Sheshshayee et al., (2005) reported that six contrasting genotypes selected and examined in a separate study showed good correspondence in both WUE_p and carbon isotope discrimination (Δ) between the experiments, indicating that WUE_p is genetically controlled in rice, and hence can be exploited

through breeding. So, we should attempt to improve crop drought-resistance and WUE for use in areas that are short of water and seasonal drought areas. On the basis of the above, it is easy to point out that three indices (yield, WUE, drought resistance) can be combined together in a suitable breeding program. By controlled experiments in the lab and in the field, high physiological WUE plants with higher drought resistance and yield are selected and analyzed at the chromosome and molecular level. On this basis, genomic and proteomic and metabolomic methodology is applied to find the targeted genes for breeding.

WUE gene location

It is difficult to measure the WUE of different breeding lines in large numbers under field conditions. Thus, there is a need to find an alternative (neutron probe and growth analysis) to the conventional approach (soil drilling) to measure the amount of water used and the WUE of field crops. Farquhar and Richards (1984) proposed that stable carbon isotope composition ($\delta^{13}\text{C}$) and carbon isotope discrimination (Δ) in the leaf during photosynthesis be measured in a C3 plant. The $\delta^{13}\text{C}$ in leaf tissue is negatively correlated with WUE in many crop species. A less negative $\delta^{13}\text{C}$ value implies an increased leaf WUE. Thus $\delta^{13}\text{C}$ or Δ can act as a representative for WUE.

Handley et al., (1994) combined the approaches of whole-shoot $\delta^{13}\text{C}$ and Chinese Spring-Betzes addition lines genetics and found that chromosome 4 controls potential water use efficiency ($\delta^{13}\text{C}$) in barley. In wheat-barley 4H(4D) disomic substitution lines, the genes located on the 4H chromosome of barley were able to increase the water use efficiency of the wheat substitution line, which is suitable for improving wheat drought tolerance through intergeneric crossing (Molnar et al., 2007).

An evaluation of the disomic additions of Imperial rye chromosomes to Chinese Spring wheat indicated that chromosome 2R increased the efficiency of water use and improved the rooting characteristics of the recipient wheat cultivar (Lahsaiezadeh et al., 1983; Shah, 1992). The translocation line 2AS.2RL of bread wheat Chinese Spring and Imperial rye surpassed Chinese Spring for grain yield, shoot biomass at maturity, root biomass, and efficiency of water use, especially under drought conditions (Lahsaiezadeh et al., 1983; Ehdaie et al., 1991; 1998). The 2AS.2RL translocation showed positive effects on WUE and on field performance, although different bread wheat and rye cultivars (Chinese Spring wheat and Imperial rye) were used to develop these translocations (Lahsaiezadeh et al., 1983; Ehdaie et al., 1991; 1998; 2003). Gorny et al., (1999) studied a complete set of the D-genome chromosome substitution lines in durum wheat and found that genes located on almost all the D-genome chromosomes could improve WUE only in vegetative tissues, and the strongest effects were related to chromosome 7D. Zhang et al., (1998, 2000a,b,c) reported that the order of flag leaf

WUE₁ of different chromosome genomes is AA > BB > DD > RR. Among 20 Chinese Spring ditelosomic lines, the flag leaf WUE₁ of the A ditelosomic group is the highest, and high WUE₁ genes are located on the 1AL, 2AS and 7AS chromosome arms. Among seven wheat-rye addition lines, the high WUE₁ genes were located on 4R chromosome, and the flag leaf WUE₁ of 5R addition lines is the lowest. The China Spring-Egyptian red substitution line was employed to identify the position of the genes controlling WUE_p, and located in genes on chromosomes 5A and 5D (Zhang et al., 2005a,b,c). Shahram (2005) reported that chromosome 1D is a possible location of a gene(s) controlling variation between wheat varieties for Δ and ET efficiency under water-stress conditions.

Molecular markers for the WUE gene

Martin et al., (1989) were the first to combine molecular markers and $\delta^{13}\text{C}$ technology to identify genes potentially associated with WUE in tomato. They found that 70% of the variation in Δ between *Lycopersicon esculentum* Miller (drought-sensitive tomato with high Δ) and *Lycopersicon pennellii* (Cor.) D'Arcy (drought-tolerant wild tomato with low Δ) was associated with three restriction fragment length polymorphisms (RFLP), loci that were mapped on linkage groups B, F and Q.

Masle et al., (1992) reported a negative relation between WUE and leaf ash across a range of C3 species. This response would depend on the maintenance of constant concentration of minerals in the transpiration stream. Mian et al., (1996) combined leaf ash and RFLP technologies to identify quantitative trait loci (QTL) associated with WUE in soybean. They found that six RFLP markers were associated with WUE_p, of the six, one is on LG15 (G), two are on LG17 (H), and three are on LG 18(J); marker cr497-1 (LG18) explained the largest amount of variation (13.2%) of all markers, and the parent "Young" allele at this locus contributes to the greater WUE of progeny lines. Ten markers are associated with leaf ash. Seven of these markers are on chromosome LG 15(G), one is on LG18 (J), one is on Lg 20 (L), and one is on LG26 (R). Leaf ash is significantly and negatively correlated with WUE_p, and two QTLs are associated with both WUE_p and leaf ash. For each of these QTLs, the allele for increased WUE_p is associated with reduced leaf ash. Mian et al., (1998) identified two previously unreported additional QTLs (one on LG C1, and one on LG L) in another population, and one QTL on LGL that explains 14% of the variation of WUE_p.

The QTLs for the rate of leaf appearance in seedlings at Bmag318 and stable isotope composition of seedling shoot ($\delta^{13}\text{C}$) and root ($\delta^{15}\text{N}$), and grain nitrogen composition were all located at *sdw1* on chromosome 3H in barley (Ellis et al., 2002).

Zhang et al., (2000–2002) reported that 114 wheat recombinant inbred lines (RIL) derived from Opata 85 and W7984 were used to study QTL of WUE in a growth chamber. Two QTL controlling WUE₁(P/T)

were detected, one on chromosome 1A and one on chromosome 6D, which explain 11.48% and 14.84% of the variation of WUE_p , respectively. Ten QTLs had a significant effect per plant WUE_p (or TE_p), and two of them are located on the A genome (4A, 7A), four on the B genome (3B, 5B) and four on the D genome (3D, 6D). Six QTLs significantly control WUE_{is} , two of them are on the A genome (2A, 4A), three on the B genome (3B), and one on chromosome 6D. Two pairs of interacting QTL affecting WUE_{is} were identified on chromosomes 1A-1D and 4A-5A. Five QTLs significantly control WUE_r , three of them (2A, 3A, and 4A) are on the A genome, and two (2B and 5B) are on the B genome. Three pairs of interacting QTLs influencing WUE_r were identified on chromosomes 3A-3D, 3A-6A, and 7A-7B. Most of the QTLs controlling different kinds of WUE were detected on the A genome. There are gene clusters consisting of two or four QTLs linked closely on chromosomes 1A, 3B, 4A, and 6D.

A doubled haploid (DH) population of 150 lines, derived from a cross between two Chinese common wheat varieties, Hanxuan10 and Lumai14, was used in this study to map QTLs for WUE and related traits, at the seedling stage under conditions of water stress. The result revealed that a total of 14 QTLs with an additive effect for the target traits were mapped on chromosomes 2A, 3A, 4A, 5A, 6A, 7A, 1B, 3B, and 3D, most of them located on the A genome. Moreover, some QTLs, such as CWM461.1 and P8422-170, may be used to perform marker-assisted selection (MAS) in wheat breeding (Zhou et al., 2005; Sylvester et al., 2009).

Hausmann et al., (2004) showed that genetic variation and genotype of *A. thaliana* to be related significantly to water use by mapping QTL in two recombinant inbred populations. These studies documented QTL effects on stomatal conductance, whole-plant TE , and patterns of whole-plant water loss. Juenger et al., (2005, 2006) reported that five QTLs affect flowering time and five QTLs affect $\delta^{13}C$, but two genomic regions contained QTLs with effect on both traits in *A. thaliana*. These experiments revealed allelic effects on $\delta^{13}C$ through the upstream trait of stomatal conductance, with consequences for both TE_p and water loss.

There are many reports of complex relations between $\delta^{13}C$ or Δ and WUE, as well yield under different water stress conditions in different ecotypes and genotypes (Johnson and Rumbaugh, 1995; Condon et al., 2002). In several crop species, Δ has been shown to be linked to drought tolerance in terms of WUE and yield stability in drought-prone environments and has been used successfully to identify genotypes with better drought tolerance. For example, eight out of ten barley QTLs that co-segregated for Δ co-located with QTLs for physiological traits related to plant water status and/or osmotic adjustment (Teulat et al., 2002). In *B. oleracea*, stable carbon isotope discrimination had QTL located with WUE_p , photosynthetic capacity with leaf thickness, and nitrogen content and stomatal density with leaf thickness

(Hall et al., 2005). In peanuts, WUE_i is correlated with the SPAD chlorophyll meter reading (SCMR), specific leaf area (SLA), and Δ . These traits can be used as surrogates for selecting for WUE (Chuni et al., 2006).

In other crop species, there is a lack of association of $\delta^{13}C$ and Δ with WUE or yield. For example, Lin et al., (2002) identified QTL associated with WUE and related traits in *Lycopersicon*; they found 28 QTLs for WUE and related traits located on 11 chromosomes, and among them a few markers were linked to two or three traits, suggesting either pleiotropic effects or closely linked genes for different traits; but a common marker for WUE and Δ was not detected. In cotton, among 11 QTLs conferring genetic differences in $\delta^{13}C$, three (LGD04, LGD05, and Chr 22) were specific to the water-limited environment and one (Chr 15) affected relative $\delta^{13}C$ (GH allele conferring higher stability across environments). The lack of association of $\delta^{13}C$ with productivity under the water-limited environment warrants special attention. We cannot discount the possibility that more severe water deficits may confer a selective advantage to $\delta^{13}C$. Although such an advantage may improve the fitness of wild plants, its economic value in crops may be small or even non-existent (Saranga et al., 2001).

Sometimes, for wheat under moderate levels of drought stress, there is a positive correlation between Δ and yield; if a higher WUE occurs due to lower stomatal conductance, water use can be restricted, leading to smaller yields in spite of a higher WUE (Condon et al., 2002; Shao et al., 2007a,b).

WUE-related gene cloning and identification

The linkage between Rubisco activase and plant WUE may provide additional insight into the role of the biochemistry of photosynthesis in the control of plant WUE. Four cDNA clones have been isolated from a high WUE cDNA library of tomato (Zhu et al., 1998); however, these results have not been confirmed. Masle et al., (2005) reported the isolation of a gene that regulates TE in *A. thaliana*, *ERECTA*. a putative leucine-rich repeat receptor-like kinase (LRR-RLK), known for its effects on inflorescence development (Torii et al., 1996; Lease et al., 2001) is a major contributor to a locus for Δ on the *Arabidopsis* chromosome 2, which, depending on growth conditions, explained 21–64% of the total phenotypic variation in Δ . Mechanisms include, but are not limited to, effects on stomatal density, epidermal cell expansion, mesophyll cell proliferation, and cell-cell contact. The *alx8* mutant in *A. thaliana* is drought tolerant, exhibits improved water-use efficiency, and a number of drought-tolerance genes are up-regulated. Additionally, *alx8* demonstrates the complexity of ABA-dependent and ABA-independent transcriptional networks, as some components in both pathways are up-regulated in *alx8*. This study provides evidence for common steps in both the drought and high light (HL) stress response pathways (Rossel et al., 2006; Reynolds et al., 2009; Richards et al., 2010).

Xu et al., (2006) used a microarray to identify genes related to drought resistance and TE in wheat. The expression levels of 11 of the 18 genes were positively correlated with the high TE trait, measured as Δ . These data indicate that some of these TE differentially expressed genes are candidates for investigating processes that underlie the high TE trait or for use as expression quantitative trait loci (eQTLs) for TE.

If major loci that directly control WUE in crops are identified in future, more WUE genes will be cloned through chromosome walking cloning, chromosome microdissection and microcloning, orthologous gene cloning and other gene cloning methods. More WUE genes will be available as transgenes for improving crop WUE and yield in the future.

Transgenic crops for improving WUE

To date, there are six transgenic cases of improving plant WUE directly. The ABA-responsive barley gene *HVA1* was introduced into spring wheat. The transgenic *HVA1* lines had significantly higher WUE_p (Sivamani et al., 2000). A second candidate gene, *Asr1*, a putative transcription factor, as explained was co-linear with drought tolerance QTLs and modified CO_2 fixation rates in leaves through changes of the activity of C_4 phosphoenolpyruvate carboxylase (C_4 -PEPC). The highest C_4 -PEPC over-expressing maize line exhibited an increase (+30%) in intrinsic WUE_i accompanied by a dry weight increase (+20%) under moderate drought conditions. A third candidate gene, *mrp5-1*, is associated with stomatal movement; transgenic *mrp5-1 Arabidopsis* plants have increased WUE_i (Klein et al., 2003, Ghasem et al., 2009). A fourth candidate gene, encoding an NAPD-malic enzyme linked to a guard cell or an epidermal cell promoter, can modulate WUE or productivity in the plant. A fifth candidate gene, *HRD*, an AP2/ERF-like transcription factor identified by a gain-of-function *Arabidopsis* mutant *hrd-D* having roots with enhanced strength, branching, and cortical cells, exhibits drought resistance and salt tolerance. Although *HRD* over-expression in *Arabidopsis* produces thicker leaves with more chloroplast-bearing mesophyll cells, in rice there is an increase in the leaf biomass and bundle sheath cells that probably contributes to the enhanced photosynthesis assimilation and efficiency. The results exemplify application of a gene identified from the model plant *Arabidopsis* for the improvement of WUE_p coincident with drought resistance in rice (Karaba et al., 2007). A sixth candidate gene, the transcription factor *DREB1A* from *A. thaliana*, was transferred into peanut. All transgenic events except one achieved higher TE_p under well-watered conditions, and this appeared to be explained by a lower stomatal conductance. Under conditions of limited water, one of the selected transgenic events showed 40% higher TE_p than the untransformed control (Pooja et al., 2007)

As we know from <http://www.plantstress.com/biotech/index.asp>, there are about more than 200 transgenic

cases for improving plant abiotic stress tolerance; meanwhile, many multi-function genes may improve tolerance of drought stress, WUE, and tolerance of other abiotic stress. For example, transcription factor *DREB* was transferred into wheat in CMMYT, and the transgenic wheat showed good drought tolerance (Pellegrineschi et al, 2002). In future, we believe that more and more genes directly controlling WUE and associated with drought resistance and yield will be transferred into crops for improving productivity in both favorable and unfavorable environments.

Discussion on practices and theory of crop breeding for improving WUE

In future water shortage will be a major factor for irrigated field crops like rice (Fischer et al., 2003) and maize (Banziger et al., 2000; Yoo et al., 2009; Zhang et al., 2009), limiting the amount and frequency of irrigation. Therefore, there will be a demand for new varieties with drought resistance and high WUE and yield, which will reduce the requirements for irrigation.

Meanwhile, in semiarid areas, new agronomic methods for water and soil conservation have been adopted in rainfed fields in the last decades, such as plastic and crop stubble mulching, and water-conserving tilling technology, to retain more rainfall and decrease evaporation from the soil. Here also, new drought-resistant varieties with medium or medium high yields and high WUE will be necessary for dryland farming (Zhang and Wan, 1992; Sheshshayee et al., 2003; Zhang et al., 2001, 2009; Zhang, 2003, 2006; Shan et al., 2006; Wang et al., 2010).

A high WUE breeding program should aim to combine high-yield traits, high WUE, and drought-resistance traits in one variety, and such water-saving crop breeding will be important for both limited irrigated land and dryland farming (Zhang and Wan, 1992; Zhang, 1998, 2003; Zhang et al., 2007). Trethowan et al., (2002) also suggested that plant breeders should select for drought resistance as well as the ability to respond to high rainfall.

Besides the breeding and introduction of new cultivars, yield-increasing methods such as improvements in agriculture, more irrigation, and increased application of fertilizers have contributed some important effects on improving crop WUE and yield in dryland and limited irrigated land (Zhang and Wan, 1992; Zhang, 1998, 2003; Zhang et al., 2005a,b,c; Zhang et al., 2001, 2003, 2007, 2009).

Many new wheat varieties have been introduced, and high-yield breeding programs are in place. There are many wheat varieties that have been bred under high-input irrigated conditions but planted in semiarid dryland environments. For example, the high-yield wheat variety Zhengyin 24 was introduced from Canada in the 1970s, and was widely planted in semiarid dryland farms in China before the 1980s. From the 1960s until now, many new winter wheat varieties with good cold stress resistance and high yield have been bred in Beijing under irrigation; for example, Fengkang 13, which has high yield

and multiple resistance to cold, drought, salinity, and other abiotic stresses, was planted in many provinces to the south of Beijing (Zhang and Wan, 1992; Zhang, 1998, 2003, 2006; Zhang et al., 2007).

This situation is somewhat similar in Australia. The proportion of Australian wheat area used for growing varieties carrying some CIMMYT (in Mexican) semi-dwarf germplasm rose from approximately 5% in 1975 to 65% in 1986. These CIMMYT spring wheat varieties grown in Australia do not lack drought tolerance, although they were developed under high input and non-stress conditions. Furthermore, the residual effects of high yield potential appear to have conferred an advantage to this germplasm over locally selected cultivars, although there is an obvious difference between Mexican and Australia environments (Edmeades et al., 1989).

It is interesting, but not surprising, that genetic gains in yield potential made by selection in predictable irrigation environments, have resulted in broadly adapted wheat cultivars that are often well suited to both favorable and low-yield rainfed environments (Sayre et al., 1995; Cooper et al., 1996; Trethowan et al., 2002). This occurs because genetic variation in traits that contribute to a high yield in all environments, such as high HI, is greater in predictable, high-output environments, and thus more likely to be selected under favorable conditions (Richards et al., 2002).

Modern crop breeding technology for the selection of water-saving varieties is in place in China. The varieties from irrigated land with a high yield and high WUE are often crossed with dryland varieties with good drought resistance but low yield, in order to combine high yield genes and high WUE and drought resistance genes in one new variety. In the water-saving wheat breeding program, generations are selected alternatively under irrigation and under dryland conditions to develop varieties with high yield and high WUE in a favorable environment as well as a stable yield with good drought resistance in an unfavorable environment (Zhang and Wan, 1992; Zhang, 1998, 2003, 2006).

Many studies have shown that although high yield potential is the target of most crop breeding programs, it might not be compatible with superior drought resistance. High yield potential can contribute to yield in moderate stress and irrigation environments (Zhang and Wan, 1992; Bray, 1997; Richards et al., 2002; Condon et al., 2002; Sheshshayee et al., 2003; Zhang, 2003; Blum, 2005; Passioura, 2006, 2007). A higher WUE is generally achieved by changing plant traits and environmental responses that reduce yield potential, thus improved WUE is expressed in improved yield under water-limited conditions only when there is a need to balance crop water use against a limited and known soil moisture reserve (Blum, 2005; Shao et al., 2007–2009).

In semiarid area, it is difficult to improve the high yield potential in dryland, but wheat breeders pursue the aim to select for medium or medium-high yield potential and high WUE in wheat improvement programs under

dryland and water-saving irrigation conditions. Many studies have shown that wild or ancient wheat species and landraces have better drought resistance than modern cultivars under water stress conditions, but do not have higher yield potential and WUE, and are not planted widely in dryland farming (Richards et al., 1987; Johnson, 1987; Zhang and Wan, 1992; Zhang, 2003). So, we think that field WUE is a quantitative index; it includes the traits of drought resistance, drought tolerance, water saving, high WUE, more yield, and so on. WUE breeding can unify drought resistance and high yield; therefore crop breeding for medium or medium-high yield and high WUE may be more important than selective breeding for drought resistance in semiarid dryland and water-saving irrigation conditions. In the past, both medium-high yield and WUE traits have been improved more than drought resistance in drought resistance crop breeding in semiarid area (Zhang and Wan, 1992; Zhang, 1998, 2003, 2006; Shao et al., 2007a,b, 2008a,b,c; Khan et al., 2010).

Drought resistance is a complex adaptation trait associated with grain yield under water stress conditions, but it may not be a component of crop yield potential. So, we must select a new genotype that has drought resistance in a water shortage season and uses water efficiently in a rainfall season and produces biomass quickly in the vegetative stage in the short rainfall period, and moves more carbohydrate, protein or oil into grain yield in the reproductive stage. We believe that improving the yield potential in terms of large ears of grain and high HI, and traits underlying WUE, especially morphological traits such as deep root systems that can absorb more water from the deep soil, and meanwhile increase grain yield greater than the amount of water used, which has had a key role in wheat breeding under both favorable and unfavorable conditions in the past (Office of Technology Assessment 1983; Edmeades et al., 1989; Zhang 1998; Banziger et al., 2000; Araus et al., 2002; Richards et al., 2002; Sheshshayee et al., 2003; Fischer et al., 2003; Condon et al., 2004; Warwick et al., 2005; Blum, 2005; Slafer et al., 2005; Tambussi et al., 2007; Tuberosa et al., 2007; Cao et al., 2009; Shao et al., 2007–2010; Salekdeh et al., 2009).

Concluding remarks

Food production must be increased to meet the projected global demands. The global climate change tendency and decreasing fresh water resources have led plant breeders to target drought-resistant varieties for dried environments. WUE is indeed an important index for evaluating plant resistance at physiological and potential yield scale. Increasing crop drought tolerance is the core issue for modern agriculture under global climate change. Crop WUE is one of the most agronomic characters, which is under the control of multi-genes and is not easily measured (Shao et al., 2009). $\delta^{13}\text{C}$ or Δ , ash of different organs of the plant as representing traits that stand for different levels of WUE have been studied widely in many plants

Table 1. Some potential genes for increasing plant WUE. (see <http://www.plantstress.com/biotech/index.asp> for concrete reference examples)

Gene	Gene action	Species	Phenotype performance
adc	Polyamine synthesis	Rice	Drought resistance/better WUE
Apo-Inv	Apoplastic invertase	Tobacco	Salt tolerance, high "osmotic pressure"
AtGolS2	Galactinol and raffinose accumulation	Arabidopsis	Reduced transpiration, better WUE
AHAL3	Phosphoprotein phosphatase	Tobacco	Improved salt, osmotic and Lithium tolerance of cell cultures
AHAL3a	Phosphoprotein phosphatase	Arabidopsis	Regulate salinity and osmotic tolerance and plant growth
ATPS1	Trehalose-6-phosphate synthase	Tobacco	Drought resistance/better WUE/sustained photosynthesis
BADH-1	Betaine aldehyde dehydrogenase	Tomato	Maintenance of osmotic potential
betA	Choline dehydrogenase (glycinebetaine synthesis)	Maize	Drought resistance at seedling stage and high yield after drought/better WUE
CMO	Choline monoxygenase (glycine betaine synthesis)	Tobacco	Better in vitro growth under salinity and osmotic (PEG6000) stress
codA	Choline oxidase (glycine betaine synthesis)	Arabidopsis	Increased stress tolerance
codA	Choline oxidase (glycine betaine synthesis)	<i>Brassica juncea</i>	Tolerance to stress induced photoinhibition/better WUE
COX	Choline oxidase (glycine betaine synthesis)	Rice	Salt and 'stress' tolerance
Ect A...ect C	Edtoin accumulation in chloroplasts	Tobacco	Salt and cold tolerance
IMT1	Myo-inositol o-methyltransferase (D-ononitol synthesis)	Tobacco	Better CO2 fixation under salinity stress. Better recovery after drought stress
LWR1,	Solute accumulation (proline)	Arabidopsis	Increasing growth, osmotic adjustment, water status
LWR2			
mt1D	Mannitol-1-phosphate dehydrogenase (mannitol synthesis)	Tobacco	Increased plant height and fresh weight under salinity stress/better WUE
mt1D	Mannitol-1-phosphate dehydrogenase (mannitol synthesis)	Wheat	Drought and salinity tolerance of calli and plants
Osm1...Osm4	Osmotin protein accumulation	Tobacco	Drought and salt tolerance in plant water status and proline accumulation/better WUE
Osmyb4	Cold induced transcription factor	Arabidopsis	Accumulation of compatible solutes
Osmyb4	Cold induced transcription factor	Tomato	Drought but not cold resistance
otsA	Trehalose-6-phosphate synthase (trehalose synthesis)	Tobacco	Increased leaf dry weight and photosynthetic activity under drought/Increased carbohydrate accumulation/ better WUE
otsB	Trehalose-6-phosphate synthase (trehalose synthesis)	Tobacco	Increased leaf dry weight and photosynthetic activity under drought/Increased carbohydrate accumulation/ better WUE
P5CS	Pyroline carboxylate synthase (proline synthesis) (tomato)	Citrus	Osmotic adjustment and drought resistance/better WUE
P5CS	Pyroline carboxylate synthase (proline synthesis)	Petunia	Drought resistance and high proline/better WUE
P5CS	Pyroline carboxylate synthase (proline synthesis)	Rice	Increased biomass production under drought and salinity stress/better WUE
P5CS	Pyroline carboxylate synthase (proline synthesis)	Rice	Resistance to water and salinity stress
P5CS	Pyroline carboxylate synthase (proline synthesis)	Soybean	Resistance to osmotic stress and heat
P5CS	Pyroline carboxylate synthase (proline synthesis) (tomato)	Soybean	Drought resistance, high RWC, high proline
P5CS	Pyroline carboxylate synthase (proline synthesis) (tomato)	Sugarcane	Drought resistance via antioxidant role of proline
P5CS	Pyroline carboxylate synthase (proline synthesis)	Tobacco	Increased biomass production / enhance flower development under salinity stress /better WUE
P5CS	Pyroline carboxylate synthase (proline synthesis)	Wheat	Drought resistance due to antioxidative action
pdcl	Pyruvate decarboxylase overexpression	Rice	Increased submergence tolerance
PPO	Polyphenol oxidase suppression	Tomato	Drought resistance
SPE	Spermidine synthase	Arabidopsis	Chilling, freezing, salinity, drought hyperosmosis
TaCRT	Ca2+-binding protein	Tobacco	Better water status/WUE / membrane stability
TPS; TPP	Trehalose synthase	Arabidopsis	Drought, freezing, salt and heat tolerance
TPS1	Trehalose synthase	Potato	Delayed wilting under drought
TPS1 & TPS2	Trehalose synthase	Tobacco	Maintenance of water status under drought stress/better WUE

(Zhang, 2003, Bacon, 2004, Araus et al., 2008, Cao et al., 2009, Agbicodo et al., 2009) for the study of genetic diversity, gene location, and molecular markers of plant WUE. However, the relationships between different kinds of WUE and these factors are not always consistent in different crop varieties or under different conditions (Salekdeh et al., 2009; Ruan et al., 2010). So, a reliable technology that can measure plant WUE directly and quickly needs to be developed, which should include physiological, biochemical, genetic, and related stressed indicators. So, a combination of more disciplines such as soil science, crop physiology, plant molecular biology, biotechnology, and agricultural management is necessary. Genes controlling the different kinds of WUE need to be located and cloned. Thus more attention should be focused on functional genomics and proteomics, as well as gene networks of plant WUE and drought resistance (Sinozaki et al., 2007; Shao et al., 2007–2009; Trethowan and Mujeeb-Kazi, 2008; Deyholos, 2010). We look forward to finding more major genes controlling WUE directly, and to gene cloning and production of transgenic plants as well as MAS breeding for improving WUE, but all this work must be combined with field experiments. Now, gene transfer for transcription factors will play potential roles in solving the above issue. Some potential genes for increasing plant WUE have been summarized in Table 1 for further work reference. It is predicted that rapid progress in biological water saving will be made, and crop WUE can be improved substantially under both favorable and unfavorable water-limited environments by traditional breeding and new biotechnology with global attention and input. Traditional breeding will also play roles in this aspect. Modern biotechnology will shorten the breeding cycle. However, most basic disciplines are also important to explore the refined details for plants and soil environment at different levels. Microarrays provide a combination of technical and practical advantages that make them the most widely used platform for transcriptional profiling, and these advantages justify their continued use until major improvements are made in the efficiency of proteomics technology and until the availability of the next-generation sequencing technology expands. Microarrays have had significant effects on collecting drought-resistant gene resources and provided insights physiological antidrought mechanisms. No doubt, a biotechnological concrete service for world agriculture to increase food supply and quality is the greatest challenge for scientists in the 21st century.

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Declaration of interest

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the article.

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