Why has breeding for water use efficiency not been successful? An analysis and alternate approach to exploit this trait for crop improvement

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Despite the realization about the importance of water use efficiency (WUE) in crop improvement, the available genetic variability in this trait has not been successfully exploited through breeding. Selection for high WUE often resulted in decrease in crop growth rates (CGR) and this was perhaps the most significant setback for further improvement in this trait. Variations in WUE are brought about by stomatal diffusive characteristics ($g_s$) and/or intrinsic photosynthetic capacity ($g_m$). Most often plants have evolved to maximize WUE through a reduction in transpiration that is linked with the $g_s$. Since dry matter production is strongly associated with total transpiration (T), any reduction in T results in reduced CGR. Since $g_s$ is associated both with T and internal CO₂ partial pressure (Pi), WUE and T become strongly inter-dependent. However, if the variations in Pi and hence WUE are brought about by $g_m$, the inter dependency between T and WUE will be lesser and selection for WUE from such types will result in higher crop growth rates. Determination of $g_m$ is difficult unlike estimation of $g_s$. We propose a novel approach to estimate the time averaged mesophyll efficiency based on the carbon and oxygen isotope discrimination that occurs during photosynthesis and transpiration. And we discuss the importance of $g_m$ types (capacity types) in improving WUE as well as total dry matter (TDM).

Plants have naturally evolved several adaptive mechanisms for survival under water-limited conditions. However, from the agronomical point of view, the concept of drought resistance is linked to superior crop growth rates (CGR) under water-limited conditions. Hence, water harvesting and its utilization assume greater importance. The latter is often referred to as water use efficiency (WUE) or transpiration efficiency (TE). In view of its importance in crop improvement, especially for rain-fed situations, significant progress has been made in recent years in understanding the physiological basis and the variability in WUE and its environmental control.

Though the existence of genetic variability in WUE was shown as early as in 1914 by Briggs and Shantz, tremendous progress in assessing the genetic variability in WUE was possible after the advent of carbon isotope discrimination ($\Delta$) as a powerful technique that can be employed as a dependable, time averaged, surrogate estimate of WUE. Several of our studies as well as those elsewhere have, since then confirmed the existence of significant genetic variability in WUE and its association with $\Delta$ (Figure 1).

With the advent of this rapid technique, several attempts were made to exploit the available genetic variability in WUE in crop improvement. Though WUE is an important component of yield in the model proposed by Passiouros, (seed yield = WUE×T×HI), genetic variability in WUE could not be exploited through breeding. Many such attempts were not successful since improvement in WUE was often associated with reduced dry matter accumulation and yield. This lack of success arises primarily due to a strong interdependency between transpiration and WUE. Since WUE and T directly influence growth rates, interdependency between these traits is not desirable. Therefore it is essential to identify types where this interdependency is lower.

WUE is predominantly regulated by the stomatal diffusive factors ($g_s$) and/or mesophyll efficiency ($g_m$) that regulates carbon assimilation. In this paper we provide evidences to show that variability in WUE brought about by intrinsic differences in $g_m$ is desirable. In such cases WUE and T will be less dependent on each other. We also provide evidences to show that WUE is indeed a potential trait for crop improvement if $g_m$-dependent

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Figure 1. Relationship between whole plant WUE (g kg⁻¹) and carbon isotope discrimination ($\Delta$) in a few genotypes of three grain legume crops. WUE was estimated by gravimetric methods between 35 and 65 days after sowing (DAS).
types are identified. However, determination of $g_m$ is difficult. We propose a novel approach based on stable isotope discrimination to assess the physiological traits ($g_s$ and $g_m$) associated with WUE. If high $g_s$ types are identified, it would be possible to improve WUE as well as crop growth rates.

Before answering the question, why breeding for WUE could not improve crop yields, one needs to adequately analyse the relative significance of the physiological factors that regulate WUE. The two important physiological traits that determine the variability in WUE are photosynthetic rate ($A$), responsible for the dry matter production and the transpiration rate. $A$ is regulated by the intrinsic mesophyll efficiency ($g_m$) and the CO$_2$ diffusive process associated with the stomata ($g_s$). Transpiration rate, on the other hand, is predominantly controlled by the differences in $g_s$ at a given vapour pressure difference ($v$). These two physiological traits also determine the CO$_2$ partial pressure in the mesophyll inter-cellular spaces (Pi) which is directly related to WUE (Figure 2). Depending on the extent of contribution of $g_s$ or $g_m$ to Pi and hence WUE, species and genotypes can be classified as $g_s$-dependent (conductance types) or $g_m$-dependent (capacity types) (Table 1).

Since $g_s$ is strongly related to both transpiration rate and Pi, the total water used (T) and WUE become inter-dependent. This inter dependence will be stronger when Pi and hence WUE, are predominantly regulated by the $g_s$. In such types, increase in WUE results in lower T and hence total biomass (Figure 3).

Evidences available in literature also suggest that the genetic variability in WUE is predominantly controlled by the stomatal factors in several species (wheat$^4$, tomato$^1$, phaseolus$^2$, forage grass species$^{13,14}$ and cotton$^5$). Further, the moisture stress-induced increase in WUE is often associated with greater reduction in $g_m$ than in $A$.$^{16-20}$

In $g_s$-dependent types, the stomatal conductance is invariably lower, resulting in lower transpiration rate. This moisture conservation trait, though relevant under stress, often results in a significant decline in total transpiration. Low $g_s$ in these types also decreases the carbon flux and hence $A$ will be less.$^{14}$ Therefore, in $g_s$-dependent types, total transpiration per unit leaf area will be low and so will be the net assimilation rate (NAR).

In our studies with conductance types such as cowpea and chickpea, both in container and mini lysimeters, mean transpiration rate was inversely related to WUE (Figure 4). Since the variation in leaf area in these experiments was not marked, the low $g_s$ types also showed low total transpiration. Since stomatal control of WUE is predominant in these species, total transpi-

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**Table 1.** The WUE traits associated with TDM in two contrasting groups of genotypes

<table>
<thead>
<tr>
<th>Author</th>
<th>Crop</th>
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<tbody>
<tr>
<td>Wright et al.$^{24}$</td>
<td>Groundnut$^a$</td>
<td>Condon et al.$^{42}$</td>
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<td>Martin and Thorstenson$^{41}$</td>
<td>Tomato$^c$</td>
</tr>
<tr>
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<td>Groundnut$^d$</td>
<td>White et al.$^{12}$</td>
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</tr>
<tr>
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<td>Groundnut$^d$</td>
<td>Johnson and Tieszen$^{44}$</td>
<td>Grasses$^{ac}$</td>
</tr>
<tr>
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<td>Cowpea$^c$</td>
<td>Acevedo$^{44}$</td>
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<td>White$^a$</td>
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<td>Ehdai et al.$^{41}$</td>
<td>Wheat$^a$</td>
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<td>Johnson and Bisset$^{41}$</td>
<td>Grasses$^{ac}$</td>
<td>Richards and Condon$^{46}$</td>
<td>Wheat$^a$</td>
</tr>
<tr>
<td>Matus et al.$^{5}$</td>
<td>Canola$^d$</td>
<td>Meinzer et al.$^{41}$</td>
<td>Coffee$^c$</td>
</tr>
<tr>
<td>Sun et al.$^{55}$</td>
<td>Spruce$^c$</td>
<td>Gutierrez and Meinzer$^{23}$</td>
<td>Coffee$^c$</td>
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<td></td>
<td></td>
<td>Porter and Farquhar$^{46}$</td>
<td>24 wild species$^c$</td>
</tr>
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<td></td>
<td></td>
<td>Lu et al.$^{15}$</td>
<td>Cotton$^c$</td>
</tr>
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$^a$, $A$ is positively related to $g_s$.

$^{ac}$, TDM is negatively related to WUE.

$\dagger$, TDM is positively related to $A$.

$\ddagger$, TDM is inversely related to $A$.

$\star$, TDM and $A$ have no relationship.
rion was inversely related (Figure 5). Because of the relationship between total transpiration and total dry matter (TDM), a weak association or a negative trend between TDM and WUE can be expected in conductance types (Figure 5).

In view of this, selection for high WUE in g\(_m\)-dependent types often resulted in lower biomass (Table 1). This could have been the major bottleneck in achieving success while improving WUE.

In capacity types, mesophyll factors (g\(_m\)) associated with \(\Delta\) determine the variability in WUE. So, in these capacity types, WUE is independent of g\(_s\), and hence T will not be associated with WUE. Selection for high WUE from such types will result in high CGR (Figure 3). WUE as well as yield levels can be increased only when capacity types are used in breeding programmes.

Reviewing the existing literature on these aspects considering the physiological traits associated with WUE, we could categorize species as capacity types and conductance types (Table 1). Among the capacity types, often, the TDM was linearly related to WUE at comparable light interception efficiencies.

In several of our recent studies where genetic variability was examined at whole plant level in groundnut and in navybean (Wright, 1995—pers. commun.), we noticed that WUE was independent of g\(_s\). Mean transpiration rate was not related either with \(\Delta\) or with WUE. However, NAR was strongly related to WUE (Figure 6). We recently demonstrated an inverse relationship between Ribulose 1,5-bisphosphatecarboxylase/oxygenase (RuBisCO) content and \(\Delta\) among groundnut genotypes (Figure 7). This suggests that the variability in \(\Delta\) and hence in WUE could be brought about by the mesophyll capacity\(^{21}\). Similar relationships were noticed by others when RuBisCO content was altered in transgenic plants with an antisense construct for the small sub unit of RuBisCO\(^{22}\) or by increasing the leaf nitrogen status\(^{23}\), reiterating the role of mesophyll efficiency in determining \(\Delta\) and WUE.

In groundnut, a capacity type, at a given leaf area, we observed that total water use will not be lower despite selection for higher WUE. Hence, total dry matter and WUE were positively related (Figure 8) and an inverse relationship was noticed between \(\Delta\) and TDM (data not shown). Similar results were also reported by Hubick and Gibson\(^{24}\) in groundnut genotypes both under stress and nonstress conditions and more recently in spruce\(^{25}\). It implies both from our results as well as of several others elsewhere that WUE can be determined

![Diagram](image_url)

**Figure 3.** Inter-relationship between WUE, T and stomatal and mesophyll conductances in determining biomass production.

![Graph](image_url)

**Figure 4.** Genetic variability in WUE as a function of mean transpiration (g of water dm\(^{-2}\) leaf area day\(^{-1}\)) in cowpea and chickpea. Each data point is a mean of at least two replicates. The experiment was conducted with plants grown in containers or mini lysimeters at 100% field capacity.
by $g_m$. In such types, selection for higher WUE will be accompanied by higher growth rates and yield.

It is evident from the foregoing discussion that the capacity types are desirable. To identify the capacity types we need to accurately determine $g_m$ besides quantifying the variations in WUE. Unlike stomatal conductance, determination of $g_m$ is difficult because of the complexity in its regulation.

Often, $g_m$ is indirectly estimated by determining the initial slope of the CO$_2$ response curves ($dA/dP_i$)$^{25}$ or by determining certain mesophyll components such as RuBisCO content and its activity$^{22,27}$. Yet another

![Figure 5. Plot illustrating the association of WUE with total transpiration (g LAD$^{-1}$ over a period between 35 and 65 DAS) and TDM in a few genotypes of cowpea and chickpea.](image-url)
approach to arrive at the carboxylation efficiency is to assess the change in Pi at a given g. Since Pi is a function of g and g_m, variations in Pi at a given g should reflect the g_m. That is, at a given g, Pi will be less if the mesophyll efficiency is higher. Recently we have shown that the ratio of Pi to g significantly correlates with mesophyll efficiency and hence can be considered as a good reflection of g_m (refs 28, 29).

However, da/dCi or Pi/g_s ratios being gas exchange measurements, cannot be used as estimates of mean g_m integrated over a period of time. Therefore, a time averaged determination of Pi and g_s becomes essential. Carbon isotope discrimination by plants (Δ) has been shown to be an estimate of Pi, integrated over time6,30. We propose here an alternate approach to determine the mean g_s by determining the tissue 18O composition.

Recently it has been shown that the leaf water and CO₂ are enriched with the heavy isotope of oxygen (18O) during transpiration31,33. The extent of 18O enrichment in leaf or chloroplast water has been shown to be dependent on transpiration rate31,33. Initial experiments conducted at our centre also revealed that leaf water was enriched with 18O when plants were exposed to higher vapour pressure deficits (Table 2).

Depending on the 18O composition of leaf water, cellulanic 18O also changes. This is brought about by the dynamic equilibrium between leaf water and intercellular CO₂ which, in turn, is used as a substrate for primary carboxylation31,36. The other possibility of 18O entering into cellulose is the carbonyl hydration reaction of the intermediate of cellulose biosynthesis37.

Irrespective of the reactions that lead to the entry of 18O into cellulose, it is certain that the 18O enrichment in leaf water results in a high cellulosic 18O composition. From this point, 18O composition in cellulose could be a reflection of transpiration rate. In a recent study.
**Table 2. Effect of vapour pressure deficit on $^{18}O$ enrichment ($^{18}O$ in parts per mil) in leaf water in sunflower and cowpea**

<table>
<thead>
<tr>
<th>Species</th>
<th>18 mbars</th>
<th>12.5 mbars</th>
<th>7.2 mbars</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sunflower</td>
<td>31.20</td>
<td>28.05</td>
<td>26.42</td>
</tr>
<tr>
<td>Cowpea</td>
<td>32.51</td>
<td>29.15</td>
<td>27.65</td>
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Farquhar et al.\textsuperscript{31} demonstrated an increase in $\Delta H^{18}O$ in leaf water at enhanced transpiration rates altered by $v$. This also resulted in an increase in $\Delta^{18}O$, further substantiating the relationship between transpiration and $^{18}O$ composition. The difference in $g_s$ at a given $v$ may enrich differently the leaf chloroplast water $^{18}O$ and, hence possibly, cellulose $^{18}O$ composition. Among genotypes with similar canopy architecture, cellulose $^{18}O$ at a given $v$ may still reflect the variations in $g_s$. From this context, leaf water or cellulose $^{18}O$ composition could be a potential tool to arrive at the mean $g_s$ of the canopy integrated over time.

As explained earlier, though several approaches exist for the determination of $g_m$, recently our observations support the hypothesis that the mesophyll efficiency can be arrived at by determining the ratio of $P_i$ to $g_s$ (ref. 28).

Change in $P_i$ levels is an integrated function of both $g_s$ and $g_m$ at steady state levels. However, at a given $g_s$, changes in $P_i$ are brought about by $g_m$. Therefore, the ratio of $P_i$ to $g_s$ can be considered as a good reflection of $g_m$. This warrants the simultaneous determination of time-averaged estimates of both $P_i$ and $g_s$.

$P_i$ integrated over time can be quantified by measuring the $\Delta^{13}C$. From the recent evidences of the inter-relation between $^{18}O$ enrichment and transpiration, it can be inferred that the leaf cellulose $^{18}O$ composition could be a good time-averaged estimate of $g_s$ at a given $v$. Since $\Delta^{13}C$ and $^{18}O$ are time-integrated estimates of $P_i$ and $g_s$, respectively, the $\Delta^{13}C/\Delta^{18}O$ ratio is a good reflection of $P_i/g_s$ integrated over time.

As the dual isotope discrimination ratio reflects the physiological traits integrated over a period of time, this can be used as a potential tool to quantify the physiological traits contributing to the variations in WUE. With appropriate corrections for the leaf energy budgets and $v$, the leaf $^{18}O$ and $^{13}C$ composition can give a good estimate of $g_s$ and $g_m$.

Though the importance of WUE has long been felt, greater success was not encountered in breeding for improvement in this trait. Most often, such attempts resulted in reduced biomass yields, since WUE was more regulated by transpiration. It is clear from the foregoing discussion that breeding for WUE is relevant only if WUE and $T$ are independent of each other. Lower interdependency between these traits is possible only when WUE is determined by mesophyll capacity, thus signifying the importance of capacity types. We hypothesize that the ratio of dual isotope discriminator that occurs during photosynthesis and transpiration, can be a dependable time-averaged estimate of $g_m$.

Analysis of habitat-use using ordination: The Nilgiri tahr in southern India

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Most conventional analyses relating to habitat use by large vertebrates are built on a univariate framework. Although they afford the major advantage of being simple to perform and interpret, they fail to take into account the interacting effects of multiple habitat variables on animal habitat use. Using data on the Nilgiri tahr (Hemitragus hylaecorus), an endangered mountain goat of the Western Ghats, this paper presents a simple ordination technique, Principal Components Analysis, to analyse habitat use. We demonstrate that the distribution of all-male herds correlate with better foraging opportunities, and the distribution of female herds correlate with better security from predation. We also present an index to assess the relative importance of different variables in determining habitat use in the tahr. Finally, we encourage a wider application of such simple multivariate analyses in large animal ecology.

Knowledge of how an animal selects and uses its habitat is essential to many ecological studies, particularly in planning conservation strategies for rare and endangered species. Biologists studying large vertebrates have traditionally described animal habitat-use by considering individual resources in an animal’s habitat such as food, water, and cover as variables. Then, using various measures of resource selection, they examine degrees of selection of these variables, or their proportionate use relative to availability in the animal’s habitat. Resource selection indices and availability-use analyses adequately describe whether an animal selects or avoids individual features of its habitat. These analytical techniques, however, suffer from certain drawbacks in describing animal habitat-use.

First, they assume an a priori knowledge of what constitutes significant habitat variables for an animal—a precondition that is seldom satisfied for many species. Second, knowledge of an animal’s apparent preference or avoidance of a particular habitat variable yields no indication of its importance relative to another habitat variable that is similarly preferred or avoided. Finally, in describing habitat-use, these analytical techniques consider individual variables as disparate features of an animal’s habitat. Animal habitat-use, however, is a multi-dimensional concept involving several interacting variables in the organism’s physical and behavioural

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