

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 CO_2 partial pressure (P_i), WUE and T become strongly inter-dependent. However, if the variations in P_i 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 conditions¹⁻⁴. 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 Shantz⁵, tremendous progress in assessing the genetic variability

in WUE was possible after the advent of carbon isotope discrimination (Δ) as a powerful technique that can be employed as a dependable, time averaged, surrogate estimate of WUE⁶. 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 Δ (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 Passioura⁷, (seed yield = WUE \times T \times 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 yield⁸. 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

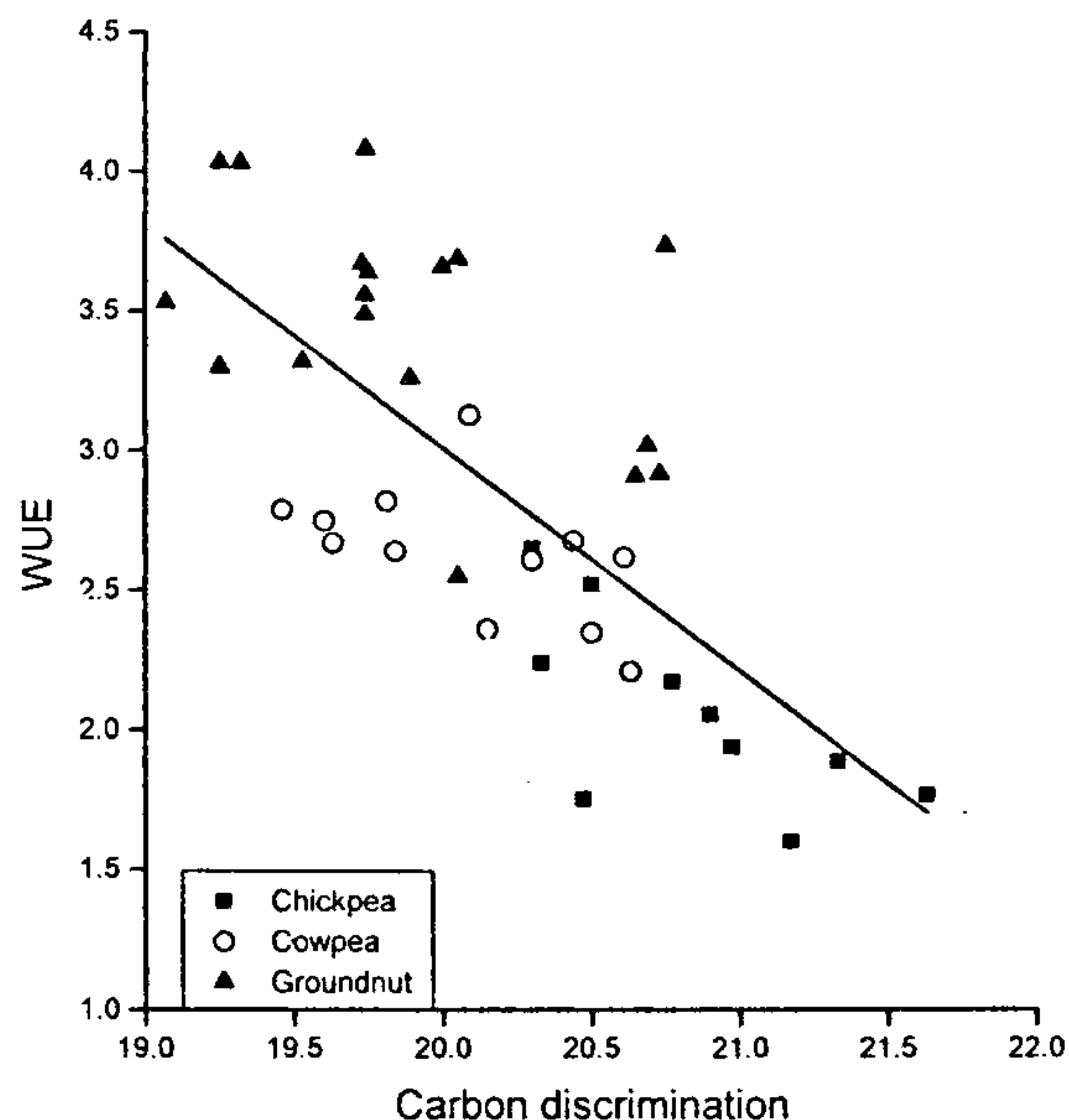


Figure 1. Relationship between whole plant WUE ($g\ kg^{-1}$) and carbon isotope discrimination (Δ) in a few genotypes of three grain legume crops. WUE was estimated by gravimetric methods between 35 and 65 days after sowing (DAS).

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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_m 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

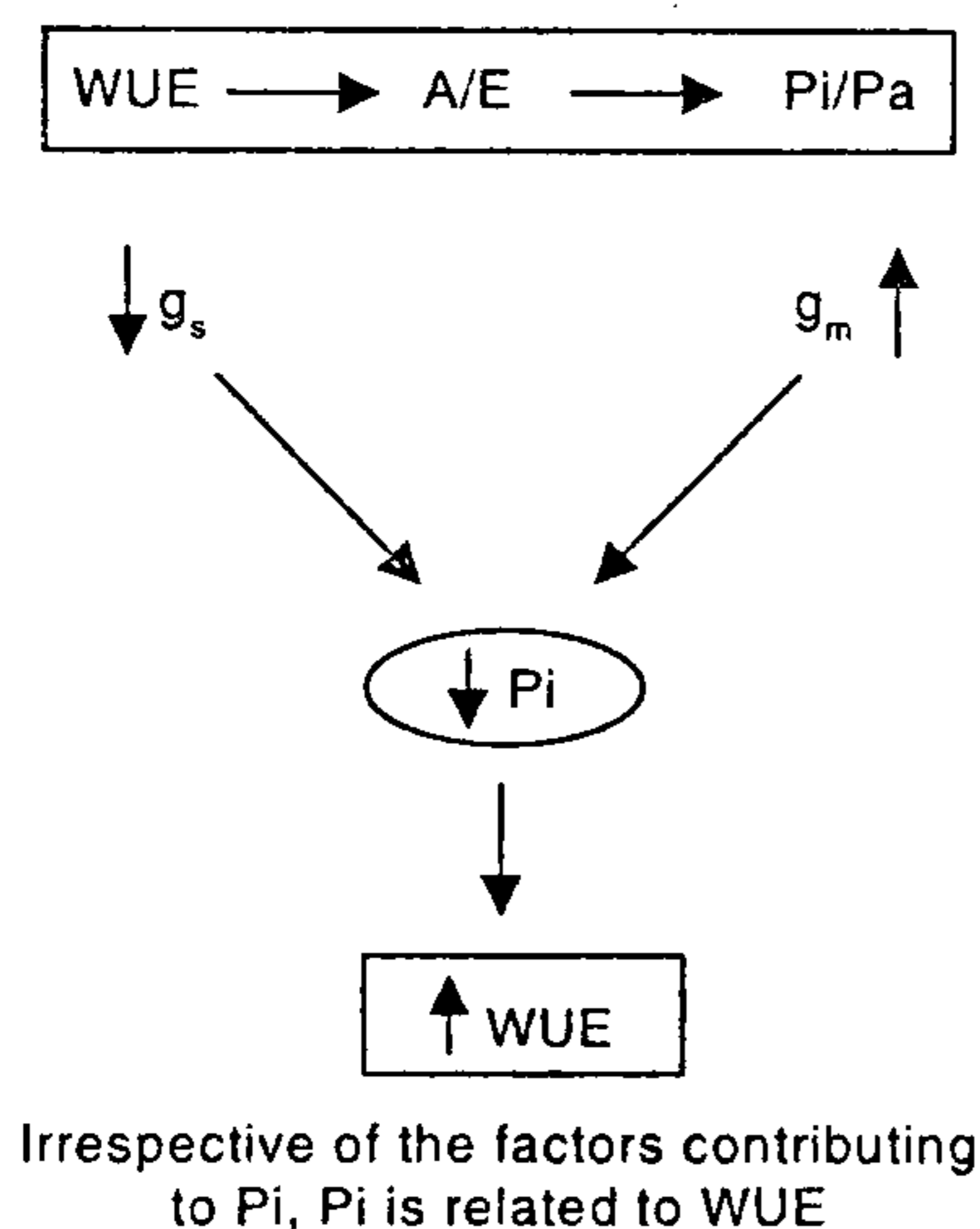


Figure 2. Schematic representation of the factors that determine inter-cellular CO_2 partial pressure (P_i) and hence WUE.

difference (v). These two physiological traits also determine the CO_2 partial pressure in the mesophyll inter-cellular spaces (P_i) which is directly related to WUE (Figure 2). Depending on the extent of contribution of g_s or g_m to P_i 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 P_i , the total water used (T) and WUE become inter-dependent. This inter dependence will be stronger when P_i 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 (wheat⁹, tomato¹¹, phaseolus¹², forage grass species^{13,14} and cotton¹⁵). Further, the moisture stress-induced increase in WUE is often associated with greater reduction in g_s 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 less¹⁴. 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-

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

Capacity types		Conducance types	
Author	Crop	Author	Crop
Wright <i>et al.</i> ³⁸	Groundnut ^d	Condon <i>et al.</i> ⁴²	Wheat ^{ac}
Condon <i>et al.</i> ¹⁰	Wheat ^d	Farquhar <i>et al.</i> ⁹	Wheat ^c
Hubick <i>et al.</i> ³⁹	Groundnut ^d	Martin and Thorstenson ⁴³	Tomato ^c
Hubick <i>et al.</i> ³⁹	Groundnut ^d	White <i>et al.</i> ¹²	Phaseolus ^c
Wright <i>et al.</i> ¹⁸	Groundnut ^d	Johnson and Tieszen ¹⁴	Grasses ^{ac}
Hall <i>et al.</i> ⁴⁰	Cowpea ^c	Acevedo ⁴⁴	Barley ^c
White ⁴⁹	Beans ^c	Ehdaie <i>et al.</i> ⁴⁵	Wheat ^b
Johnson and Bessett ⁴¹	Grasses ^d	Richards and Condon ⁴⁶	Wheat ^c
Matus <i>et al.</i> ⁸	Canola ^d	Meinzer <i>et al.</i> ⁴⁷	Coffee ^c
Sun <i>et al.</i> ²⁵	Spruce	Gutierrez and Meinzer ²³	Coffee ^c
		Porter and Farquhar ⁴⁸	24 wild species ^c
		Lu <i>et al.</i> ¹⁵	Cotton ^c

^a, Δ is positively related to g_s .
^b, TDM is negatively related to WUE.
^c, TDM is positively related to Δ .
^d, TDM is inversely related to Δ .
^e, TDM and Δ have no relationship.

ration 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_s -dependent types often resulted in lower bio mass (Table 1). This could have been the major bottleneck in achieving success while improving WUE.

In capacity types, mesophyll factors (g_m) associated with A 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 Δ 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 Δ among groundnut genotypes (Figure 7). This suggests that the variability in Δ and hence in WUE could be brought about by the mesophyll capacity²¹. Similar relationships were noticed by others when RuBisCO content was altered in transgenic plants with an antisense construct for the small sub unit of RuBisCO²² or by increasing the leaf nitrogen status²³, reiterating the role of mesophyll efficiency in determining Δ and WUE.

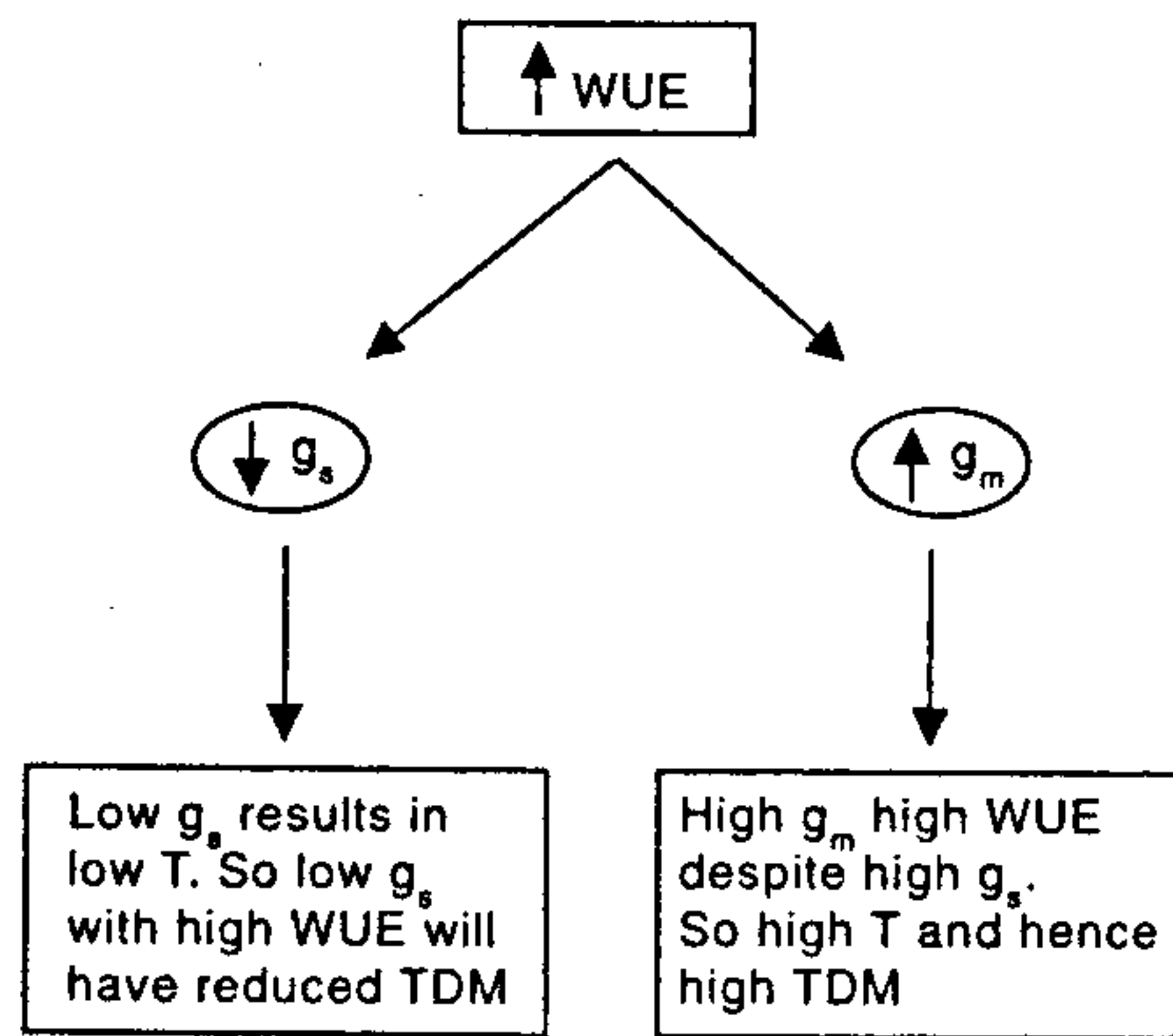


Figure 3. Inter-relationship between WUE, T and stomatal and mesophyll conductances in determining biomass production.

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 Δ and TDM (data not shown). Similar results were also reported by Hubick and Gibson²⁴ in groundnut genotypes both under stress and nonstress conditions and more recently in spruce²⁵. It implies both from our results as well as of several others elsewhere that WUE can be determined

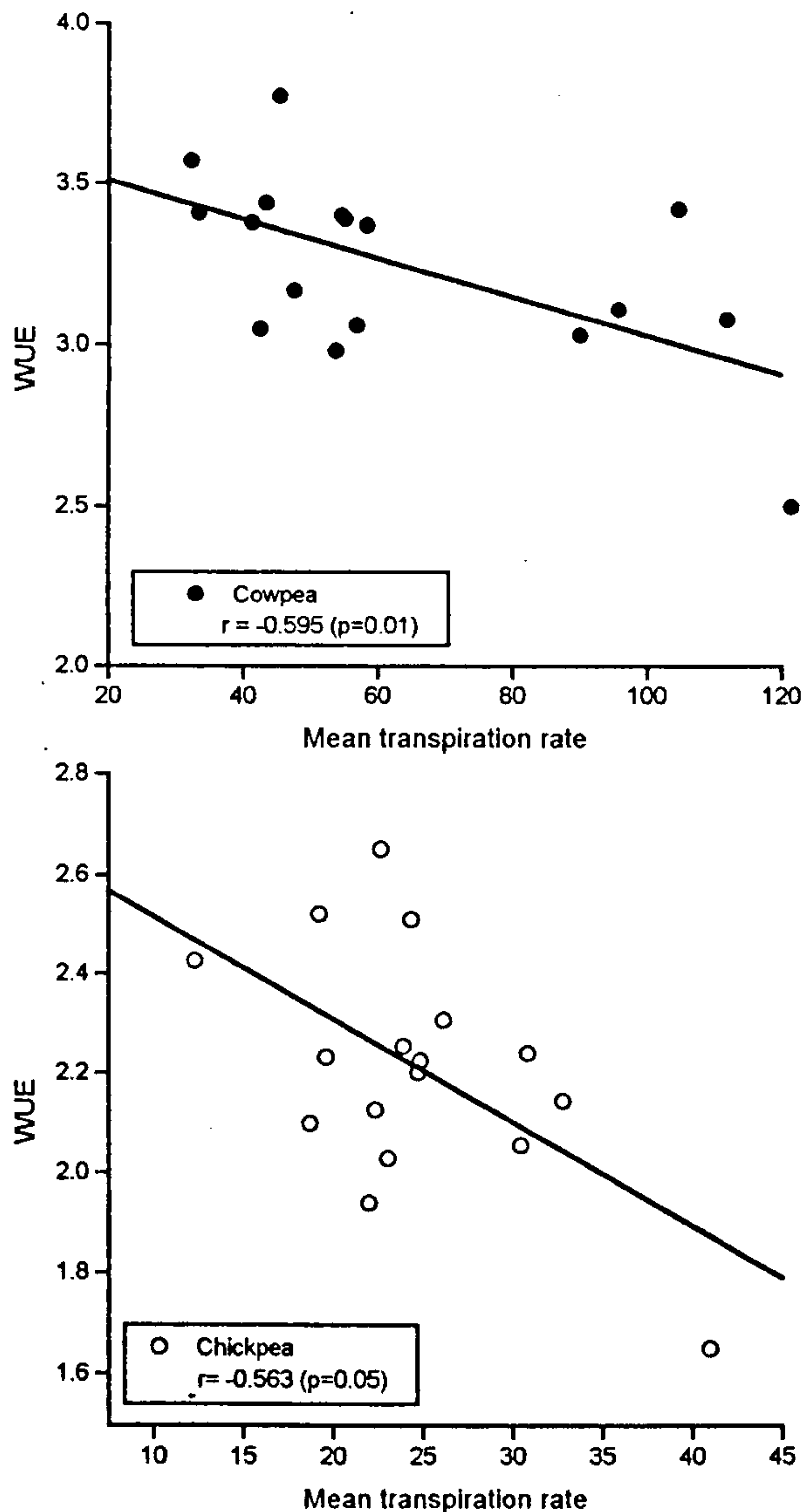


Figure 4. Genetic variability in WUE as a function of mean transpiration (g of water dm^{-2} leaf area day^{-1}) in cowpea and chickpea. Each data point is a mean of at least two replicates. The experiment was conducted with plants grown in containers or mini lysimeters at 100% field capacity.

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 conduc-

tance, 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 $(dA/dP_i)^{26}$ or by determining certain mesophyll components such as RuBisCO content and its activity^{22,27}. Yet another

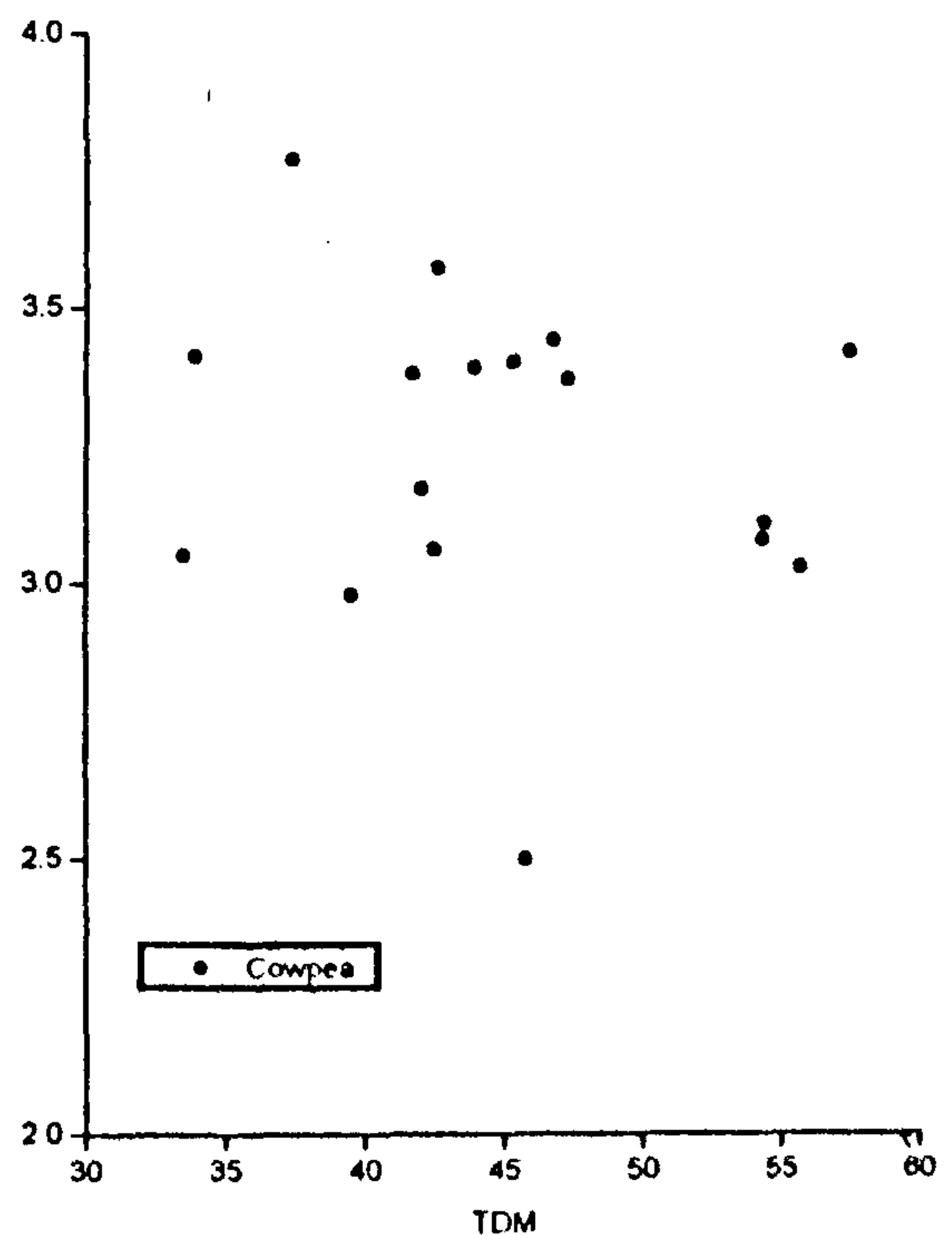
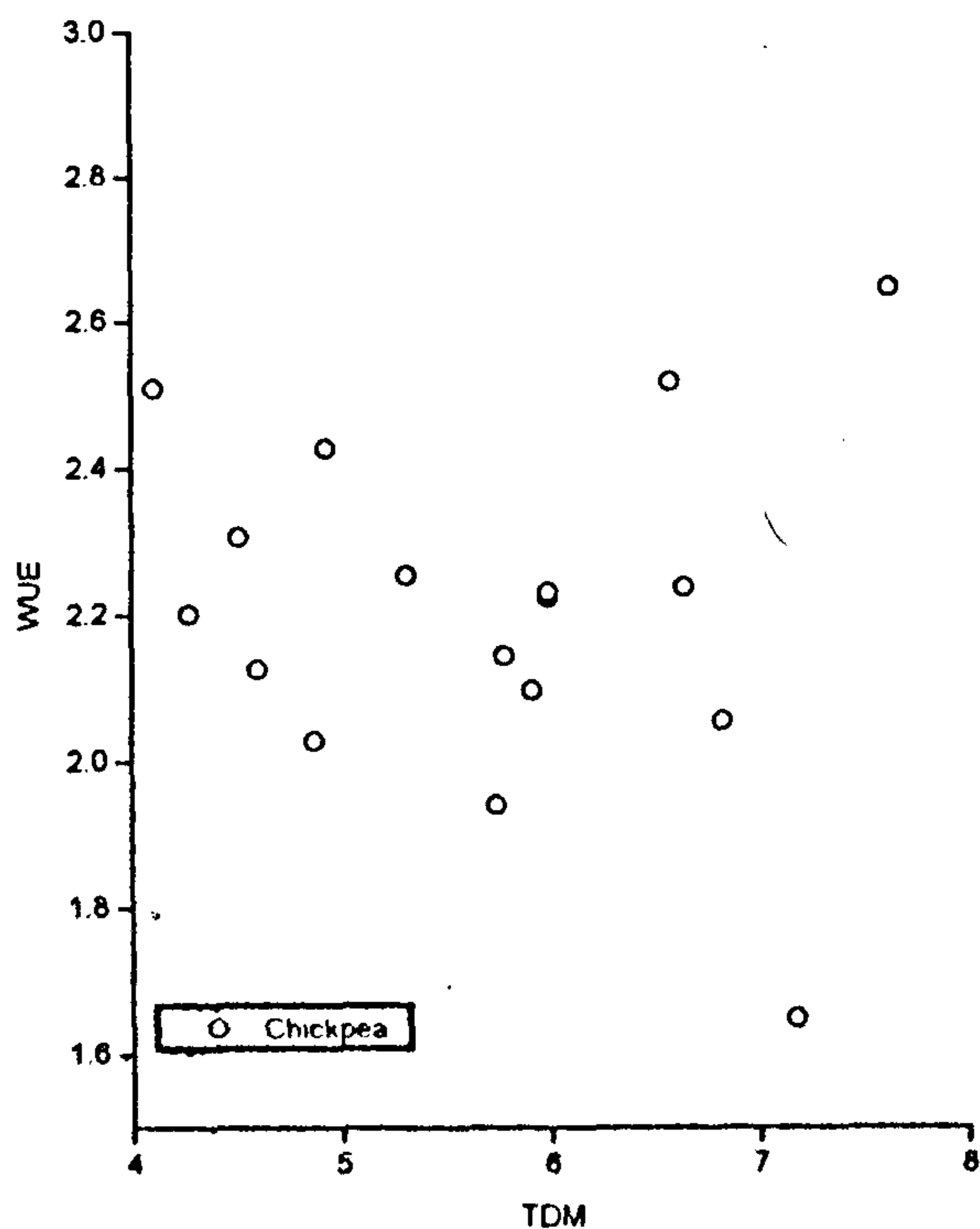
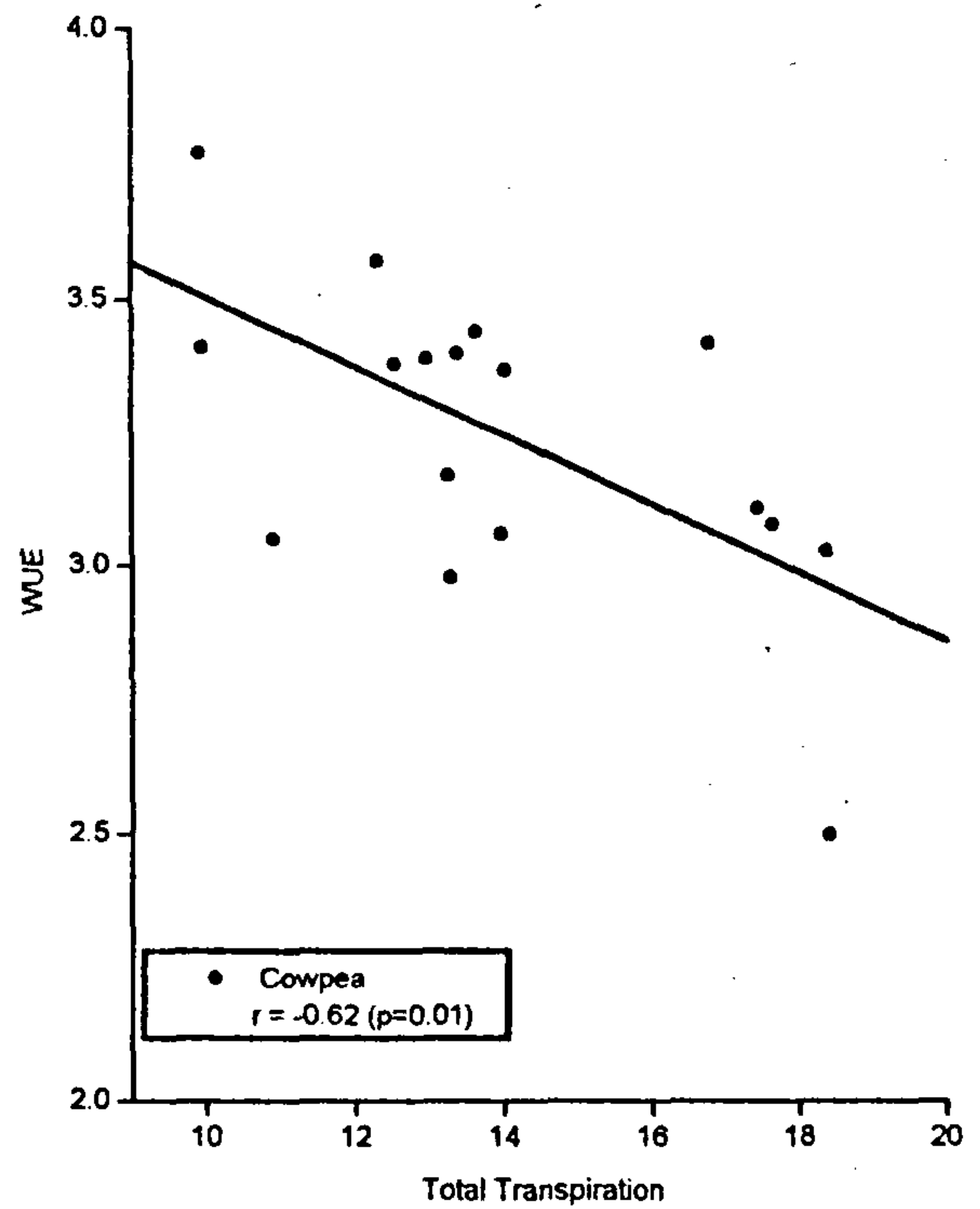
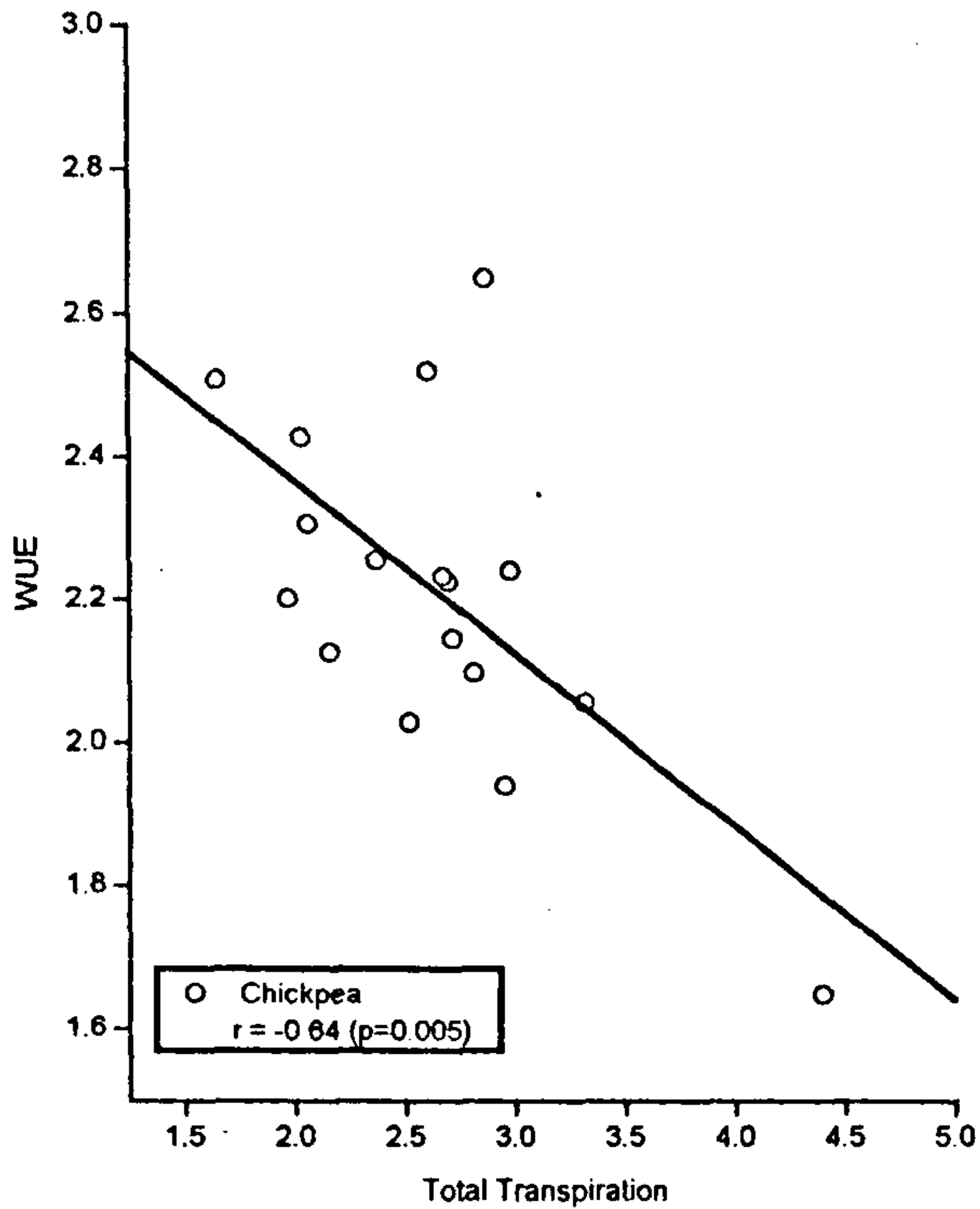


Figure 5. Plot illustrating the association of WUE with total transpiration ($g\text{LAD}^{-1}$ over a period between 35 and 65 DAS) and TDM in a few genotypes of cowpea and chickpea.

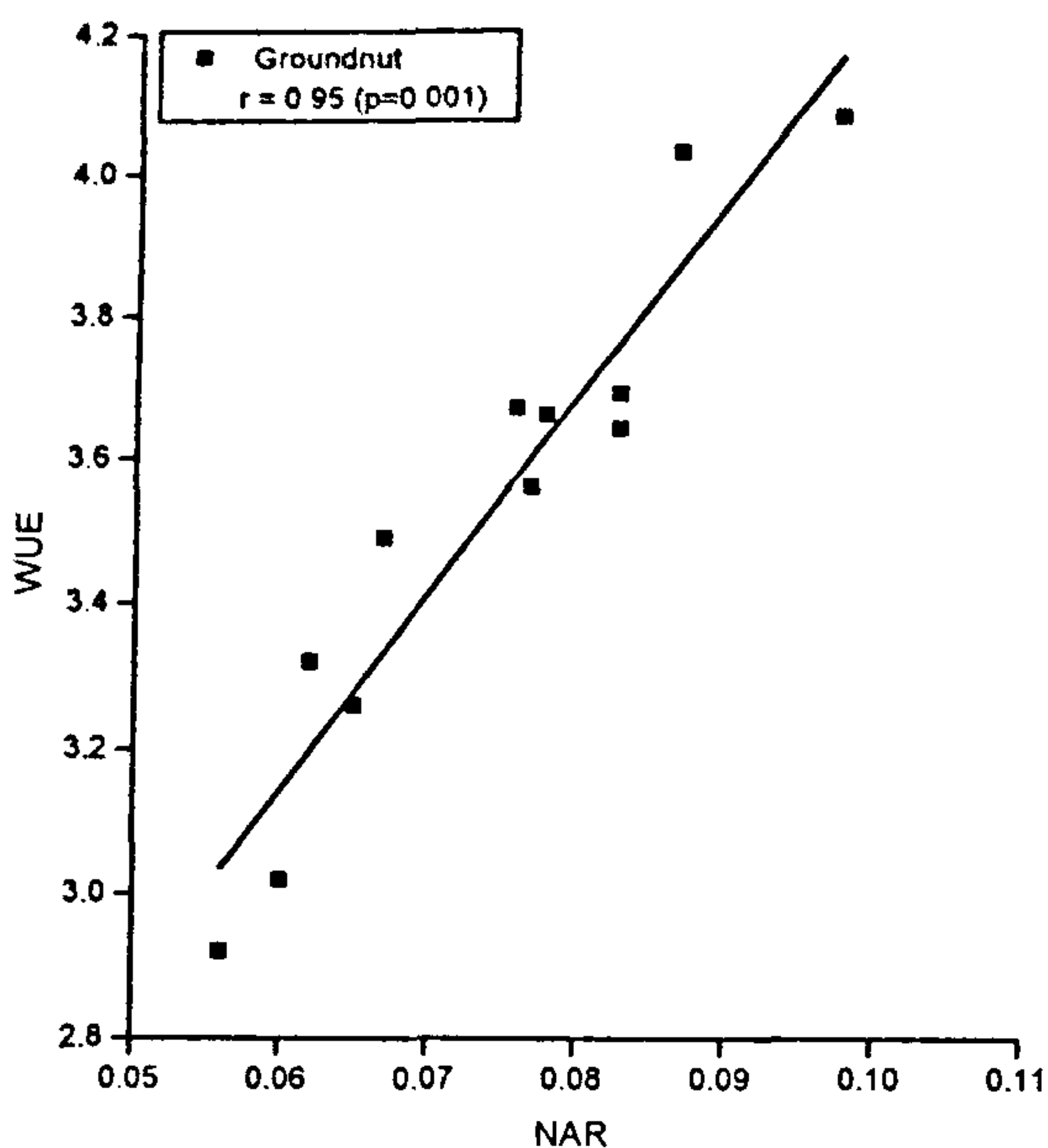


Figure 6. Genetic variability in a WUE as a function of net assimilation rate ($\text{g dm}^{-2} \text{ day}^{-1}$) in groundnut. Different genotypes were grown in containers at 100% field capacity and NAR, a reflection of mean photosynthetic rate was determined by gravimetric method.

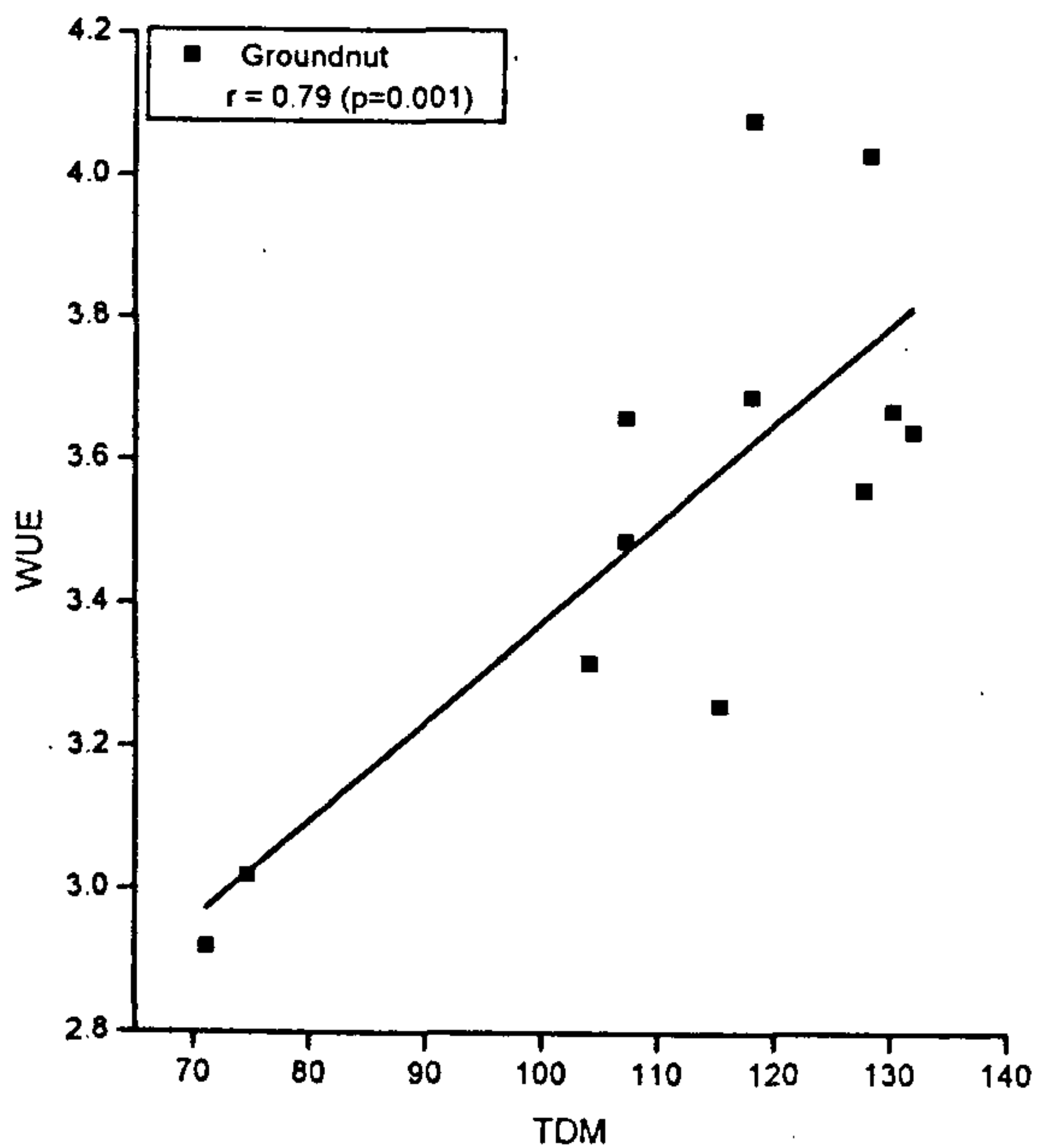


Figure 7. Relationship between TDM and WUE among genotypes of groundnut.

approach to arrive at the carboxylation efficiency is to assess the change in P_i at a given g_s . Since P_i is a function of g_s and g_m , variations in P_i at a given g_s should reflect the g_m . That is, at a given g_s , P_i will be less if the mesophyll efficiency is higher. Recently we have shown that the ratio of P_i to g_s significantly correlates with mesophyll efficiency and hence can be considered as a good reflection of g_m (refs 28, 29).

However, dA/dC_i or P_i/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 P_i and g_s becomes essential. Carbon isotope discrimination by plants (Δ) has been shown to be an estimate of P_i , integrated over time^{6,30}. We propose here an alternate approach to determine the mean g_s by determining the tissue ^{18}O composition.

Recently it has been shown that the leaf water and CO_2 are enriched with the heavy isotope of oxygen (^{18}O) during transpiration^{31,33}. The extent of ^{18}O enrichment in leaf or chloroplast water has been shown to be dependent on transpiration rate^{34,35}. Initial experiments conducted at our centre also revealed that leaf water was enriched with ^{18}O when plants were exposed to higher vapour pressure deficits (Table 2).

Depending on the ^{18}O composition of leaf water, cellulosic ^{18}O also changes. This is brought about by the dynamic equilibrium between leaf water and inter cellular CO_2 which, in turn, is used as a substrate for primary carboxylation^{31,36}. The other possibility of ^{18}O entering into cellulose is the carbonyl hydration reaction of the intermediate of cellulose biosynthesis³⁷.

Irrespective of the reactions that lead to the entry of ^{18}O into cellulose, it is certain that the ^{18}O enrichment in leaf water results in a high cellulosic ^{18}O composition. From this point, ^{18}O composition in cellulose could be a reflection of transpiration rate. In a recent study,

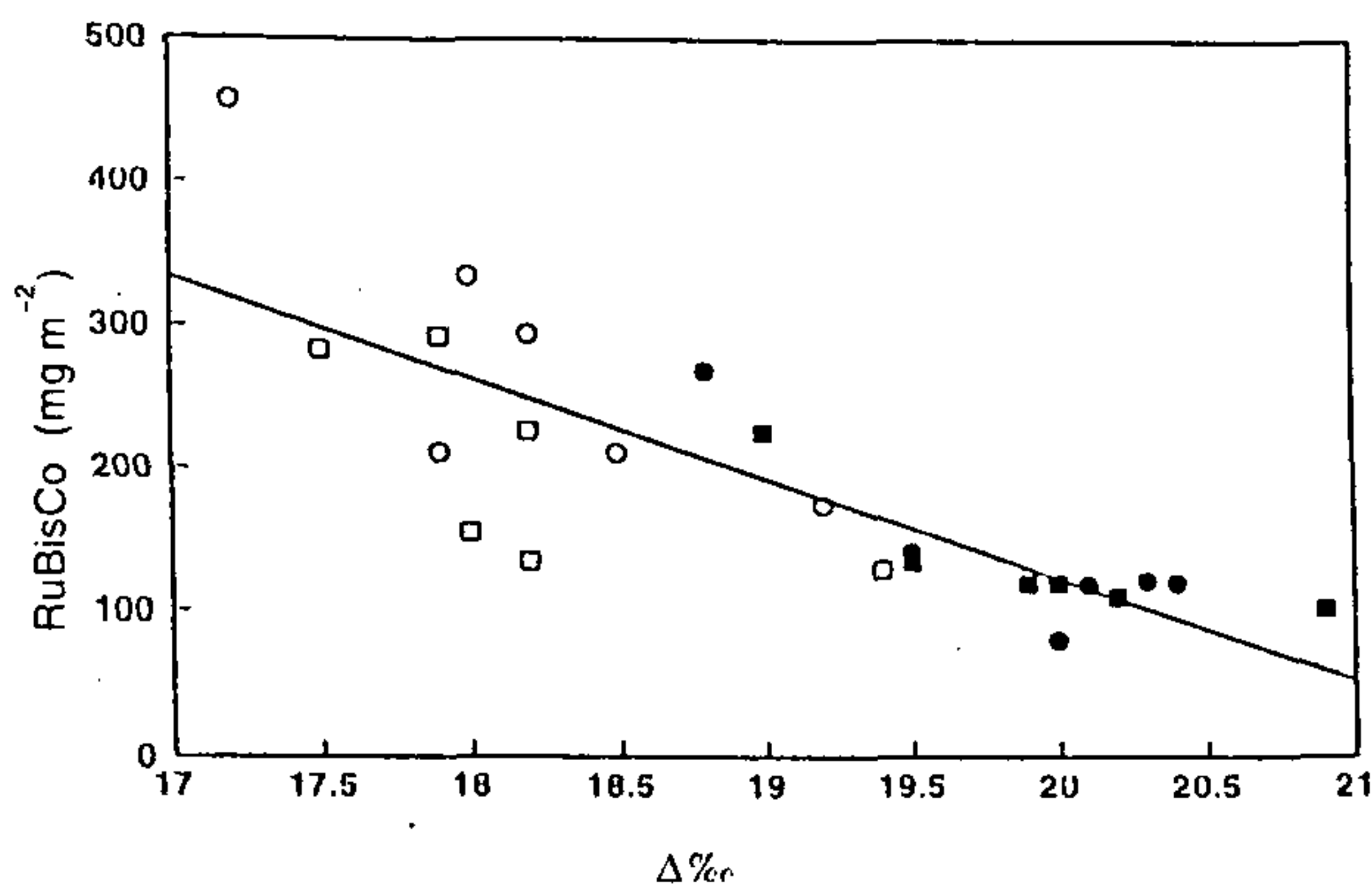


Figure 8. Relationship between RuBisCO content and Δ in upper (\square, \circ) and lower (\blacksquare, \bullet) of six groundnut genotypes grown under irrigated (\square, \blacksquare) and water-deficit conditions (\circ, \bullet). Δ was determined from field-grown groundnut plants either in top or bottom leaves. In another set of leaves RuBisCO content was quantified by immuno assay techniques (adapted from Rao *et al.*²¹).

Table 2. Effect of vapour pressure deficit on ^{18}O enrichment ($\delta^{18}\text{O}$ in parts per mil) in leaf water in sunflower and cowpea

Species	18 mbars	12.5 mbars	7.2 mbars
Sunflower	31.20	28.05	26.42
Cowpea	32.51	29.15	27.65

Farquhar *et al.*³¹ demonstrated an increase in $\Delta\text{H}_2^{18}\text{O}\%$ in leaf water at enhanced transpiration rates altered by v . This also resulted in an increase in $\Delta\text{C}^{18}\text{O}^{16}\text{O}$, further substantiating the relationship between transpiration and $\text{C}^{18}\text{O}^{16}\text{O}$ composition. The difference in g_s at a given v , may enrich differently the leaf chloroplast water ^{18}O and, hence possibly, cellulosic ^{18}O composition. Among genotypes with similar canopy architecture, cellulosic ^{18}O at a given v may still reflect the variations in g_s . From this context, leaf water or cellulosic ^{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 $\Delta^{13}\text{C}$. From the recent evidences of the inter-relationship between ^{18}O enrichment and transpiration, it can be inferred that the leaf cellulosic ^{18}O composition could be a good time-averaged estimate of g_s at a given v . Since $\Delta^{13}\text{C}$ and ^{18}O are time-integrated estimates of P_i and g_s , respectively, the $\Delta^{13}\text{C}/\Delta^{18}\text{O}$ 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 discrimination that occurs during photosynthesis and transpiration, can be a dependable time-averaged estimate of g_m .

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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 hylocrius*), 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 habitat¹⁻⁶. 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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