POTENTIAL FOR IMPROVING THE DROUGHT RESISTANCE OF SOYBEAN (GLYCINE MAX (L.) MERR.) USING THE TRANSPIRATION EFFICIENCY TRAIT

by

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ABSTRACT

Improving the drought tolerance of commercial soybean varieties is a priority issue for the Australian soybean industry. In this study, a series of glasshouse and field experiments were conducted to investigate the potential for improving the drought tolerance of soybean, via indirect selection for transpiration efficiency (TE) in breeding programs. Initial surveys were conducted on three soybean germplasm collections to determine the variation in carbon isotope discrimination (Δ), and presumably TE, based on empirical associations documented for many other C_3 crops. Significant variation (*c.* 15%) in Δ was found in a collection of 98 diverse soybean lines randomly sampled from the Queensland Department of Primary Industries Genetic Resources Centre's international soybean germplasm collection. A subset of these lines, representing the observed range in Δ, were then selected for use in a series of subsequent glasshouse experiments.

Three glasshouse experiments, grown with non-limiting water supplies, were conducted to measure the magnitude of variation in TE and the mechanisms responsible for genotypic variation in TE. Accumulation of total dry matter (TDM; including roots), cumulative transpiration (T), Δ, specific leaf area (SLA; cm^2/g), specific leaf nitrogen (SLN; $g\ N/m^2$) and leaf mineral content (m_a) were measured during vegetative growth. TE was calculated as the ratio of TDM to T. Instantaneous gas exchange equipment was used to measure rates of leaf photosynthesis and transpiration in order to investigate the leaf-level mechanism/s responsible for the genotypic variation in TE among the soybean genotypes.

Significant variation in TE was measured in all of the three experiments, with genotypic ranking in TE being maintained in experiments 1 and 3 ($r = 0.66$), but breaking down in experiment 2. Significant correlations between TE and Δ were observed in experiments 1 ($r = 0.58$) and 3 ($r = 0.98$), but not in experiment 2. It was hypothesised that the lower incident radiation prevailing in experiment 2 strongly influenced the expression of genotypic differences in TE. Other significant results relevant to the development of TE as a selection trait included:-

- differences in both TDM and T contributed to the genotypic variation observed for TE. This suggests that selection for high TE will not be at the expense of relatively high T, which is equally important as high TE for grain yield via its association with TDM.
- While the association between TE and other proposed surrogate measures of TE (ie. SLA, SLN and m_a) showed promising trends, the degree of correlation was less than that observed for Δ, and less than that required to reliably predict TE in selection and evaluation studies.

These results represent the first published report on the extent and nature of variation for TE among soybean genotypes. The experiments were however, conducted under well watered conditions in the glasshouse. To confirm these results in the field under contrasting water stress conditions, a subsequent field experiment was conducted.

Using mini-lysimeter pots and a rainout shelter in the field, TDM, T, TE and potentially correlated traits such as Δ, SLA, SLN and m_a, were measured for the same genotypes examined in the pot experiments. Half the lysimeters were subjected to two water stress

cycles to simulate a typical intermittent drought pattern, while the other half were kept fully irrigated.

Large variation in TE was observed among genotypes (*c.* 45%). Although TE increased under water stress (13%), the genotypic ranking in TE was maintained across water stress treatments (r = 0.80). TE values measured among genotypes in this experiment were strongly associated with those recorded in pot experiments 1 and 3 conducted in the glasshouse. Δ was strongly correlated with TE under both water stress treatments (r = 0.89 and 0.79 for water stress and non-limiting water conditions, respectively), but other potential surrogate measures were not. Harvest Index (HI) was approximated from the bulk crop surrounding the lysimeter pots to determine whether TE was negatively associated with HI in soybean. Of the six genotypes examined no correlation was observed, suggesting that at the crop scale, improvement in TE may be achieved without compromising the relatively high HI of current commercial varieties.

This study provided evidence that large genetic variation in TE is present in soybean. TE does not appear to be negatively associated with either T or HI suggesting that increasing TE will be a beneficial strategy to improve soybean grain yield at the crop level. It was demonstrated that TE is a stable trait over contrasting water stress environments in both the field and glasshouse. Similar stability was observed for the strong correlations between TE and Δ across these environments, which suggests indirect selection for TE using Δ as a surrogate measure could be strongly advocated in soybean breeding programs aimed at improving drought tolerance.

Declaration of Originality

This thesis reports original work, except where acknowledged in the usual manner, and has not been submitted previously for a degree at any university. I give consent to the thesis being available for photocopying and loan when accepted for the award of the degree.

D. S. White

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CHAPTER ONE

Introduction

Soybean first emerged as a domesticated species in China, somewhere around the 11th to 7th century B.C. The wild annual soybean (*Glycine soja* Siebold & Zucc.) is thought to be the ancestor of the cultivated soybean (*Glycine max* (L.) Merr), but precise details of its origin are unknown (Hymowitz and Singh, 1987). In general terms, soybean may be described as a summer-growing, annual legume ranging from 75 to 125 cm in height. The diverse commercial genotypes used today are best suited to sub-tropical climates, with growth habit ranging from determinate to indeterminate (Carlson and Lersten, 1987).

In Australia, soybean was introduced as early as the 1940's but production was generally unsuccessful until the late 1950's, when expansion into southern Queensland occurred (Lawn *et al.*, 1986). Currently, soybeans are grown predominantly as an irrigated crop in the sub-tropics where rainfall is unpredictable (Lawn *et al.*, 1977). Grain production varies around a mean of 80 000 tonnes per annum (Coombs, 1994), with a little over 70 000 tonnes produced during the 1995-1996 summer (Colton, 1996). Australian production falls well below the domestic requirement for soybean, and has been steadily decreasing since peak plantings during the mid to late 1980's. This decline can be largely attributed to the fact that the current soybean industry is based on irrigated or high rainfall production systems (Lawn *et al.*, 1986) where soybean gross margins and efficiency of water use are uncompetitive relative to other irrigated crops (eg. cotton and vegetables; Colton, 1996). With irrigation resources at maximum utilisation (Richards, 1991), the only avenue for soybean production to increase is by expansion into rain-fed regions.

In Australia, there is approximately 750 000 ha of non-irrigated, arable land which is potentially suitable for soybean production (Lawn *et al.*, 1986). However the poor drought tolerance of current soybean varieties (Carter, 1989) has prohibited the expansion of soybean production into such regions (Lawn and Imrie, 1991). A typical example of yield limitation under rainfed conditions in comparison to irrigated production is shown for the sub-tropics of Queensland (Table 1). These data illustrate the importance of developing soybean cultivars specifically adapted to dryland conditions before any future expansion in rain-fed regions can occur.

Table 1 - Average yield and gross margin of soybean under fully-irrigated or rainfed conditions in the South Burnett Region of south-eastern Queensland, Australia.

	Irrigated Production System	**Dryland Production System**
Yield (t/ha)	3.0	1.3
Gross Margin ($/ha)	530	136

Source : - (Crosthwaite, 1995)

The physiological basis of differing genotypic responses of soybeans to water availability is poorly understood (Lawn *et al.*, 1986). Until recently, drought adaptation studies have received less attention than research on daylength and temperature because the soybean industry has been located in high rainfall and irrigated areas. The research reported in this thesis focuses on understanding the physiological basis of soybean yield under water-limited conditions. More specifically, it investigates the possibility of developing soybean cultivars with better adaptation to water deficit patterns typical of the sub-tropics, where two major drought patterns exist (Lawn and Imrie, 1991). The first is where the crop is planted on a full

profile of stored soil moisture and receives little in-crop rainfall, resulting in an end-of-season (terminal) drought. The second is an intermittent drought pattern (Fischer *et al.*, 1982) which is typical of summer dominant rainfall areas. In this situation, the crop is dependent on in-season rainfall, which varies widely in terms of timing and intensity.

Different approaches to improving crop yield and the efficiency of water use under such water-limited conditions have been proposed (Hubick *et al.*, 1986). These can be divided into agronomic or genetic options. Agronomic options include management of tillage and farming systems to intercept rainfall more efficiently by, for example, improving soil structure. At the same time, agronomists and plant breeders have made substantial advances in decreasing the proportion of total evapo-transpiration (ET) that is due to soil evaporation (E_s). This has been achieved by designing plant types that more rapidly develop leaf area, by optimising row spacing and plant population to achieve earlier canopy coverage and by advocating surface stubble retention (Hanks, 1983; Tanner and Sinclair, 1983; Loss and Siddique, 1994).

Most of the genetic improvement of soybean yield under drought has largely been achieved by better matching of phenology to terminal drought stress patterns. The shorter duration lines developed from these studies have produced higher yields in dryland environments on soils with limited water-holding capacity, however, this strategy is not suited to all drought patterns likely to occur in the sub-tropics (Williamson, 1974; Lawn *et al.*, 1977). While both agronomic and genetic components have undoubtedly improved dryland crop performance, the potential for further improvement via these strategies may be limited (Turner *et al.*, 1997).

Recently, a relatively unexplored avenue for improving yield under drought has been proposed. This involves improving the efficiency of rainfall utilisation by increasing the transpiration efficiency (TE), defined as the amount of biomass production per unit of water transpired, in crop plants through genetic improvement (Stanhill, 1986; Richards, 1991; Loss and Siddique, 1994; Wright and Nageswara Rao, 1996). Inter- and intra-specific variation in TE was first reported in the early 1900's (Briggs and Shantz, 1916) but there has been little subsequent work on the extent of intra-specific variation in TE in crop plants. It was not until the early 1980's, when Farquhar and Richards (1984) reported approximately two-fold variation in TE among wheat genotypes grown in pots under glasshouse conditions, that renewed interest in this subject was generated. At the same time, Farquhar *et al.* (1982) demonstrated, on the basis of theory, that TE should be negatively related to carbon isotope discrimination (Δ) in C_3 species, raising the possibility that Δ could be used as a surrogate measure of TE in large-scale breeding programs. Since these initial studies on wheat, numerous crops have been shown to exhibit large genotypic variation in TE (see review by Hall *et al.*, 1993).

There are no published reports of the extent of TE variation among soybean genotypes. This thesis reports experiments designed to investigate the extent of genotypic variation in TE and the potential of using TE as a selection criteria for identifying drought tolerant soybean varieties. To do so, the following questions were considered.

1.) Is there significant variation for TE within soybean germplasm ?

2.) What are the physiological mechanisms controlling genotypic variation in TE among soybean ?

3.) Is TE a stable trait across contrasting environments typical of the sub-tropics ?

4.) Are correlated surrogate measures of TE available to enable indirect screening of the difficult to measure TE trait in soybean breeding programs ?

5.) If suitable surrogate measures are found, are their correlations with TE consistent across contrasting environments ?

6.) Is TE associated with other physiological traits (ie. transpiration and harvest index) which are equally as important to dryland yield ?

7.) Can glasshouse studies on TE provide similar rankings of genotypic performance as under field conditions ?

A series of controlled glasshouse and field experiments using weighing lysimeters were conducted to address these questions.

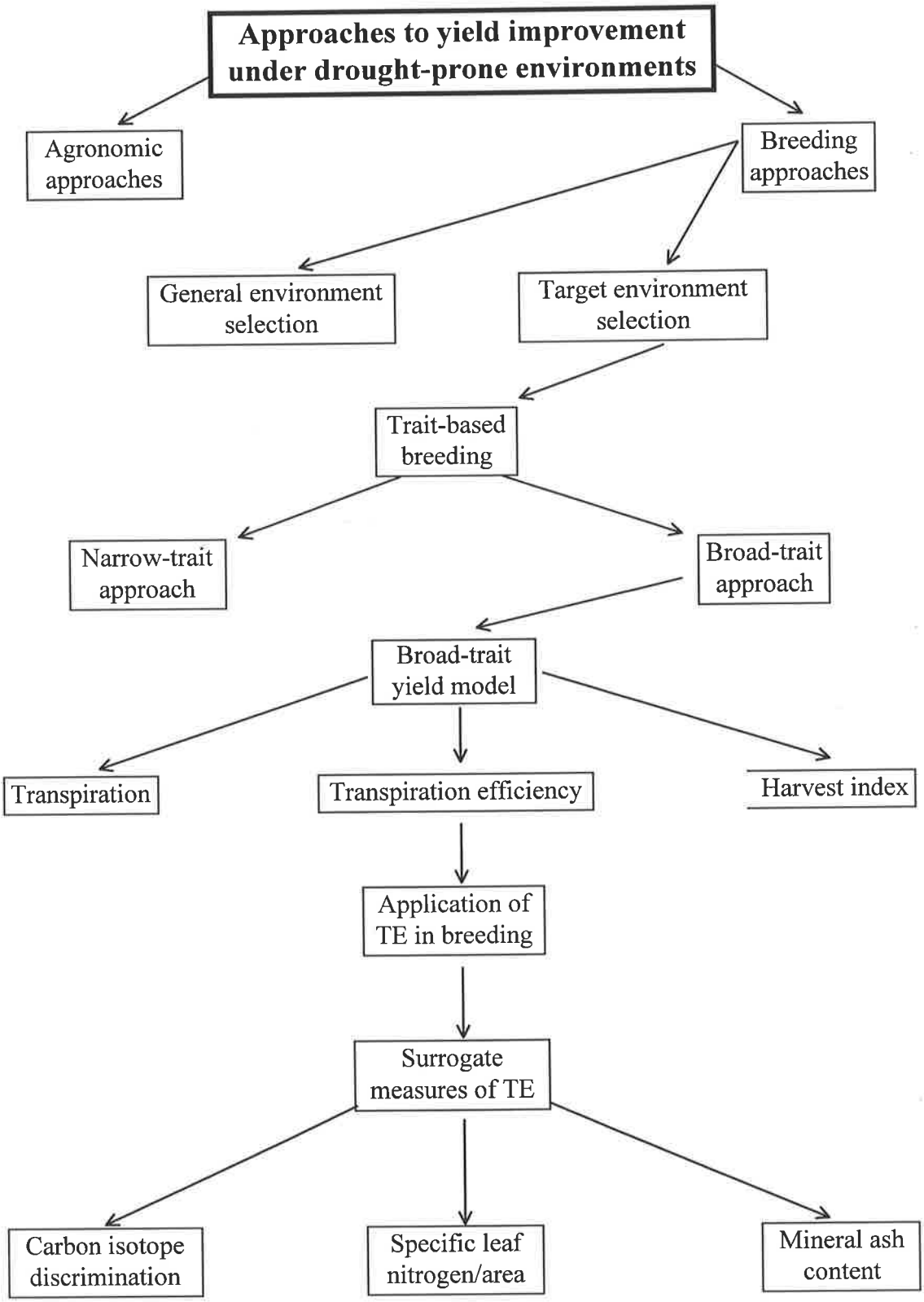
CHAPTER TWO

Review of literature

2.1 Introduction

Throughout Australia, water is the major abiotic constraint limiting both yield potential and agricultural expansion into new regions of dryland agriculture (Richards, 1991; Muchow and Carberry, 1993; Loss and Siddique, 1994). With a relatively low crop value, soybean cannot compete with high value crops such as cotton and various horticultural crops, for the limited irrigation areas available in southern Queensland and northern New South Wales. Consequently, soybean production has declined in the past decade and it has been suggested that future increases are only likely to be through expansion into rainfed regions (Lawn *et al.*, 1986). If this expansion occurs, drought will emerge as a major constraint to grain yield under dryland production systems. Despite being a relatively new problem to the soybean industry, evidence from other species grown under rainfed conditions (such as peanut, maize and sorghum) suggests there are several approaches to reduce the effects of drought on yield. These approaches and their relationship to one another are summarised in Figure 1. This chapter discusses each approach using the framework of Figure 1.

Figure 1 - Schematic illustration of literature review: Approaches to yield improvement for drought-prone environments.



2.2 Yield improvement of soybean for drought-prone environments

There are two major approaches to improving yield performance under rainfed environments - genetic and agronomic (Richards, 1991). The genetic approach aims to increase the genetic potential of a crop through breeding (Marshall, 1991), while the agronomic approach focuses on optimising the production environment to allow full expression of the inherent genetic potential (Lawn and Imrie, 1991).

2.2.1 Agronomy

Evaporative losses as great as *c.* 20-25% of the total evapo-transpiration have been measured during summer for a sorghum crop in the pulse producing environments (Singh and Russell, 1979). Such losses suggest there is scope for reducing soil evaporation (E_s) by adoption of agronomic practises. Agronomic approaches have been successful in maximising the yield potential of various tropical grain-legume species under water-limited environments. Soil evaporation depends largely on the amount of radiation reaching the soil surface when it is wet. By optimising planting date and nutrition to promote early canopy growth, more rapid ground cover can be achieved thus ensuring that less of the subsequent rainfall is lost as evaporation and instead, used for crop transpiration (Ludlow and Muchow, 1990). For soybean, these agronomic options have been extensively studied (Freebairn and Boughton, 1985; Rowland and Freebairn, 1985; Felton *et al.*, 1987). Complex cultivar x sowing date x spatial arrangement interaction is well understood (eg. Douglass, 1981) and for late planting situations, appropriate agronomic recommendations (ie. narrower row spacings and higher plant populations as suggested by Lawn *et al.* (1977)) have led to superior yield performance of soybean (Lawn *et al.*, 1986). The "reduced tillage" revolution has also reduced the impact of evaporative losses and increased available soil moisture by using crop stubble to shade the

soil and increase the boundary layer resistance to water vapour transfer. However, it could be argued that such agronomic modifications are short-term solutions (Richards, 1991) and because soybean agronomy has been studied in great detail (Lawn and Imrie, 1991), the large gains of the past are unlikely to be equalled. In spite of the fact that agronomic studies will continue to play an important role in soybean crop improvement, future efforts to improve soybean yield under drought should also explore the potential offered by breeding approaches.

2.2.2 Breeding

Conventional breeding is based on empirical methodologies where promising genotypes are identified through selection for grain yield under specific environments. The resultant genotype variation in yield (more simply known as phenotypic variation) is due to four components: variation due to genotype, environment, genotype x environment interaction (G x E) and experimental error (Byth, 1981). Genetic progress is dependent upon the phenotypic variation consisting of a large genetic component, relative to the other components (Edmeades *et al.*, 1989).

There are two major derivatives of the empirical breeding methodology which have been used to increase the genetic yield potential of the major crop species (Blum, 1979) - general environment selection and target environment selection. The former involves selecting superior genotypes under optimum conditions assuming that they will also perform well under any environment, while the latter involves selecting for superior performance under target production environments. Both general and target environment-based selection efforts have been successful in improving soybean yield potential under non-water limited environments (see review by Frederick and Hesketh (1994)) and have also been used to improve yield under

water limited environments in other crops (eg. cereals; Fischer *et al.*, 1982). The potential of using these breeding approaches to improve dryland adaptation of soybean will now be evaluated.

2.2.2.1 *General environment selection*

General environmental selection has historically been the major breeding approach used for soybean improvement. Known as the 'universal cultivar' approach, broadly-adapted cultivars were developed across contrasting environments (ie. a narrow range in yield was observed across the different environments). However, since the 1970's the yield variation across different environments has increased dramatically (1000-6700 kg/ha; Cooper, 1985) with improved agronomic management. In order to maintain yield performance in the lower yielding environments breeders had to accept a compromise at both ends of the yield curve (Cooper, 1981; Cooper, 1985) and no longer could one variety provide acceptable yield performance using this approach. For example, the superior yields of soybean genotypes developed through 70 years of selection using the universal cultivar approach were not expressed under water stress conditions (Frederick and Hesketh, 1994). This example highlights one of the potential shortfalls of using the general environment selection approach - traits which confer superior yield under water-non-limiting environments may be of little use under water-limited conditions (Blum, 1979). Therefore, although this approach has made considerable progress in increasing soybean yield performance in the past, it is becoming difficult to sustain similar rates of genetic improvement, especially within water-limited environments. This has prompted consideration of an alternate breeding philosophy where selection is conducted under a specific target environment.

2.2.2.2 *Target environment selection*

Target environment selection has also been successfully used in soybean improvement programs. Examples include an irrigated soybean breeding program where Cooper (1985) selected for specific adaptive traits conferring high yield under water non-limiting conditions (ie. lower plant height and lodging resistance). Combined with appropriate agronomic practises (row spacing and plant population), the resulting progeny out-yielded the conventional control varieties by *c.* 23% over 10 years of experimentation. Another study targeted high rainfall production environments, which experience large variation in the timing of planting rains. For this environment Douglass (1981) exploited genotypic variation in cultivar x sowing date x spatial arrangement interactions to develop a suite of varieties which permit the flexibility in sowing date which was essential in this environment. Such evidence suggests the possibility of using the target environment approach to develop soybean cultivars with specific adaptation to drought-prone environments.

There are several examples where this breeding approach has been used with mixed success in crop species other than soybean. For example, a maize improvement project in Columbia achieved yield increases of only 25% after three cycles of selection for yield under dry-season conditions (Blum, 1979). In contrast, Ceccarelli *et al.* (1995) compared yields of two groups of barley lines grown under water stress. One group had been selected for superior yield under non-water limited conditions while the other had been selected under water-stress conditions. Owing to superior performance of the water-stress-selected lines, they concluded that when selecting for drought resistance, selection is most efficient under drought conditions. One major reason for these variable results from using the target environment

selection approach may be associated with the variability in both the timing of onset and severity of individual drought episodes.

Under the influence of variable drought episodes, stability in yield performance is difficult to achieve because the effects of a multitude of potential drought tolerance and escape mechanisms on yield differs with the intensity and duration of the water stress (McWilliam, 1989). For example, Dashiell *et al.* (1994) observed wide and inconsistent variation in yield of 18 soybean genotypes grown over five locations, resulting in low heritabilities for yield (22.6 to 45.3%) and large G x E interactions affecting grain yield. Specht *et al.* (1986) also noted that the variance attributed to G x E interactions in regional soybean variety trials was often as large as, or larger than, the genotypic variance. Other reports have also identified the presence of large G x E interactions for drought resistance in soybean, determined by comparing yields measured under several droughts differing in intensity and duration (Frederick and Hesketh, 1994). Therefore, G x E interaction for yield of soybean has been a major impediment for empirical yield selection under droughted environments (Lawn and Imrie, 1991).

As a result of the problems and associated inefficiencies of empirical breeding methods (both the general and target environment selection), it has been suggested that breeding for improved drought tolerance could be made more efficient if specific physiological attributes (or traits) which improve yield under drought could be identified and used, in conjunction with yield, as selection criteria (eg. Table 2; Morgan, 1989). The incorporation of physiological traits into target environment selection approaches has followed two paths which can be summarised into the narrow-trait and broad-trait based approaches.

2.2.2.3 Narrow-trait based breeding

The narrow-trait approach is based on specific physiological traits (Table 2), with each conferring drought resistance via one of the three strategies; drought escape, desiccation tolerance or dehydration avoidance (Levitt, 1972). Drought escape is the ability of a crop to avoid prolonged crop water deficits; desiccation tolerance is the ability to maintain assimilate production during periods of reduced plant water status; and drought avoidance is the ability to maintain high plant water status during periods of reduced soil moisture availability (Fischer *et al.*, 1982).

Analysis of climate variability allows us to quantify the variation in drought between and within the sub-tropical pulse production regions. This is critical to the understanding, merits or otherwise, of each of the three drought resistance strategies. For example, in a comparison of the two main dryland pulse cropping regions of Queensland - the South Burnett and the Atherton Tableland - it has been shown that in the South Burnett terminal drought is likely in >60% of years, compared to <5% of years in the Atherton Tableland region (Wright, 1997). Furthermore, the time of onset of terminal droughts within the South Burnett ranged from 50-90 days after planting (dap) and there was large variation in the intensity of the drought. These analyses indicate that the sub-tropical, rain-fed pulse production regions of Australia are subject to unpredictable droughts that vary in both timing and intensity. It is likely that the specific drought patterns of a region will favour different approaches to yield improvement under drought. For example, drought escape is likely to be most important in the South Burnett, while drought tolerance characteristics will be more important on the Atherton Tableland. The following section summarises the results from several attempts to improve soybean drought resistance using the narrow-trait breeding approach.

Table 2 : Plant responses to water deficits.

RESPONSE	**RESISTANCE TO DROUGHT TYPE** (terminal/intermittent)
Drought Escape	
phenology (early flowering)	terminal
photoperiod sensitivity	terminal
developmental plasticity	intermittent
remobilisation of assimilates	terminal
Drought tolerance	
dehydration avoidance (postponement)	
root depth and density	both
low hydraulic resistance in roots	terminal
high hydraulic resistance in roots	terminal
early seedling vigour	both
osmotic adjustment (osmoregulation)	both
reduced leaf conductance	intermittent
leaf movements (rolling, parahelitropic)	intermittent
increased leaf reflectance (glaucousness)	both
high transpiration efficiency	both
desiccation tolerance	
low lethal water status	intermittent
maintenance of leaf area	intermittent
high temperature tolerance of leaves	intermittent
high relative leaf and stem elongation rates	intermittent

Source : (McWilliam, 1989)

(i) *Drought escape*

There would appear to be considerable scope for the application of drought escape strategies in soybean by exploiting the considerable variation in photo-thermal characteristics that exist in the crop (Lawn, 1989). This strategy has been used in a northern New South Wales soybean improvement program. Rose *et al.* (1992) evaluated early maturing soybean lines under a terminal drought treatment and found that crop duration was negatively correlated to drought resistance.

However, the use of a drought escape strategy to improve drought resistance is limited to regions that are subject to terminal droughts of consistent timing of onset, duration and intensity (Chapman, 1989; Fischer, 1981; Muchow and Carberry, 1993). For instance, in the situation where a short season variety is grown in a season which receives high rainfall and experiences no terminal drought, farmers may forgo considerable potential yield by not exploiting the extended growing period (Turner *et al.*, 1997).

(ii) *Drought tolerance*

There are numerous examples of significant variation in several drought tolerance traits among soybean genotypes. These include phenological adjustment to water stress and osmotic adjustment (Rose *et al.*, 1983), pubescence and leaf morphology (Baldocchi *et al.*, 1985), deeper rooting (Coale and Grove, 1986), growth habit (Specht *et al.*, 1986) and stomatal characteristics, including density (Santos, 1978). There has also been work conducted to evaluate whether such traits are linked with drought tolerance, as proposed by McWilliam (1989).

For example, better exploration of available soil moisture by deep rooting characteristics is a trait which may confer yield advantages during drought (Carter, 1989). Studies have shown that a reported drought-sensitive cultivar extracted less soil moisture from deep in the soil profile than a control cultivar under water stress conditions (Cortes and Sinclair, 1986b). Similarly, Sloane *et al.* (1989) found that a putative drought-tolerant variety extracted soil moisture to greater depths than the control cultivar Forrest. Inter-species comparisons between sunflower and soybean (Cox and Joliff, 1987), and between maize and soybean (Lorens *et al.*, 1987), demonstrated that sunflower and maize suffered less yield loss under drought than soybean due to their ability to exploit soil moisture deep in the profile via deeper rooting. Whilst these studies suggest that the deeper rooting trait may be a beneficial trait for drought resistance, there have been no attempts at selection for deeper rooting to confirm this hypothesis, mainly due to the difficulties in accurately measuring the characteristic (Wright *et al.*, 1993). In fact it has been argued that the deeper rooting trait will be of little use in environments where significant sub-soil moisture reserves are not available (Richards, 1991), as in many parts of the Australian sub-tropics (Lawn *et al.*, 1986).

Osmotic adjustment (OA) is another physiological trait that has been suggested as a possible drought tolerance character (Table 2). It is a biochemical response to drought which confers drought tolerance by allowing the stomata to remain open for relatively longer periods than normal (Morgan, 1984), thus allowing maintenance of higher rates of CO_2 fixation during the onset of drought (Lawn, 1982b). Cortes and Sinclair (1986) studied the association between OA and drought tolerance and found that OA was negatively associated with drought tolerance. This was in contrast to Sloane *et al.* (1989) who found that OA and grain yield

under drought were positively associated. Such conflicting reports make it difficult to assess whether OA is a beneficial drought tolerance trait, or not.

Deep rooting and OA are just two of the many characters which have been investigated as possible drought tolerant traits (Carter, 1989). However, there have been few attempts to specifically incorporate such traits into breeding programs targeting improved drought tolerance of soybean. One example, however, comes from a current Australian project, which is seeking to improve soybean drought tolerance via selection for stomatal control of water loss, OA and low critical plant water status. To date, despite significant genetic variation being present for all three traits, limited progress in improving yield under drought has been observed (James *et al.*, 1996).

In order to determine whether yield under drought is associated with some of the physiological characters listed in Table 2, two major concerns are apparent. Firstly, despite extensive investigations, there remains conflicting evidence regarding the value of drought tolerance traits. Thus their usefulness as selection criteria in dryland breeding programs has been questioned (eg. Passioura, 1981). Secondly, while much work has been done to understand the physiology of such traits, their incorporation as indirect selection criteria to assist empirical breeding programs aiming at improved drought resistance has not occurred to any great extent in soybean, or in any of the major crop species (Passioura, 1981; Ludlow and Muchow, 1990). An alternative approach has been proposed whereby "bulkier" traits conferring drought adaptation are selected for in breeding programs. This method is known as the broad trait breeding approach.

2.2.2.4 Broad-trait based breeding

Passioura (1981) argues that attempts at trait-based selection have failed due to disparity between the drought traits and yield in terms of the scale of biological organisation. Grain yield operates at the community level of plant organisation, where it is the final integration, over the entire life of the crop, of many complex biological functions - from cellular through to plant and community processes. In contrast, many drought stress response mechanisms operate at lower levels of plant organisation during short periods of time relative to the crop life, and may evoke a response on only a part of the plant. Any short-term response that these mechanisms produce may be masked by many other biological processes and therefore result in little influence on final grain yield. Additionally, because these mechanisms may be strictly triggered by specific environmental conditions, any yield benefits may be inconsistent from year to year and between environments (ie. G x E interaction). It has recently been argued that to overcome these problems, crop physiologists should develop traits that operate at, or as close as possible to, the community level of plant organisation (Passioura, 1977; Muchow and Carberry, 1993). In the process of identifying better integrated physiological characters, a number of analytical models describing the determinants of yield under drought have been proposed (Turner *et al.*, 1997). These integrate the effects of numerous drought responses into a few "bulky" parameters operating at the canopy level (Wright and Nageswara Rao, 1996). It is suggested (Williams, 1992) that selection on the basis of such bulky traits should be more efficient than either sole selection for yield under drought conditions as discussed in section 2.2.2.2 or for selection of 'lower level' drought adaptive traits discussed above. Further issues regarding the potential for improving yield of rainfed soybeans by utilising these types of yield component frameworks are discussed below.

2.3 Broad-trait yield model

Grain yield (Y) of a water-limited crop was proposed by Passioura (1977) to be a function of the amount of water transpired by the crop (T), the efficiency of use of that transpired water to produce dry matter (TE) and the proportion of total plant dry matter produced as harvestable grain or harvest index (HI; see equation 1). Assuming that interactions between model components are minimal (Passioura, 1981), improvements in any one component should result in an increase in yield. In the following sections each functional component is further discussed in relation to relevant physiological and morphological traits and their interactions, and potential for exploitation in breeding programs.

$$Y = T * TE * HI \quad \text{.....[equation 1].}$$

2.3.1 Transpiration

Biomass accumulation is a linear function of cumulative transpiration (T) in most crop species under water-limited environments (Tanner and Sinclair, 1983). This means that improvements in T will lead to increases in biomass, and therefore yield (assuming a constant TE and HI). Efforts to improve yield via increases in T have followed two strategies;

a) to increase T by minimising soil evaporation (E_s), and

b) maximising extraction of soil moisture through more extensive rooting systems (Ludlow and Muchow, 1990).

2.3.1.1 Minimising soil evaporation

Section 2.2.1 discussed the scope for reducing the large evaporative water losses (E_s) of crops grown in the sub-tropics and the agronomic management options to reduce E_s. In conjunction with agronomic management, reductions in E_s can also be achieved genetically by selecting for plant characteristics that minimise E_s, such as prostrate growth habit, fast early growth or high above-ground biomass (Richards, 1991). However, rainfed soybean crops often have to rely on limited amounts of stored soil moisture that need to be effectively metered out over the entire growing season (Turner *et al.*, 1997). In this instance, early vigour and rapid canopy development may cause rapid exhaustion of soil moisture reserves during reproductive development (Ludlow and Muchow, 1990). Therefore, selection for early vigour in an attempt to minimise E_s may not always be the best strategy for producing consistent yield improvements under conditions of variable rainfall. Consequently, its usefulness may be confined to environments where rainfall is more evenly distributed throughout the growing season (Ludlow and Muchow, 1990) and evaporative losses are considerably higher. For example, E_s can be as high as up to 40% of total ET for crops grown in temperate climates (Richards, 1991). However, in the tropical to sub-tropical regions where soybeans are mainly produced, E_s is relatively low because rainfall events are more infrequent, soils have higher water-holding capacity and the high prevailing temperatures naturally promote more rapid early canopy development (Turner *et al.*, 1997). Based on this reasoning, Ludlow and Muchow (1990) suggested that there may be little scope for further reducing E_s to improve T, and to therefore increase yield of soybean in rainfed production regions of Queensland.

2.3.1.2 Increasing soil moisture extraction by roots

The second avenue for increasing crop T is by maximising the extraction of soil moisture, especially from deep in the soil profile. This can be achieved genetically by extending the vegetative period to provide more time for the roots to grow deeper into the soil profile, or by selecting for genotypes with more vigorous root growth (Richards, 1991). In many grain legumes, large genotypic variation in rooting depth and ability to extract water at depth has been reported (Turner *et al.*, 1997). Yield increases of soybeans achieved over several decades of soybean improvement under irrigated conditions have mainly been attributed to greater ability of roots to exploit soil moisture reserves which has allowed increased T (Cortes and Sinclair, 1986a). However, it was also noted that these yield benefits were not present under rainfed environments prone to drought stress (Frederick *et al.*, 1991). Therefore the value of a deeper rooting system for enhanced soybean yield in the rainfed sub-tropics is uncertain.

In addition to the uncertainty regarding the scope for increased T to contribute to soybean yield improvement there are practical problems associated with screening and selecting for this trait. It is very difficult to accurately partition total evapo-transpiration (ET) into its two components - E_s and T (Wright *et al.*, 1994). Measuring ET in surface soil layers in genotype screening trials is limited to non-destructive sampling procedures such as lysimetry or soil moisture measuring devices such as neutron moisture probes. However the use of lysimeters for the screening of large numbers of lines is impractical, and large errors are commonly encountered when measuring soil moisture loss in the surface layers with the neutron moisture probe. These practical limitations, combined with large errors involved in measuring T (Turner, 1986), severely restrict large-scale screening and selection for high T. Such is the

extent of the problem that Baker (1989) identified development of better methods to measure T as a priority research objective. As a result, soybean breeders have not used T or genotypic root traits as indirect selection criteria for improving grain yield under water-limited environments.

Despite the discussions for and against the strategies for increasing T to effect yield improvement under dryland environments, it is vital to note that relatively high T is essential to maintain current levels of biomass production, and consequently, grain yield in the rain-fed sub-tropics.

2.3.2 Harvest Index

Harvest index (HI) has been defined in a number of ways (Schapaugh and Wilcox, 1980), but the generally accepted definition is that HI is the ratio of economic yield to above ground biological yield (Donald and Hamblin, 1976; Gifford *et al.*, 1984; Salado-Navarro *et al.*, 1993).

By definition, HI will be positively correlated with grain yield and negatively correlated with biological yield and this has been confirmed in soybean (Schapaugh and Wilcox, 1980). Therefore, assuming biological yield is stable (Snyder and Carlson, 1985) selecting for high HI should increase grain yield. There have been attempts by breeders to select directly for enhanced HI which have had mixed results (see review by Lawn, 1989). However, regardless of whether HI improvement has come about through direct selection or indirectly through its association with grain yield, HI of commercially released soybean varieties has increased from <0.25 in the 1950's to greater than 0.50 in modern varieties (McWilliam and Dillon,

1987; Lawn, 1989), and has been the single most important factor responsible for grain yield increases over this period (Gay *et al.*, 1980). It has similarly been argued that increased HI has also been responsible for the major genetic improvements in yield observed in peanut (Mozingo *et al.*, 1987), small grains (Donald and Hamblin, 1976; Austin, 1988), rice, sorghum, millet, and cotton (Evans, 1980). This evidence highlights the importance of HI, although there are some problems associated with its accurate measurement in soybean.

While HI is relatively easy to measure, large measurement errors can occur in senescent crops such as soybean. Soybean continually senesces leaves and petioles throughout the podfilling period, with reductions in total above-ground biomass of up to 40% being reported when these losses have not been taken into account (Schapaugh and Wilcox, 1980). These losses need to be accounted for if total above-ground biomass is to be measured accurately. HI in soybean is usually measured at maturity after most of the leaves have been shed, so significant errors in final HI must occur in many reported studies. This methodology may also cause problems when comparing genotypic performance, particularly if there is genotypic variation in the extent of leaf senescence at maturity. Definitive studies on the measurement of HI in many of the grain legumes that senesce large proportions of dry matter during reproductive growth are needed to clarify this issue.

Additionally, HI is not a stable trait because it is strongly dependent upon environmental (eg. water stress and photoperiod) and management effects (eg. variation in plant population, and time of planting). Differing sensitivities to such factors can result in large G x E interaction for HI, which can make reliable selection decisions very difficult (Donald and Hamblin, 1976). Finally, it has been suggested that the genetic limit to further improvement

in HI may have been approached in soybean (Austin, 1988), which raises the question of the extent to which HI can further contribute to yield improvement in water limited environments?

Finally, obtaining consistent and reliable estimates of HI is essential if the analytical framework (equation 1) proposed by Passioura (1977) is to be applied in soybean improvement programs. This is particularly so if researchers wish to examine possible interactions between model components. To overcome the problems with measuring HI as defined earlier, it is suggested that better estimates of HI can be obtained by measurement of maximum above-ground biomass achieved prior to leaf drop (Rose, 1992). This definition and measurement procedure is used in the following experimental chapters.

2.3.3 Transpiration Efficiency

Of the three model components, transpiration efficiency (TE) is perhaps the least studied in most major crop species, including soybean. It is often confused with the term "evapo-transpiration" efficiency, which includes evaporation from the soil in the denominator (Passioura, 1986). This confusion has been associated with the common use of the term "water use efficiency" to describe both ET efficiency and TE. Many reviews (Tanner and Sinclair, 1983; Passioura, 1986; Stanhill, 1986; Ludlow and Muchow, 1990) have covered this controversial subject in more detail. It is important that crop improvement studies aiming to increase yield under rainfed environments clearly make this distinction, as improvement in ET efficiency by way of modification of agronomic practices (eg. row spacing, or stubble retention for ground-cover) will have no effect on TE, which is a measure of intrinsic crop performance (Tanner and Sinclair, 1983).

Recent evidence suggests that variation in TE does exist within crop species, and that it may be possible to breed for improved TE (Farquhar *et al.*, 1982; Hubick *et al.*, 1986; Martin and Thorstenson, 1988; Wright *et al.*, 1988; Farquhar *et al.*, 1989b; Hubick and Farquhar, 1989; Virgona *et al.*, 1990; Dingkuhn *et al.*, 1991; Condon and Richards, 1992b; Hall *et al.*, 1992; Ismail and Hall, 1992; Wright, 1995a). However, there have been no reports of genotypic variation in TE for soybean.

2.4 TE in crop improvement

The potential role for increased TE to improve crop yields under water-limited conditions has long been recognised (Briggs and Shantz, 1916; Fischer *et al.*, 1982; Passioura, 1986; Richards, 1991; Muchow and Carberry, 1993; Turner, 1993). However the likelihood of this potential being realised has often been questioned, due to inherent conflicts between two essential biological processes posed by an increase in TE (Stanhill, 1986). On one hand, plants require rapid gas exchange between the atmosphere and the interstitial leaf space for maximum carbon fixation, but on the other hand, plants also need to conserve water by minimising the rate of water vapour diffusion from inside the leaf to the atmosphere. These processes are in conflict because both share the stomata as their diffusion pathway. In considering this problem, Farquhar *et al.* (1989b) described a 'shrewd plant' as one which would determine it's optimal stomatal conductance by examining the marginal benefit of assimilation to the marginal cost of transpiration. Thus TE provides researchers with a tool to identify these 'shrewd plants'.

A basic understanding of the mechanisms determining TE is critical for successfully exploring the possibilities of increasing TE within the context of crop improvement research. TE can be examined at the leaf level over a very short time scale, using equation 2.

TE = CO_2 assimilation (A) / transpiration (E).....[equation 2]

where A is proportional to the concentration gradient of CO_2 from the bulk air to the site of carboxylation within the leaf and is inversely proportional to the sum of resistances to CO_2 diffusion into the leaf (Hsiao and Acevedo, 1974). Similarly, E is directly proportional to the concentration gradient of water vapour between the intercellular leaf space and the atmosphere, and inversely proportional to the total resistance to water vapour transport from inside the leaf to the bulk air outside (Hsiao and Acevedo, 1974). TE variation is predominantly driven by either a relative increase in A, a relative decrease in E, or a combination of both. The nature of any TE variation is an important issue to determine the likely impact on crop yield.

2.4.1 C_3 and C_4 photosynthetic pathway differences in TE

Large differences in TE exist between C_3 and C_4 plants because of the basic biochemical differences between photosynthetic pathways. C_4 plants utilize a four-carbon dicarboxylic acid pathway (Salisbury and Ross, 1992), which means that C_4 plants usually have low CO_2 compensation points and low resistances to CO_2 transport between the intercellular air space and the CO_2 sink in the chloroplasts (mesophyll resistance to CO_2 uptake). C_3 plants, which utilize the Calvin-Benson photosynthetic pathway (Hsiao and Acevedo, 1974), require higher CO_2 compensation points and higher resistances to CO_2 transport. Therefore the CO_2 concentration gradient in C_4 plants is greater than C_3 plants, accounting for higher rates of assimilation (A), and consequently higher TE.

2.4.2 Intra-specific variation in TE

Briggs and Shantz (1916) reported differences in TE between C_3 and C_4 species, and between and within species utilizing the same photosynthetic pathway, at the beginning of this century. Such observations allowed the authors to rank species in terms of TE and also to speculate that it may be possible to develop high TE lines through selection (Farquhar *et al.*, 1989b). Despite these early findings, little research was subsequently conducted on TE variation within species. The reasons for this lack of follow-up research are unclear, but probably relate to the fact that workers were sceptical (Ludlow and Muchow, 1990) and simply did not believe that variation in TE existed within a particular photosynthetic pathway (Tanner and Sinclair, 1983; Farquhar *et al.*, 1989b).

Due to the increasing interest and application of the model that described yield under water-limited environments as a function of T, TE and HI (equation 1), there has been growing interest in the potential benefits from selecting for improved TE. The observation of two-fold variation in TE among wheat genotypes made by Farquhar and Richards (1984), proved to be the catalyst for further extensive investigations into the variation of TE within a species.

TE can be investigated at two levels of organisation. Some studies examine TE at the leaf level by measuring fluxes of CO_2 and water vapour over very short time scales (Baldocchi *et al.*, 1985; Frank *et al.*, 1987; Dingkuhn *et al.*, 1991). Encouragingly, such studies have revealed variation in TE for crops such as rice, cowpea, grasses and soybean. While this 'instantaneous' TE approach is a useful indicator of basic mechanistic differences in TE, it does not provide any insight into how the plant will perform in a canopy situation over a crop

life-cycle. Therefore, measurements of short term differences in TE have been criticised because they do not integrate the impacts of morphological, phenological and other influences on TE (Richards *et al.*, 1993). Additionally, the large coefficients of variation of such measurements make them unsuitable for selection studies (Subbarao *et al.*, 1994). Empirical evidence from studies on tomato support this belief, and conclude that instantaneous TE was a poor indicator of crop TE (Martin and Thorstenson, 1988). As this review is targeted at crop improvement via a broad-trait approach, whole-plant TE measured over a long time scale will need to be the trait investigated.

2.5 TE as a selection criteria in breeding programs

Since the initial studies on wheat (Farquhar and Richards, 1984), a number of workers have conducted container and field experiments to investigate the range in TE among genotypes of many crop species (Richards *et al.*, 1993). The results of a number of these studies are summarised in Table 3 and provide strong evidence of genotypic variation in TE for a range of species. Despite these numerous reports of TE variation, and the increasing interest in the subject, there are no published reports of TE variation in soybean.

Table 3 - Summary of experiments which measured significant ($P<0.05$) genotypic variation in TE for several crop species.

Author	Crop	Type of Study
Farquhar *et al.* 1984	Cowpea	Pot
Hubick *et al.* 1986	Peanut	Pot
Hubick *et al.* 1987	Cotton	Pot
Wright *et al.* 1988	Peanut	Field
Condon *et al.* 1990	Wheat	Field
Virgona *et al.* 1990	Sunflower	Pot
Wright *et al.* 1991	Peanut	Pot
Donatelli *et al.* 1992	Sorghum	Pot
Condon *et al.* 1993	Wheat	Pot
Ismail *et al.* 1994	Cowpea	Pot

To date, most research into TE has been confined to pot studies due to the constraints imposed by the available methods of measuring TE in the field. While the TE variation reported in these studies is encouraging, such results also need to be confirmed under field conditions before advocating TE as a viable trait (Subbarao *et al.*, 1994), as a number of complications may arise when scaling up from studies on individual plants to crop canopies (Farquhar *et al.*, 1989a).

A phenomenon known as 'coupling' is a major component of this scaling up effect. The degree of coupling is determined by several factors, but is dominated by the depth of the internal boundary layer. The internal boundary layer is the envelope of relatively moist, still air immediately adjacent to the leaf (in the case of an isolated plant) or crop canopy (in the case of a field-grown crop), which provides a shield against the external ambient conditions (Oke, 1990). The more closely a plant/canopy is coupled to the surrounding atmosphere, the more sensitive the stomata are to changes in vapour pressure deficit of the prevailing

atmospheric conditions. An isolated plant, due to its smaller boundary layer, is more closely coupled to the atmosphere than a plant grown within a canopy. In a genotype/species in which stomatal control factors are responsible for variation in TE, any large differences in TE measured on individual plants may not necessarily be expressed at the canopy level due to the 'uncoupling' effect associated with canopy boundary layer resistances (Jarvis and McNaughton, 1985). Conversely, in species where photosynthetic capacity differences control TE variation (eg. peanut, Wright *et al.*, 1988), the degree of coupling seems to have little effect on TE and scaling from the plant to the canopy level has been shown to have little effect on TE variation (Farquhar *et al.*, 1989a; Subbarao *et al.*, 1994).

2.5.1 Measurement of TE

Precise measurements of T and root biomass have been required to accurately measure TE (Wright *et al.*, 1994) and have been extremely difficult to achieve in field studies (Tanner and Sinclair, 1983). Such constraints probably led to the misconception that selection for TE in breeding programs was a practical impossibility (Hall *et al.*, 1993). However, recent reports of strong correlations between TE and the relative abundance of C^{12}:C^{13} isotopes in plant dry matter (referred to as carbon isotope discrimination or Δ), have recently altered these perceptions.

2.6 Carbon isotope discrimination (Δ) as an indirect measurement of TE

2.6.1 Background

There are two naturally occurring, stable isotopes of carbon in atmospheric CO_2. The most abundant is $^{12}CO_2$, accounting for 98.9% of atmospheric CO_2, while $^{13}CO_2$ accounts for only 1.1%. During photosynthesis plants actively discriminate against the heavier $^{13}CO_2$ (Farquhar

et al., 1989a), thus fixing proportionally more $^{12}CO_2$ than the atmospheric ratio of the two isotopes would suggest. The magnitude of this discrimination varies with photosynthetic pathway, environment and genotype. It is this variation that allows scientists to apply isotopic methodologies to the study of plant physiology (Ehleringer *et al.*, 1993).

To measure the carbon isotopic discrimination of a plant, carbon in plant dry matter is converted to CO_2 prior to analysis, usually by combustion (O'Leary, 1993). Fundamental to the determination of discrimination is the molar abundance ratio (R; Hall *et al.*, 1993), which is determined using the following equation :-

$$R = {}^{13}C / {}^{12}C \quad \text{.....[equation 3]}$$

An isotopic ratio mass spectrometer is used to precisely measure R to an accuracy of ± 0.1 per mil (‰). These R values are then converted to an index ($\delta^{13}C_p$) of isotope abundance of plant material by the following formula :-

$$\delta^{13}C_p = [R \text{ (plant sample)} / R \text{ (PDB standard)} -1] * 1000‰ \quad \text{.....[equation 4]}$$

The PDB standard refers to the abundance ratio (R) of a specific limestone source from a formation in South Carolina (O'Leary, 1993). To calculate the final, non-dimensional discrimination values (expressed as 'per mil' or Δ), the following relationship is used.

$$\text{discrimination } (\Delta) = (\delta^{13}C_a - \delta^{13}C_p) / (1+[\delta^{13}C_p/1000]) \quad \text{.....[equation 5]}$$

where $\delta^{13}C_a$ = atmospheric CO_2 substituted for plant sample CO_2 in equation 4.

Discrimination expressed in this manner reflects the isotopic differences between the source (CO_2 in the atmosphere) and the product (carbon in plants: O'Leary, 1993).

2.6.2 Relationship between TE and Δ

Farquhar and Richards (1984) proposed that Δ could be used as an indirect measure of TE. This was based on the assumption that TE and Δ should be correlated at the leaf level in C_3 plants, via independent links to the ratio of intercellular and ambient CO_2 partial pressures (p/p_a) (Hubick and Farquhar, 1989). The ribulose-1,5-bisphosphate carboxylase-oxygenase (rubisco) enzyme, which fixes CO_2 in the first step of photosynthesis (Salisbury and Ross, 1992), actively discriminates against the heavier $^{13}CO_2$ molecule (Farquhar *et al.*, 1989a). As the internal partial pressure of CO_2 in the sub-stomatal cavity (p_i) gradually decreases, $^{13}CO_2$ becomes concentrated relative to $^{12}CO_2$ in the intercellular spaces (Subbarao *et al.*, 1994), with the net effect being an increased proportion of $^{13}CO_2$ fixed. The carbon fixed by plants which achieve lower p_i (through either stomatal closure to prevent water loss, or increased CO_2 fixation due to a higher photosynthetic capacity per unit leaf area (Wright *et al.*, 1988)), will consequently have a lower Δ value. It follows that Δ should be positively correlated to p/p_a in C_3 plants where the discriminating rubisco enzyme is present as the major CO_2 fixation pathway. In subsequent studies of poplar, cotton, bean (Brugnoli *et al.*, 1988), peanut, barley (Farquhar *et al.*, 1989b) and wheat (Condon *et al.*, 1990), Δ has indeed been shown to be positively correlated with p/p_a.

It has also been shown in a number of studies (Ehleringer, 1990; Dingkuhn *et al.*, 1991; Hall *et al.*, 1992; Chen *et al.*, 1993; Comstock and Ehleringer, 1993; Tan and Buttery, 1994) that genotypes differ in how they balance relative rates of leaf conductance of water vapour (E) with the uptake of CO_2 for photosynthesis (A; see equation 2). These differences produce variation in p/p_a that is subsequently reflected in Δ values of plant dry matter. Some studies (Dingkuhn *et al.*, 1991; Hall *et al.*, 1992) have empirically confirmed the negative

relationships between A/E and Δ. This should also be the case over longer time scales where TE, rather than A/E, is the integrated measure of differences in conductance and/or assimilation. In this situation, the differences in TE (resulting from the cumulative effects of differences in p_i/p_a) should be correlated with the differences in Δ. At the crop scale, the correlation between TE and Δ has also been demonstrated for many different species in both the glasshouse and field (Table 4). The prospects of developing Δ as a surrogate measure of TE appear to be quite encouraging for a comprehensive range of C_3 species, however no specific studies on the correlation between TE and Δ have been reported in soybean.

Table 4 - The range in Δ and its correlation with TE in a range of crop species.

Crop Species	**Location**	**Range in Δ (‰)**	**Association (TE / Δ)**
Wheat	Glasshouse	4.1	negative
Barley	Glasshouse	2.6	negative
Rice	Field	1.8	negative
Sunflower	Glasshouse	3.0	negative
Common bean	Pots (field & glasshouse)	3.2	negative
Tomato	Pots (in field)	3.4	negative
Crested wheatgrass	Glasshouse	5.4	negative
Cotton	Glasshouse	3.3	negative
Coffee	Field	4.0	negative
Peanut	Field and glasshouse	3.8	negative

Source : extract from (Turner, 1993)

2.6.3 Advantages of using Δ as a surrogate measure of TE

There are several advantages in using Δ as a surrogate measure for TE. Firstly, it is not necessary to directly measure transpiration and dry matter production, both of which involve difficult and time-consuming methodology (Turner, 1993). In contrast, as genotypic ranking of Δ has been shown to be maintained when measured on different plant parts (Hubick, 1986 and Condon *et al.*, 1992), only a small sample of plant dry matter (eg. leaf) is required for Δ determination. In addition, Δ also provides an integrative assessment of genotypic variation in TE over the life of a crop (Condon *et al.*, 1992), enabling the integration of many lower order responses at the canopy-level (Passioura, 1981). Subsequent studies have also shown that genotypic ranking for Δ is reasonably stable across differing water stress treatments (Hubick, 1990 and Hall *et al.*, 1994), and that the broad sense heritability for Δ is generally greater than 50% for a range of species (Turner, 1993). These are particularly attractive attributes for plant breeders who consider the use TE as an indirect selection trait for improved yield performance under water-limited conditions.

2.6.4 Disadvantages of using Δ as a surrogate measure of TE

Any environmental condition that affects either stomatal conductance or photosynthetic capacity per unit leaf area will affect Δ (Condon *et al.*, 1992). For instance, decreased soil water availability (Wright *et al.*, 1988; Hubick and Farquhar, 1989) and increased VPD (Winter *et al.*, 1982) can lead to lower Δ in plants. Such environmental effects may contribute to G x E interaction for Δ, although most studies indicate the G x E for Δ has been small (Winter *et al.*, 1982; Condon and Richards, 1992a; Hall *et al.*, 1992; Hall *et al.*, 1994; Ismail *et al.*, 1994; Ngugi *et al.*, 1994). The relatively small G x E suggests Δ could be used as an early generation screen in breeding programs (Wright *et al.*, 1993).

The expense of measuring Δ may be one factor which limits its widespread adoption. An isotope ratio mass spectrometer especially set up to measure R (the ratio of ^{13}C to ^{12}C) is required to analyse plant material for Δ (Turner, 1993). Considerable expertise is required to operate such a machine and commercial sample analyses can cost upwards of $20 AUD per sample. Sample analytical costs could therefore become prohibitive in intensive breeding programs where large numbers of samples need to be analysed.

Recent studies have identified cheaper alternatives to measuring Δ, which are similarly well correlated with TE in a range of species (Masle *et al.*, 1992; Nageswara Rao and Wright, 1994). For example, specific leaf area (SLA) and leaf mineral content have been found to be well correlated with TE in a number of species. These findings are further reviewed in section 2.7.

2.7 Specific leaf nitrogen and specific leaf area

In species where photosynthetic capacity (P_n) dominates variation in TE, surrogate measures of P_n should also correlate with TE. The amount of the photosynthetic enzyme rubisco has been shown to be directly proportional to photosynthetic rates (Nageswara Rao *et al.*, 1995). Most leaf nitrogen is accounted for by such photosynthetic enzymes (Evans, 1989) so it follows that specific leaf nitrogen (g N cm^{-2}; SLN) should also be correlated with P_n. Higher SLN can be achieved through either increased N concentrations in leaves of constant thickness or relatively thicker leaves of similar N concentration. In several species, correlations between leaf thickness and P_n suggest that thicker leaves are the cause of higher SLN and consequently higher P_n rates.

Specific leaf area (SLA), defined as the leaf area per unit leaf dry weight (cm^2/g), describes the thickness and density of a leaf (Wright *et al.*, 1994). It may be used as a surrogate measure of SLN in species where C:N leaf ratios are constant (Wright *et al.*, 1988). Experimental evidence supports this hypothesis, with differences in SLA shown to be correlated with genotypic variation in P_n rates in a number of species reviewed by Nageswara Rao and Wright (1994). In species such as sunflower (Virgona *et al.*, 1990), peanut (Hubick *et al.*, 1986; Wright *et al.*, 1988; Nageswara Rao and Wright, 1994; Nageswara Rao *et al.*, 1995;), and navybean (Wright, 1995a), where differences in P_n rates determine TE variation, SLA has been shown to be highly correlated with TE. SLA is an attractive trait to plant breeders because it is easy and inexpensive to measure, requiring only a leaf area meter, dehydrator and an accurate balance. In addition, the physiological basis of the correlation between TE and SLA is well-understood (Wright *et al.*, 1993; Nageswara Rao and Wright, 1994).

While the ease of measurement of SLA suggests it is worthy of evaluation as a surrogate measure of TE in soybean, there are conflicting reports regarding the strength of the relationship between P_n rate and SLA in soybean (Ma *et al.*, 1995). These observations may be due to a number of factors that may cause the relationship between TE and SLA to break down. Firstly, if stomatal conductance was mainly responsible for TE variation (eg. as in wheat; Farquhar, 1984; Condon, 1990), SLA will not be strongly correlated with TE (Subbarao *et al.*, 1994). Secondly, the relationship may not hold where leaf N content (and presumably the amount of rubisco) is not consistent across different leaf thicknesses, although this can be avoided if SLN, rather than SLA, is used. SLN is a more direct indicator of rubisco, and hence leaf P_n (Evans, 1989), because it combines the effects of both leaf

thickness and leaf N content. To account for these possible scenarios, both SLA and SLN were investigated in the experiments reported in this thesis.

2.8 Tissue mineral content

Recent studies have shown that the mineral content of plant dry matter is negatively correlated with TE (Masle *et al.*, 1992), suggesting that leaf mineral concentration may also be a useful surrogate measure of TE. Leaf mineral concentration is easily determined. To measure mineral weight, plant samples are pre-dried at 70°C for 48 hours, ground and incinerated in a high temperature furnace. The residual ash is taken as an approximation of mineral content since chloride and sulphur disappear and other elements are oxidised during combustion. Tissue mineral concentration is then calculated as the ratio of mineral weight (mg) to the pre-furnace plant dry weight (g). The physiological basis for the negative relationship between TE and mineral content is described by the following equation of Masle *et al.* (1992).

$$m = x * [T/TDM] \quad \text{.....[equation 6]}$$

where: m = the mineral content per unit biomass (dry weight),

x = the concentration of minerals in the passive transpiration stream of the xylem, and

T/TDM = the inverse of TE.

Variation in the amount of minerals concentrated in plant dry matter (m_a) is largely the result of relative differences in transpiration and dry matter accumulation. Higher transpiration rates coupled with constant carbon assimilation rates, will cause a decrease in TE but a rise in m_a, due to the greater amount of minerals travelling in this transpiration stream that are deposited into dry matter. Conversely, if T remains relatively constant but high rates of dry matter fixation are achieved, TE would increase but m_a would decrease because the same minerals are distributed among a larger pool of dry matter. Scenario one would be typical of wheat,

where large genotypic differences in T cause variation in TE (Farquhar and Richards, 1984). Sunflower (Virgona *et al.*, 1990) provides an example of the second scenario, with large genotypic differences in P_n capacity dominating variation in TE. Significant correlations between R and m_a have been observed in both wheat and sunflower (Masle *et al.*, 1992), suggesting that m_a, like Δ, may integrate the effects of differences in CO_2 assimilation and transpiration. In this respect it could have wider application than SLA and SLN, which are only useful as a surrogate measures for TE in species where differences in P_n are responsible for TE variation (eg. peanut; Rao and Wright, 1994).

2.9 Conclusions

Selection for yield under drought conditions has produced variable results and attempts to select for specific drought adaptation traits have had only limited success. Recently, TE has been identified as a major determinant of dryland yield performance, while subsequent studies have shown that significant genotypic variation exists for TE in some crop species. There is, therefore, the potential that TE may be a useful selection criterion for breeding for improved dryland yield performance of soybean. Easy-to-measure surrogates of TE have also been reported making indirect selection for TE improvement a possibility (eg. Δ, SLA, SLN, m_a). In the work reported here, a range of soybean germplasm was initially surveyed in both field and glasshouse studies to determine the extent of variation in Δ (and by inference, TE) in soybean and to provide a preliminary examination of the relationships between TE and Δ and between TE and SLA. More detailed studies were subsequently conducted to investigate the relationships between TE and Δ, SLN, SLA and m_a, and the mechanisms responsible for TE variation in soybean. Finally, genotypic variation in TE and its relationship with Δ, SLA, SLN and m_a were examined under canopy conditions in the field.

CHAPTER THREE

Surveying for variation in carbon isotope discrimination among soybean genotypes

3.1. Introduction

The objective of any breeding program is to increase the genetic potential of a particular trait (eg. disease resistance or yield; Halloran *et al.*, 1979). The rate of improvement for a given trait, or its genetic progress, is proportional to the extent of variation for that trait within a species, so breeders screen large numbers of genotypes to obtain the widest possible variation (Byth *et al.*, 1986). Transpiration efficiency has been postulated as a potentially useful trait for enhancing drought resistance in breeding programs (Passioura, 1977; Tanner and Sinclair, 1983). Substantial genetic variation for TE has been measured in wheat (Farquhar and Richards, 1984), barley (Hubick and Farquhar, 1989), cotton (Hubick and Farquhar, 1987) sunflower (Virgona *et al.*, 1990) and tomato (Martin and Thorstenson, 1988) in pot experiments and for pea, canola, wheat (Knight *et al.*, 1994), cowpea (Abdelbagi *et al.*, 1992) and peanut (Wright *et al.*, 1988) under field/canopy conditions. While no similar work has been reported for soybean, the extensive evidence for substantial TE variation in many other C_3 species suggests there may also be exploitable variation in TE among soybean genotypes.

Practical difficulties associated with the measurement of TE (Wright *et al.*, 1994) on large numbers of genotypes prohibits wide-scale screening of soybean germplasm to determine the presence and extent of TE variation. This problem has led workers in other crops to conduct initial surveys for Δ, as a surrogate measure of TE, on large germplasm collections. Once extreme genotypes have been identified, more detailed experiments to determine the reasons for genetic variation in TE are then undertaken. This approach, which assumes TE and Δ are

well correlated, has been successfully used in a number of crops (Hubick *et al.*, 1986; Wright *et al.*, 1988).

While there are no previous reports of TE variation among soybean genotypes, a study by Kumarasinghe *et al.* (1992) has investigated the range in Δ among a limited number of USA soybean genotypes. This study observed only a small range in Δ of *c.* 0.94‰ which would equate to a 12 % variation in TE (Farquhar and Richards, 1984). While this range appears small relative to other crop species (Turner, 1993), it could be argued that these genotypes were derived from a narrow genetic base and that more extensive variation in TE may exist within the species. Consequently, a survey of the variation in Δ was conducted among a series of Australian soybean germplasm collections to enable the widest possible range in Δ to be sampled. This approach then allowed a small subset of genotypes to be studied in more detail in a preliminary glasshouse experiment to establish the existence and strength of the relationship between TE and Δ in soybean.

3.2 Methods & Materials

Three different collections of soybean germplasm were measured for variation in Δ.

3.2.1 Survey 1 - CSIRO Breeding Collection

The initial survey examined 20 soybean genotypes selected from within a CSIRO Division of Tropical Crops and Pastures drought tolerance improvement project. The collection represented a range of genotypes with differing drought resistance characteristics including epidermal conductance, critical relative leaf water content and osmotic adjustment James (1996). The plants were grown under well-watered conditions in the field at Gatton, Queensland. During the late vegetative stages a random sample of new fully-expanded leaves

was collected and oven dried at 70 °C for 48 hours. The samples were then ground to pass through a 100 μm sieve, and analysed for Δ. This process involved conversion of leaf dry matter into gas by combustion. The gas was then analysed in an isotopic ratio mass spectrometer to measure molar abundance ratios which were then converted to carbon isotope discrimination values using equations 4 and 5 presented earlier (for a detailed explanation refer to Ehleringer *et al.*, 1993). The Biological Sciences Laboratory of the Australian National University, Canberra was contracted to conduct all Δ analyses.

3.2.2 Survey 2 - QDPI Breeding Collection

The second survey sampled germplasm from the QDPI Soybean Breeding Program based at Warwick, Queensland. The collection of 19 genotypes consisted of a mixture of released commercial varieties and advanced breeding lines. Samples were collected from a QDPI variety trial located at Kingaroy (151°5′ E, 26°32′ S) in south-eastern Queensland during the summer growing season of 1992 - 93. Plants were sampled during the late vegetative stages, at which time a random sample of new, fully expanded leaves were harvested. The dried leaf samples were then ground to pass through a 100 μm sieve, and analysed for Δ using the methodology described for survey 1.

3.2.3 Survey 3 - Collection from QDPI Genetic Resources Centre - Biloela

A third survey was conducted during the summer of 1993 - 94. A diverse range of 98 genotypes was randomly selected from a soybean germplasm collection located at the QDPI Genetic Resources Centre at Biloela, Queensland. This collection of soybean genotypes was derived from a wide diversity of overseas origins that would hopefully represent a significant

variation in Δ, and presumably TE. The survey was conducted at the Kingaroy experimental site as described above. The plants were grown in 0.90m rows in 10 m x 3.6 m plots in the field under well watered conditions. At 56 DAP, 40 fully expanded leaves were randomly selected from each plot and Δ was measured on ground samples using the procedures outlined in survey 1.

3.2.4 Statistical analysis

All three experiments were analysed using RANB Randomised Block, a statistical software computer program developed by the Queensland Department of Primary Industries (Swain *et al.*, 1977). Regression equations were calculated by the method of least squares using GRAPHER for Windows software (Kecker *et al.*, 1994).

3.3 Results

A summary of results from the three germplasm collections surveyed for Δ, including the maximum and minimum genotype values and the variation in Δ are shown in Table 5.

Table 5 - Variation in Δ measured from surveys of three collections of soybean germplasm.

Germplasm Survey	Extreme values from the range in Δ (‰)	Range in Δ (‰)	Variation in Δ (%)
Survey 1	22.1 - 23.2	1.2	5.3
Survey 2	18.5 - 19.3	0.8	4.3
Survey 3	19.0 - 21.8	2.8	14.7

3.3.1 Survey 1 - CSIRO Breeding Collection

The germplasm of the Drought Tolerance Improvement Project showed a range in Δ of only 1.2‰ (Table 5). This was similar to the 0.8‰ recorded by Kumarasinghe *et al.* (1992) for a subset of soybean genotypes grown in pots and considerably narrower than the ranges recorded for many other species (Table 4). The lack of substantial variation was surprising, given that this germplasm had been shown to express significant variation in other putative drought adaptive traits.

3.3.2 Survey 2 - QDPI Breeding Collection

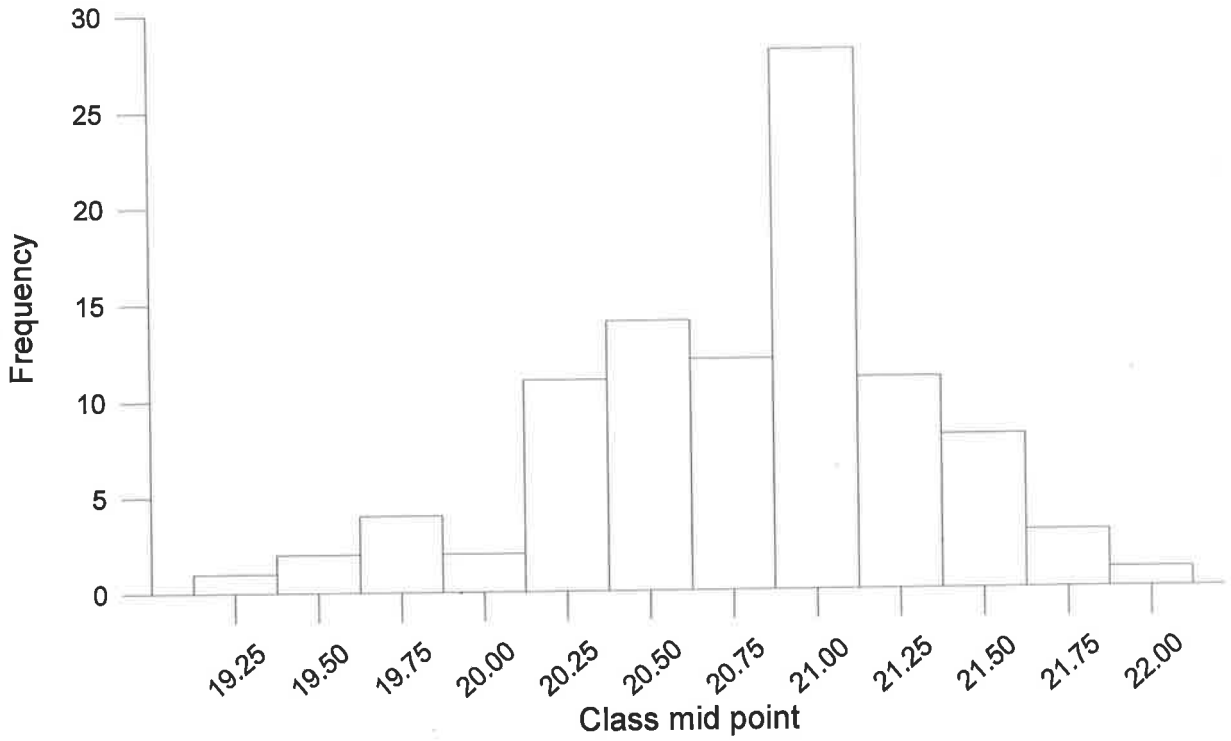
Within the QDPI breeding collection, the observed range in Δ of 0.8‰ was again relatively small and similar to the Δ range observed in survey 1 (Table 5). The fact that this collection of commercial and advanced soybean varieties did not exhibit substantial variation in Δ, and

presumably TE, is not surprising as the collection probably represented a narrow genetic base of material selected for specific adaptation to the irrigated south Queensland environments.

3.3.3 Survey 3 - QDPI Germplasm Bank Collection

Details of the 98 germplasm lines, including country of origin and Δ values, are presented in Appendix 1. Individual genotypes were plotted in a frequency distribution chart (Figure 2) to examine the spread of Δ values across the observed range. It revealed that the sample of genotypes was not normally distributed about the median, being significantly skewed towards lower Δ values (Pearsonian coefficient of skewness = -0.68). This result suggests that the bulk of the soybean genotypes lie at the high Δ (and presumably low TE) end of the total Δ range.

Figure 2 - Frequency distribution of Δ measured from 98 soybean genotypes grown under full irrigation. Class intervals are 0.24‰ and the distribution is significantly skewed to lower Δ values (Pearsonian coefficient = -0.68).



3.4 Discussion

TE was not measured in this study because of the practical problems associated with measurement of total plant dry matter and transpiration on large numbers of lines. However, assuming that TE and Δ are correlated in soybean as in many other C_3 species (Turner, 1993), an indication of the likely TE variation within a germplasm collection can be determined from Δ values. Significant variation in TE of other studies (Wright *et al.*, 1988; Abdelbagi *et al.*, 1992) was evident when the range in Δ was ≥1‰. This prompted the adoption of 1‰ as a minimum benchmark for the range in Δ, above which genotypic variation in TE could be expected (Ehleringer *et al.*, 1993). This benchmark value was therefore used as an indication of the extent of variation in TE from Δ values measured in preliminary surveys.

The measured Δ values can also be used to estimate the likely range in TE. Based on an empirical relationship describing the association between TE and Δ (Farquhar, 1982; Farquhar, 1984), a method has been used to derive approximate ranges in TE from maximum and minimum Δ values (Hubick *et al.*, 1986; Wright *et al.*, 1988). This approach was based on the identities listed over page.

For a typical leaf, the ratio of assimilation and transpiration (A/E) is inversely related to p_i/p_a in the manner:

$$TE = A/E = [p_a(1-p_i/p_a)] / 1.6v \quad \text{.....[equation 7]}$$

p_i = intercellular partial pressure of CO_2

p_a = atmospheric partial pressure of CO_2

v = water vapour pressure differences between the intercellular spaces and the atmosphere

1.6 = a factor to account for the higher diffusivity of water vapour in air than CO_2 in air

The p_i/p_a term is linearly related to Δ in all C_3 plants by equation 8 (Hubick *et al.*, 1986), in which the constants 4.4 and 22.6 are the fractionations associated with diffusion of CO_2 into the leaf and the total of the fractionation processes that occur within the mesophyll, respectively.

$$\Delta = 4.44 + 22.6*p_i / p_a \quad \text{.....[equation 8]}$$

The highest and lowest Δ values from genotypic surveys are substituted into equation 8 to calculate the corresponding $1- p_i/p_a$ values. As $1- p_i/p_a$ is proportional to TE (equation 7), the range in $1- p_i/p_a$ should equate to the likely range in TE.

Using this approach, the Δ values measured from the three soybean collections were used to indirectly estimate the ranges in TE (Table 6).

Table 6 - Estimates of TE variation made from extreme Δ values, based on the theoretical association between TE and Δ.

Germplasm Survey	$1-p_i/p_a$	TE Variation (%)
Survey 1	0.22 - 0.17	30
Survey 2	0.38 - 0.34	11
Survey 3	0.36 - 0.23	57

Compared with the benchmark Δ range of 1‰, the ranges for Δ measured in collections 1 and 2, suggest that there may not be significant genotypic variation in TE within that soybean germplasm. Furthermore, the errors of direct TE measurement in peanut studies (Hubick *et al.*, 1986; Wright *et al.*, 1988), expressed as a percentage of the total TE variation, have been shown to range from 12 - 25 %. Therefore, it could be argued that the magnitude of error would be of similar proportions to the phenotypic variation in TE shown in collections 1 and 2 and as a consequence, it would be difficult to identify genotypic variation TE.

In survey 3, there was a considerably larger range in Δ (*c.* 2.8‰) than in collections 1 and 2, which corresponded to a predicted variation in TE of 57%. This variation in Δ was similar to some of the largest ranges in Δ measured of any crop species (Table 3) and well above the 1‰ benchmark Δ value. Additionally, such a large calculated variation in TE (*c.* 57%) indicated that significant genotypic variation in TE might be detected, even in the presence of large error terms. While these results provided a strong indication that marked variation in Δ, and presumably TE, existed among the soybean genotypes, definitive experiments were needed to confirm this predicted TE range, as well as to confirm as the association between TE and Δ.

The range in Δ of surveys 1 and 2 was less than half that of survey 3. A number of hypotheses can be proposed to explain these differences. Firstly, the germplasm examined in survey 1 varied significantly in several physiological traits such as osmotic adjustment, critical relative leaf water content and epidermal conductance (James *et al.*, 1993), which are responsible for controlling dehydration tolerance in soybean (Ludlow, 1989). The fact that these genotypes showed little variation in Δ suggests that these dehydration tolerance traits may not directly impact on photosynthetic and/or stomatal conductance processes, and hence on Δ and TE.

Secondly, the fact that the ranges in Δ of the CSIRO and QDPI breeding collections were much smaller than that of the international germplasm collection suggests that the material within the respective breeding collections may have been derived from a relatively narrow genetic base. This hypothesis is supported by the results of Gizlice *et al.* (1994), who found that only six progenitors accounted for greater than half the genetic diversity of northern American soybean germplasm, and only 5 ancestors were responsible for two thirds of the southern American soybean gene pool. It is therefore relevant that most of the germplasm lines contained within the two breeding collections surveyed in this study were derived directly from these American progenitors (Lawn *et al.*, 1986). The third survey sampled the Δ trait among a much more diverse range of soybean germplasm and clearly showed that substantial genetic variation does exist. These observations also suggest that variation of Δ (and by inference, TE) may be quite limited in the soybean germplasm collections of the more developed countries.

Finally, the significantly skewed distribution towards low Δ found among the 100 soybean lines in survey 3 (Figure 2), suggests that the low Δ trait may be associated with substantially lower gene frequency in soybean germplasm. If this is true, a large sample of lines would need to be examined in order to capture the widest possible variation in Δ, and presumably TE.

The large range in Δ found in survey 3 allowed a subset of lines to be selected, on the basis of variation in Δ, for further evaluation to determine the extent of TE variation and its relationship with Δ among soybean genotypes. These experiments are described in subsequent chapters.

CHAPTER FOUR

Variation in TE among soybean genotypes under glasshouse conditions and its relationship with Δ.

4.1 Introduction

Transpiration efficiency (TE) is defined as the ratio of total plant biomass to transpiration (Wright *et al.*, 1988). From equation 1 it can be seen that improved TE can confer yield (Y) improvement under water limited environments and is therefore, an important trait for enhancing yield in dryland crop improvement programs.

A number of issues are involved in developing TE as a potential trait for selection in dryland soybean improvement programs. Firstly, significant genotypic variation must exist for the TE trait among soybean genotypes. To date there have been no reported attempts to quantify TE variation for soybean, mainly because the practical difficulties in measuring TE have limited the number of genotypes which can be surveyed (Wright *et al.*, 1994). To overcome this problem, leaf traits that are reasonably correlated with TE and easy to measure on large numbers of lines have been used to screen for potential TE variation on large germplasm collections (eg. Hubick *et al.*, 1986). In Chapter 3 we reported the use of an approach which used leaf Δ to indirectly screen a collection of soybean germplasm for TE variation. We observed *c.* 60% variation in calculated TE from a 2.8‰ range in Δ among the genotypes in that survey, which suggested that significant variation in TE may also be present provided TE and Δ are reasonably well correlated. While this assumption has been proven to be true in several other C_3 species (Turner, 1993), it has not yet been tested for soybean. The experiments reported in this chapter measured TE and Δ on a subset of the germplasm

collection sampled in survey 3 (Chapter 3) with genotypes selected on the basis of variation in Δ measured from the original survey. As well as Δ, other easily measured leaf traits such as SLA and leaf mineral content (m_a) have also been found to be reasonably well correlated with TE in a number of crop species (Wright *et al.*, 1988; Masle *et al.*, 1992). These leaf traits are also potentially attractive for use in breeding programs due to their low cost of measurement and simple analytical requirements. The relationship of these leaf traits with TE among soybean genotypes is also evaluated in the experiments reported in this chapter.

Once significant genotypic variation in TE has been demonstrated, the mechanisms responsible for TE variation need to be determined (Hubick and Farquhar, 1987). As TE is the ratio of TDM production to T, genotypic variation in TE may arise from variation in TDM, T or both. Empirical evidence shows that all three mechanisms of TE variation exist within major crop species (Condon *et al.*, 1990; Condon and Richards, 1992b; Nageswara Rao *et al.*, 1993; Nageswara Rao and Wright, 1994). If TE variation in a species is mainly due to variation in T, then high TE genotypes may have relatively lower T than the other lines (Hall *et al.*, 1992). However, high T is also essential to produce high yield under dryland conditions, due to the significant positive association between TDM and T in most crop species (Tanner and Sinclair, 1983). Therefore, in a crop where TE variation is largely due to variation in T, selecting for high TE may result in low T and ultimately, low yield.

In cowpea, for example, variation in T was shown to be largely responsible for genotypic variation in TE measured among cowpea lines (Udaykumar *et al.*, 1996). The authors warned against selection for TE unless genotypes in which TE variation was caused mainly by variation in TDM could be identified. It is therefore important that a thorough understanding

of the mechanisms responsible for TE variation be achieved in the target species under investigation. Consequently, we conducted detailed investigations into the mechanisms of TE variation in soybean at both the plant and leaf levels of biological organisation. These data allowed a determination of whether selection for high TE in soybean may cause reduced T by association, and consequently, whether high TE is likely to indirectly reduce yield under dryland conditions. Selection for high TE should theoretically be a beneficial trait to dryland soybean improvement programs if it is found that TE is not negatively associated with the other components of equation 1 (ie. T and HI).

In summary, the three experiments reported in this chapter were conducted to :

- confirm that significant variation in TE exists among soybean genotypes as observed from preliminary studies of leaf Δ, by measuring TE under controlled glasshouse conditions,
- evaluate Δ, SLA and leaf mineral content as possible surrogate measures of TE for use as potential screening techniques in soybean breeding programs,
- investigate the mechanisms underlying TE variation in soybean, in order to demonstrate that selection for high TE will not compromise high T and therefore plant size or yield,
- determine the influence of genotype x environment (G x E) interaction for TE and Δ, using data generated from the glasshouse experiments grown during different seasons.

4.2 Methods & Materials

4.2.1 Pot Experiment 1

From the germplasm collection of survey 3, a sub-set of 20 lines representing the full range in Δ were selected for investigation in a glasshouse experiment conducted at the J. Bjelke-Petersen Research Station, Kingaroy, during the summer of 1994.

4.2.1.1 Cultural conditions and experimental design

Glasshouse temperatures were allowed to fluctuate between a minimum of 18°C and a daytime maximum of 30°C, using heaters and evaporative coolers. Average daily VPD was 1.5 kPa and the average daily solar radiation outside the glasshouse was approximately 20.5 MJ m^{-2} d^{-1} over the duration of the experiment.

TE and Δ were measured during the vegetative growth phase only (from 9 to 34 days after emergence; dae) under non-limiting soil moisture conditions. Five replicates of 20 genotypes (see Table 7) were established in a randomised complete block design. Seeds were inoculated with the appropriate Rhizobium *spp.* strain and six seeds were planted into each pot on the 16th September, 1994. At 7 dae, seedlings were thinned to leave two healthy, uniform plants pot^{-1}. The pot system consisted of a 10 litre plastic bucket which was lined in the bottom with basalt gravel (10 mm diameter) to a depth of 5 cm with a shadecloth gauze partition placed on the gravel. The remaining pot volume was filled with a 2:1 mixture of red-brown light clay soil and fine sand, which had been pasteurized with a steam sterilisation treatment at 60°C for 30 minutes. Basal fertiliser (13.6% P, 13.7% K, 1.1% S and 11.3% Ca) was applied at the equivalent rate per pot surface area of 200 kg/ha and thoroughly mixed through the soil volume.

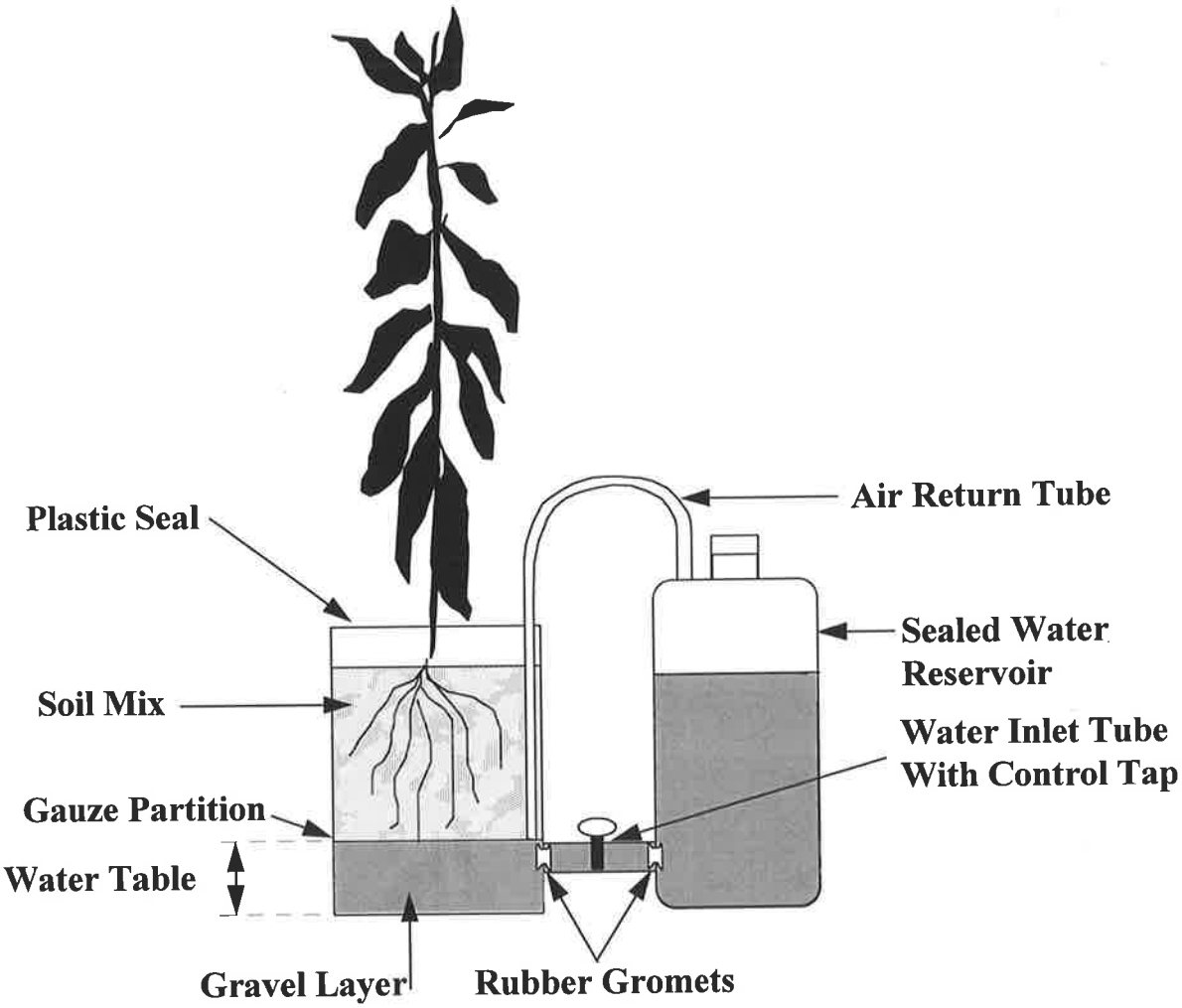
4.2.1.2 Pot system

To facilitate precise measurement of T for calculation of TE, a semi-automatic watering system (Figure 3 and Plate 1a) was developed based on the pot culture system outlined by Hunter (1981). This pot system allowed plants to grow under non-limiting soil moisture conditions, while enabling accurate measurement of plant water use.

Each pot was connected at the base to a 5 litre plastic, airtight water reservoir by means of a flexible plastic tube with an in-line PVC tap. This allowed controlled entry of water from the reservoir to the base of the pot. A water table of approximately 5 cm depth (corresponding to the depth of the basalt gravel) was maintained in the pot by fixing a return air tube, starting at 5 cm from the bottom of the pot, into the top of the water reservoir. All tube entry points to either the pot or reservoir bottle were via soft rubber grommets, cemented in place with silicon adhesive to maintain a vacuum seal.

When the water level dropped below the air feeder tube in the pot, air moved up the tube into the top of the water reservoir. This allowed water to enter the bottom of the pot until the water table reached the height of the air feeder tube (ie. 5 cm). At this point, air-flow through the air feeder tube into the top of the reservoir stopped, reinstating the vacuum in the reservoir which ceased water flow into the pot. When the reservoir bottle needed refilling, the in-line tap was turned off to stop the flow of water into the pot. Water was added via the reservoir cap, which was then replaced (to make the reservoir airtight) before again turning on the in-line tap. Each addition of water was carefully measured for subsequent calculation of pot transpiration.

Figure 3 - Schematic illustration of the automated pot watering system where fully-watered conditions are maintained, water-logging is avoided and transpiration is measured.



To minimise evaporation, a thin plastic sheet was attached to the top of all plots using a silicon adhesive, prior to the TE measurement period. Two small holes were made in the plastic sheets through which the plants could grow. The evaporative losses of the pot system were measured directly using control pots that were assembled in identical fashion to the pots described earlier, but not planted. A similar approach was used by Ismail *et al.* (1994) and Virgona *et al.* (1990) to minimise and measure evaporation. After all pots had been covered with plastic (9 dae), the measurement of T was commenced. Every few days the reservoirs of both the planted pots and the control pots were refilled and the volume required was recorded. Pots, including reservoirs, were weighed at the beginning and end of the treatment period to allow calculation of the change in soil moisture storage. Total transpiration per pot was calculated as total water use plus the change in soil water storage, less soil evaporation from the control pots.

4.2.1.3 Destructive sampling

The experiment was terminated at the early reproductive stage (34 dae). Plants were cut at ground level, and the above-ground dry matter separated into stem and leaf components which were subsequently oven dried at 70 °C for 48 hours and weighed.

To measure root dry matter, the pot contents were emptied into a large bucket containing a solution of water and dispersing agent. After gentle agitation, the larger primary roots were retrieved by hand and rinsed in clean water. The remaining slurry was passed through a sieve (0.8 cm) to trap the fine roots, which were then rinsed in clean water to remove any contaminating material. Both root samples were then bulked and oven dried at 70°C for 48 hours and weighed.

4.2.1.4 Specific leaf area (SLA) and Δ

At the time of final harvest, a random sub-sample of fresh leaves (approximately 1000 cm^2) was collected and measured for leaf area before being dried at 70°C for 48 hours and weighed. SLA was calculated as leaf area per unit weight (cm^2/g). The dried leaf sub-sample was ground to pass through a 100 μm sieve, and analysed for Δ (as described in Chapter 3).

4.2.1.5 Calculating TE

In all three pot experiments reported in this chapter, the components of TE (ie. TDM and T) were measured during only the vegetative phase of growth to minimise the potential influence of phenological effects on TE, as reported by Abdelbagi *et al.* (1992). TE is defined as the ratio of total biomass produced (g) to water transpired (kg) measured over the same period of time (Wright *et al.*, 1988). Total biomass accumulated during the period prior to commencement of T measurement ranged from 0.4 - 0.9 g. $plant^{-1}$. This was subtracted from final TDM and the resulting value was used to calculate TE.

4.2.2 Pot Experiment 2

4.2.2.1 Cultural conditions and experimental design

A glasshouse pot experiment was established at the J. Bjelke-Petersen Research Station, Kingaroy, Queensland during the Autumn of 1995 (Plate 1b). The thermal regime was regulated to between 20±3 and 30±3 °C. An average daily VPD of 1.01 kPa was calculated from daily maximum and minimum temperatures (Tanner and Sinclair, 1983).

Plate 1a (left) : Close-up view of the pot watering system used to measure TE in the glasshouse.

Plate 1b (below) : Distant view showing the layout of the second pot experiment measuring TE of soybean under glasshouse conditions.

The experiment was designed as a randomised complete block with 10 replicates of 5 soybean genotypes (Garoba Rouest, Otootan, Kabanyolo-1, Rawit, and Mensoy 6) split into two harvests. This gave a total of 100 pots, which were prepared in identical fashion to the procedure outlined in section 4.2.1. Pots were planted with six seeds each on the 6th March, 1995 and seedlings emerged in 3 days. At 13 dae seedlings were thinned to two plants pot^{-1} and weighed. A plastic sheet was applied to each pot to reduce soil evaporation.

4.2.2.2 Split harvests

This experiment was harvested in 2 stages. At 41 dae, 4 replicates were harvested and TDM, T, SLA, leaf N% and Δ were measured. Methodology was similar to the first pot trial with leaf N% determined using the Kjeldahl method (Rayment and Higginson, 1992). At 63 dae the remaining replicates were harvested and similar measurements were made. SLN (g N m^{-2}) was calculated from the product of leaf N% and SLA.

4.2.2.3 Calculation of TE and Δ over the period between initial and final harvests

TE was calculated during the period from the initial harvest at 41 dae until the final harvest at 63 dae, using the data on relative changes in TDM and T. The average Δ during this period was calculated using the following identity from Wright *et al.* (1994).

$$\Delta = (\Delta_2 TDM_2 - \Delta_1 TDM_1) / (TDM_2 - TDM_1) \quad \text{[equation 9]}$$

where Δ_1 and Δ_2 are the isotope discriminations, and TDM_1 and TDM_2 are total plant dry weights at initial and final harvests respectively.

4.2.3 Pot Experiment 3

A subset of 6 genotypes from pot experiment 1 (including the 5 genotypes used in pot experiment 2) were selected for investigation in a third glasshouse experiment conducted at Kingaroy during the summer of 1996.

4.2.3.1 Cultural conditions and experimental design

Temperature was moderated to fluctuations between a minimum of 20 ± 1°C and a maximum of 31 ± 3°C, with an average daily VPD of 1.3 kPa. The average incident solar radiation for the duration of the experiment was 19.6 MJ m^{-2} day^{-1}, from a range of 15.5-31.8 MJ m^{-2} day^{-1}. The experimental design was a randomised complete block design with a total of six varieties (Garoba Rouest, Otootan, Tai-Dung-Wu-Tou, Kabanyolo-1, Rawit and Mensoy 6), randomised within 3 replicates. Pots were set up in identical fashion to that described in section 4.2.1, including control evaporation pots. On the 12th January 1996, each pot was planted with six seeds that emerged after 4 days. At 9 dae, pots were thinned and covered with plastic to minimise evaporation. The thinned plants were not weighed as in previous pot experiments because these showed that seedlings weights were less than 1% of final dry matter and were therefore considered negligible. At 14 dae, when plants were at the 1 - 2 trifoliate stage, measurement of T was commenced.

4.2.3.2 Harvest and processing

At 44 dae, the 3 replicates were harvested and TDM (including roots) was measured using techniques described in pot experiment 1. Total dry matter, T, TE and above-ground TE

(TE_{ag}) were calculated for the period 14 - 44 dae. TE_{ag} is similar to TE, but only uses above-ground dry matter as the numerator (root dry weight is ignored). Measurements of Δ and leaf N % were made using similar analytical procedures as described for the previous experiments, both from a random sample of bulk leaves (Δ and N) and a sample of uppermost, fully expanded leaves (Δ_u and N_u). SLA was determined using leaves from a companion trial grown in the glasshouse at the same time. SLN (g N/m^2) was calculated using SLA and leaf N % values of the bulk leaf sample. Total leaf mineral content (m_a) was measured on a portion of the dried and ground bulk leaf sample used for Δ determination.

Net photosynthesis (P_n) and instantaneous transpiration (T_i) were measured on individual leaves using an open system C1 - 301PS Portable Photosynthesis System, on three cloudless days during the period from 34-40 dae. Photosynthetic photon flux density (PPFD) ranged from 1600 - 2800 μmol m^{-2} s^{-1} during measurement which took place at either mid-morning (0900 - 1030 h) or midday (1200 - 1300 h). Subsequent analysis showed no significant genotype x time of measurement interaction between the 3 data sets, so a pooled analysis of genotypic differences was constructed. Instantaneous transpiration efficiency (TE_i) was calculated as the ratio of P_n and T_i.

4.3 Results

4.3.1 Pot Experiment 1

Significant ($P<0.05$) genotypic variation of 74% for TDM and 56% for T were observed from ranges of 28.5 - 49.4 g. pot^{-1} and 11.7 - 18.3 L. pot^{-1}, respectively (Table 7). There was a highly significant ($P<0.01$) correlation between TDM and T (Figure 4) and the large range in root to shoot ratio (0.29-0.50) among genotypes indicated genotypic differences in dry matter partitioning (Table 7).

From the range in TE (2.15-3.04 g/kg) measured among genotypes, a significant ($P<0.05$) genotypic variation of *c.* 41% was observed (Table 7). This magnitude of variation compared well with *c.* 60% variation which was predicted from Δ values measured in Chapter 3 (survey experiment 3). A significant ($P<0.05$) range in Δ was also measured among genotypes (20.3-21.8 ‰; Table 7). The variation in Δ was linearly related to differences in TE, such that a significant ($P<0.05$) negative relationship between TE and Δ was observed ($r = -0.57$ in Figure 5).

There was also a large and significant ($P<0.05$) range in SLA (197-366 cm^2/g) measured among genotypes (Table 7). The variation in SLA was also negatively correlated with TE ($r = -0.65$; Figure 6).

Table 7 : Total dry matter (TDM), transpiration (T), root:shoot ratio (r:s), transpiration efficiency (TE), specific leaf area (SLA) and carbon isotope discrimination (Δ) measured from 20 soybean genotypes grown in the glasshouse under fully-irrigated conditions.

	Genotype	TDM (g)	T (l)	r:s	TE (g/kg)	SLA (cm^2/g)	Δ $*10^3$
1	Shih-shih	49.1	17.4	0.50	2.83	220	20.9
2	Taichung 3	43.0	16.0	0.45	2.69	272	21.4
3	Garoba Rouest	45.5	15.9	0.34	2.84	197	20.3
4	Oribi	40.2	13.3	0.43	3.04	216	20.8
5	Ecuador 2	49.4	16.9	0.44	2.93	299	21.0
6	G230	34.1	11.8	0.34	2.90	241	20.9
7	Otootan	35.3	14.3	0.34	2.46	304	21.2
8	Tai-Dung-Wu-Tou	29.6	13.6	0.34	2.15	335	21.7
9	Mensoy 3	40.2	15.3	0.32	2.63	206	20.8
10	C8014	48.0	18.3	0.31	2.64	237	20.5
11	Roanoke 49376	38.1	14.0	0.29	2.71	338	20.9
12	Kabanyolo -1	44.4	17.4	0.41	2.56	366	21.8
13	Ocepar 2 Iapo	45.4	15.3	0.40	2.92	244	20.8
14	G869	42.9	15.8	0.38	2.73	225	20.4
15	Mensoy 6	43.8	15.0	0.37	2.93	232	20.9
16	Karangduen 1	42.6	16.4	0.30	2.60	332	21.1
17	Fruhe Gelbe	36.7	13.5	0.29	2.72	221	20.8
18	CPAC X3-76	33.7	13.6	0.31	2.53	300	21.0
19	Bau1/4	41.7	15.8	0.39	2.65	294	21.0
20	CEP 7138	28.3	11.7	0.35	2.41	277	21.2
	LSD (P<0.05)	8.5	2.3	0.09	0.32	28	0.5

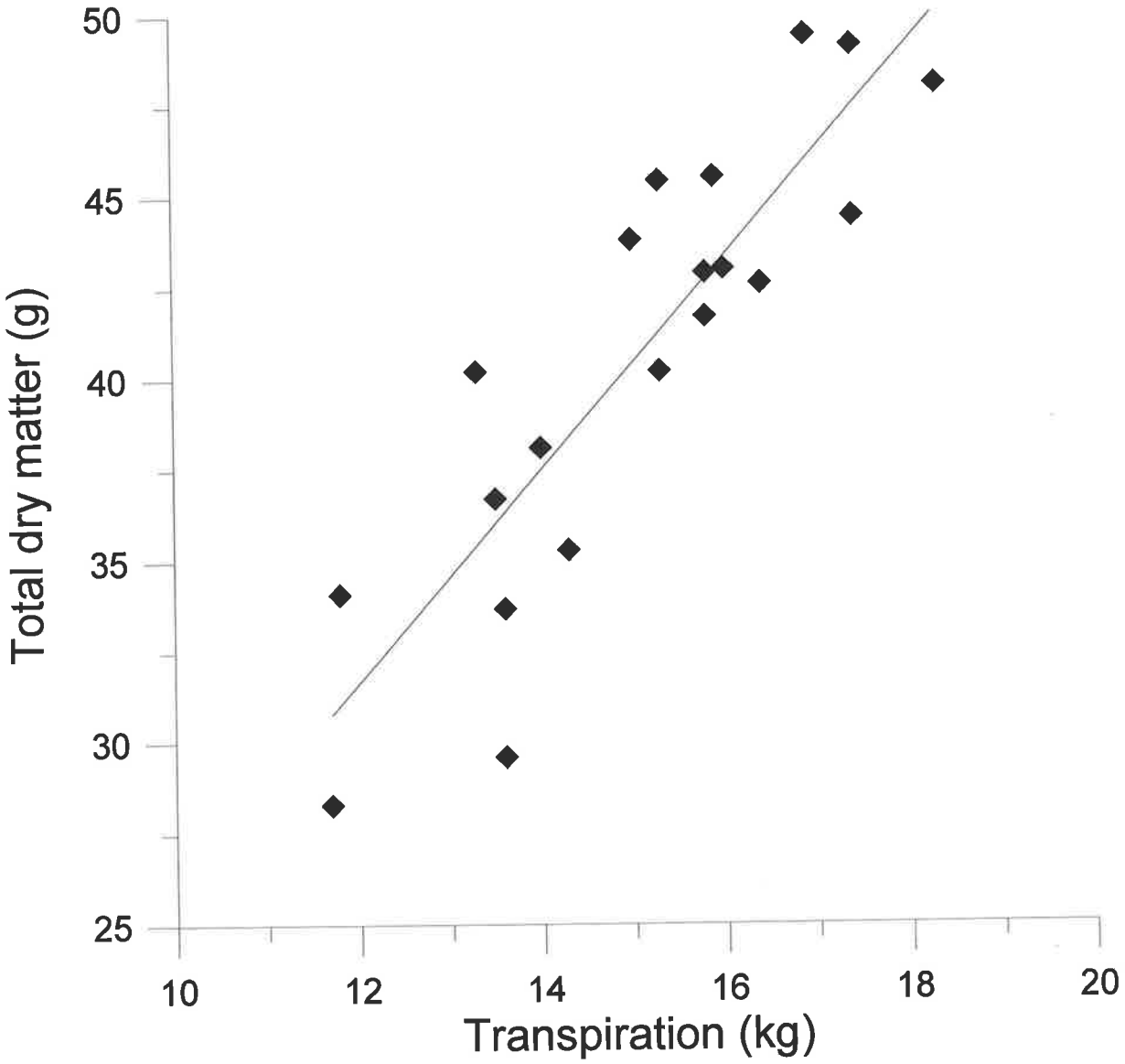


Figure 4 - Relationship between TDM and T measured from 20 diverse soybean genotypes. The regression equation for this relationship is described by: $y = 2.94x - 3.63$ ($r = 0.87$, $P<0.01$).

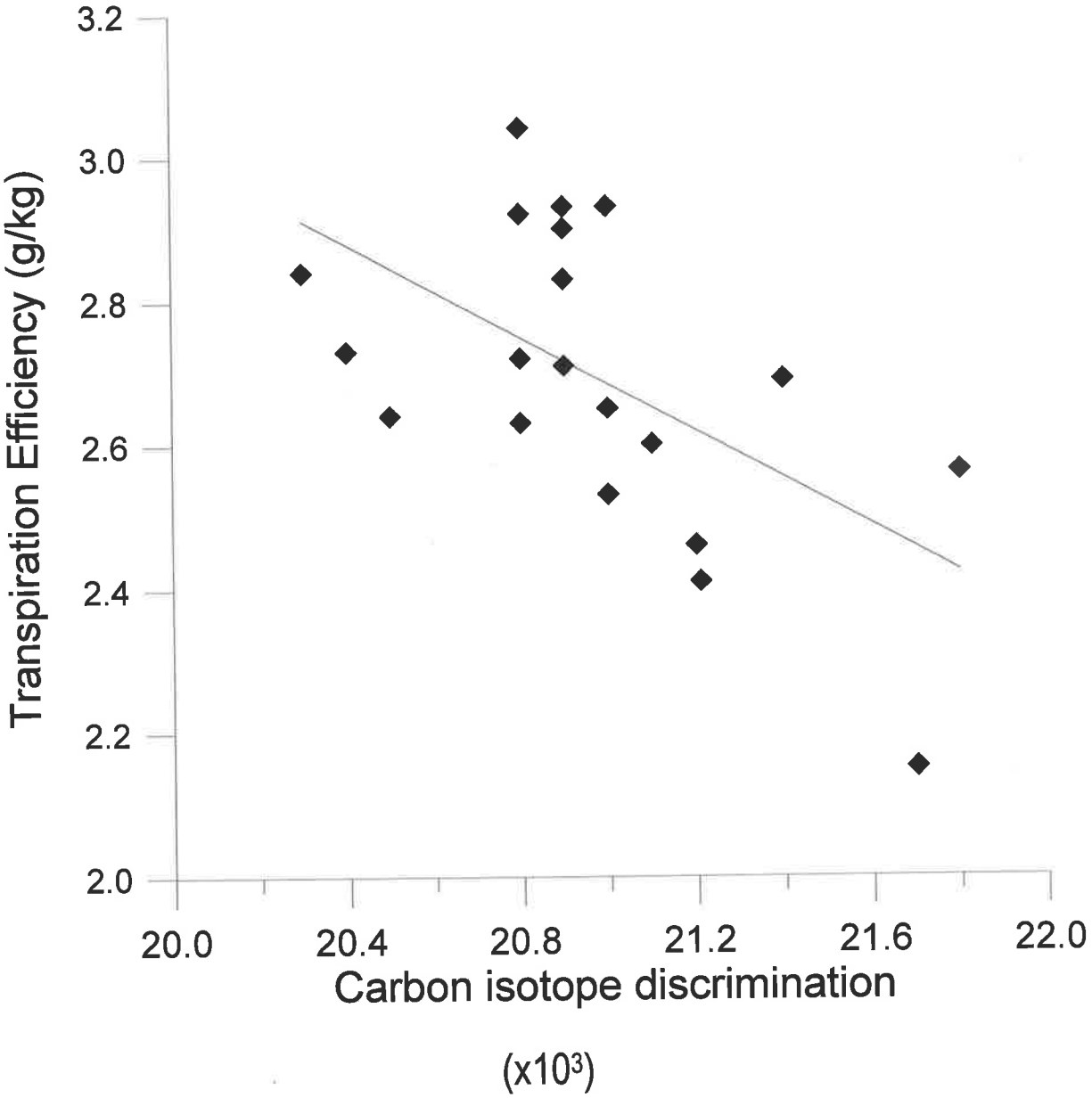


Figure 5 - The relationship between TE and Δ measured from 20 diverse soybean genotypes. The regression equation for this relationship is described by : $y = -0.33x + 9.53$ ($r = -0.57$, $P<0.01$).

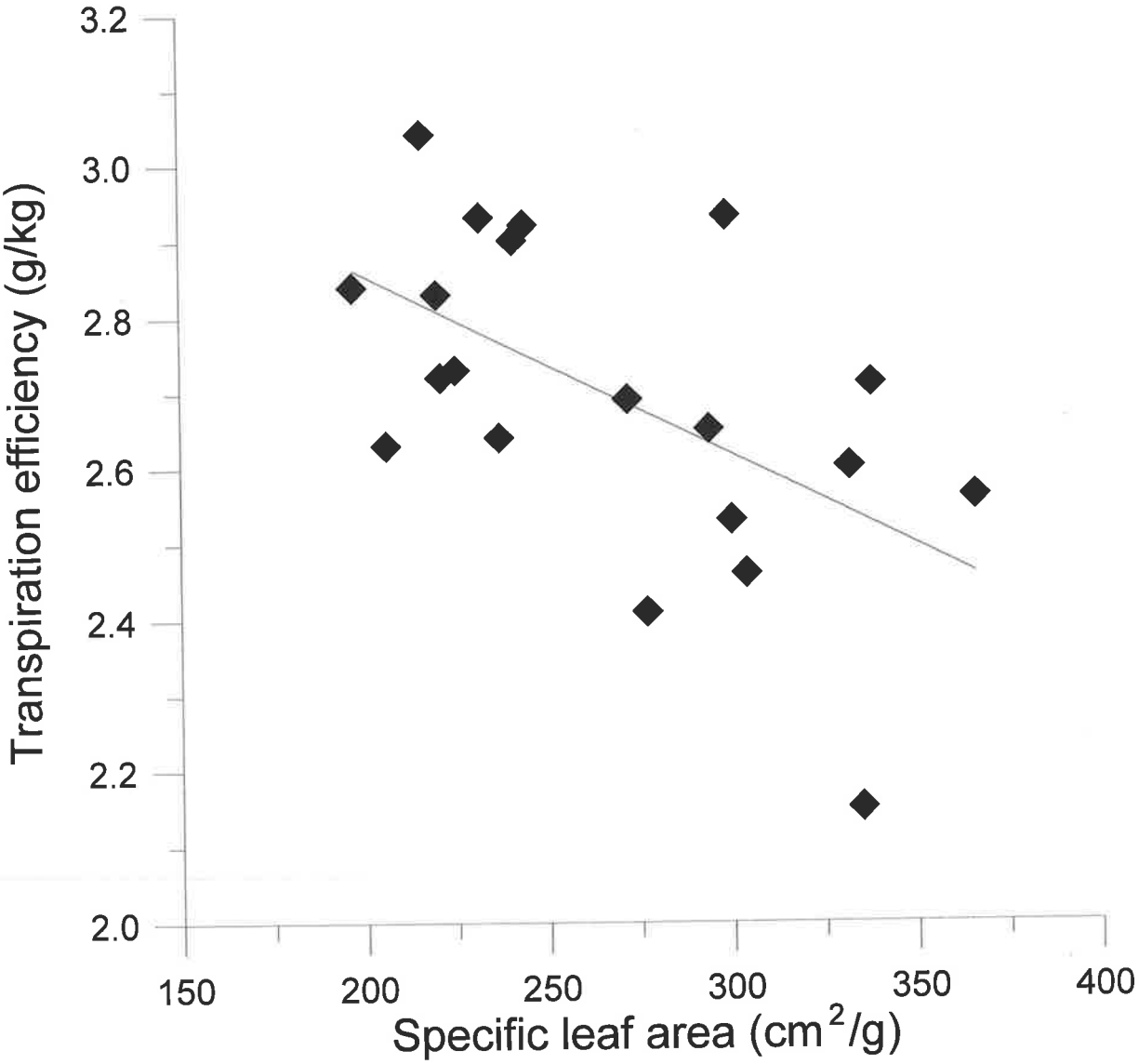


Figure 6 - The relationship between TE and SLA measured from 20 diverse soybean genotypes. The regression equation for this relationship is described by $y = -0.003x + 3.41$ ($r = -0.65$; $P<0.01$).

4.3.2 Pot Experiment 2

Components of plant DM, leaf area, root to shoot ratio (r:s), TDM, T, Δ, leaf N %, SLA and SLN measured at harvest 1 (41 dae) and harvest 2 (63 dae) are presented in Appendix 3. The change or average value of these plant traits integrated over the period between harvests 1 and 2 were of particular importance to this study and are shown in Table 8.

Table 8 - Accumulated TDM and T, TE, TE_{ag} and average Δ, SLA and SLN, measured on fully-irrigated soybean genotypes from 41-63 dae.

Genotype	TDM (g)	T (kg)	TE (g/kg)	TE_{ag} (g/kg)	Δ (‰)	SLA (cm^2/g)	SLN (g N/m^2)
Garoba Rouest	76.5	28.2	2.72	2.38	21.0	399	1.2
Ototan	119.6	36.2	3.33	2.49	21.5	453	0.9
Kabanyolo-1	125.4	40.5	3.12	2.41	21.7	501	1.0
Rawit	85.5	25.0	3.47	2.49	21.4	356	1.4
Mensoy 6	46.5	21.5	2.15	1.90	22.9	400	1.1
LSD. (5%)	*14.1*	*4.3*	*0.36*	*0.30*	*0.3*	*33*	*0.2*

4.3.2.1 TDM and T

Genotypes produced significantly ($P<0.01$) different amounts of DM (46.5 - 125.4 g pot^{-1}) over the measurement interval (Table 8), representing a genotypic variation of *c.* 170%. Similarly, highly significant ($P<0.01$) genotypic variation in T (88%) was observed, with a range of 21.5 - 40.5 kg pot^{-1} (Table 8). The relative variation in TDM and T was approximately twice that observed for the same genotypes in pot experiment 1. This was likely to be a result of the later measurement period in pot experiment 2 (41-63 dae, compared with 10-34 dae in pot experiment 1), and corresponded with a more rapid phase of growth (Fehr and Caviness, 1979). Under such conditions the genetic differences in TDM production

and T were likely to be more pronounced. TDM and T were highly correlated ($P<0.01$; $r = 0.95$).

4.3.2.2 Transpiration efficiency

Over the measurement interval TE ranged from 2.15-3.47 g/L, representing significant ($P<0.01$) genotypic variation of 61% (Table 8). While the magnitude of variation was similar to that observed in pot experiment 1, the genotypic ranking was quite different. For example, in pot experiment 1 Garoba Rouest had the highest TE, while in this pot experiment it was the lowest. This observation suggests environmental factors may have been differentially affecting the expression of TE. TE was not significantly ($P<0.05$) correlated with either TDM or T, suggesting that a combination of genotypic variation in both TDM and T contributed to differences in TE among soybean genotypes. TE and TE_{ag} were reasonably well correlated ($r = 0.93$), suggesting that under well watered conditions in pots, there was minimal genotypic variation for r:s and hence TE_{ag} may provide a reasonably accurate estimate of TE.

4.3.2.3 Potential surrogate measures of TE

The average Δ calculated over the period ranged from 21.0-22.9 ‰ among genotypes. This 1.9 ‰ range was slightly larger than the 1.5 ‰ range in Δ reported in pot experiment 1. However the correlation between TE and Δ was not significant ($P<0.05$). This was surprising considering there was a significant relationship measured in pot experiment 1, in which the genotypic variation in both TE and Δ was smaller than that recorded here. This observation supports the hypothesis that TE may have been influenced by an environmental factor. While the significant ($P<0.05$) correlation between SLN and SLA ($r = 0.83$) may justify the use of

SLA as an easily measured estimate of SLN, the correlation between TE and average SLA and SLN over the interval was poor (r = -0.01 and r = -0.10, respectively).

4.3.3 Pot Experiment 3

Root shoot ratio (r:s), TDM, T, TE, and TE_{ag} data are shown in Table 9 and Δ, Δ_u (Δ of leaves positioned at the top of the plant), leaf N, leaf N_u (% N of leaves positioned at the top of the plant), SLA, SLN and m_a data are shown in table 10. Measurements were taken over an interval of 30 days (14-44 dae).

Table 9 - Root shoot ratio (r:s), TDM, T and TE measured over a 30 day period during the vegetative growth phase for six soybean genotypes grown in the glasshouse during summer.

Genotype	r:s (g)	TDM (g)	T (kg)	TE (g/kg)	TE_{ag} (g/kg)
Garoba Rouest	0.26	76.0	24.3	3.13	2.48
Otootan	0.30	72.2	28.8	2.52	1.94
Tai-Dung-Wu-Tou	0.33	81.7	29.7	2.75	2.04
Kabanyolo-1	0.39	80.5	30.3	2.66	2.01
Rawit	0.37	76.7	27.4	2.79	2.04
Mensoy 6	0.26	74.5	24.8	2.97	1.79
LSD (P<0.05)	n/s	n/s	n/s	0.35	n/s

n/s denotes non-significance

4.3.3.1 Total dry matter and transpiration

No significant (P<0.05) genotypic differences were observed from the measured ranges in TDM (72.16-81.65 g pot^{-1}) or T (24.29 - 30.32 kg pot^{-1}). Garoba Rouest and Mensoy 6 transpired the least while T for Kabanyolo 1 was the highest. The genotypic variation for T (25%) was almost double the genotypic variation for TDM (13%).

4.3.3.2 Transpiration efficiency

The range in TE (2.52 - 3.13 g kg^{-1}) equated to a 24% variation among the six genotypes. This magnitude of variation was surprisingly lower than that predicted from Δ values measured in survey 3 (Chapter 3), and lower than variation measured in both previous pot experiments. Despite such a relatively small range, the genotypic variation in TE was significant (P<0.05). Garoba Rouest and Mensoy 6 had the highest, and Kabanyolo-1 and Otootan the lowest TE. TE was correlated with T (r = 0.86) but not with TDM.

Table 10 – Potential surrogate measures of TE; Δ, Δ_u (carbon isotope discrimination of an upper canopy leaf sample), N, N_u (nitrogen content of an upper canopy leaf sample) SLA, SLN and m_a, measured from six soybean genotypes of contrasting TE.

Genotype	Δ (‰)	Δ_u (‰)	N (%)	N_u (%)	SLA (cm^2/g)	SLN ($gNcm^{-2}$)	m_a (%)
Garoba Rouest	21.3	21.1	2.1	2.43	174	1.4	3.73
Otootan	22.4	22.6	2.1	2.67	266	1.0	5.13
Tai-Dung-Wu-Tou	22.1	21.8	2.3	2.97	337	0.9	4.83
Kabanyolo-1	22.3	22.5	2.3	2.95	278	1.1	5.79
Rawit	22.0	21.9	2.3	3.13	271	1.2	5.93
Mensoy 6	21.8	21.5	2.2	2.10	275	0.8	4.47
LSD (P<0.05)	*n/s*	*0.6*	*n/s*	*0.54*	*n.a.*	*n.a.*	*n/s*

n.a. denotes not able to be included in statistical analysis

Although not statistically significant ($P<0.05$), a 1.12 ‰ range in Δ was observed among genotypes. This was not as large as earlier reported ranges, but was above the theoretical 1.0 ‰ benchmark range used to estimate whether significant variation in TE is present. The genotypic variation in Δ was highly correlated with variation in TE ($P<0.01$; Figure 7). In addition to the measurement of Δ from the bulk canopy leaves, Δ was measured from a sample of newly-expanded, upper canopy leaves (Δ_u) in order to gain an understanding of sampling requirements for Δ determination in soybean. We observed highly significant differences in Δ_u and a strong correlation between Δ and Δ_u ($r = 0.95$; $P<0.01$). It would appear that a relatively small, non-destructive leaf sample can reliably estimate the average whole-plant leaf Δ.

Neither SLN nor SLA were significantly correlated with TE ($r = 0.42$ and 0.60, respectively). However m_a showed some promise, being reasonably ($P<0.10$) well correlated with TE ($r = -0.73$ in Figure 8), T ($r = 0.71$) and Δ ($r = 0.76$).

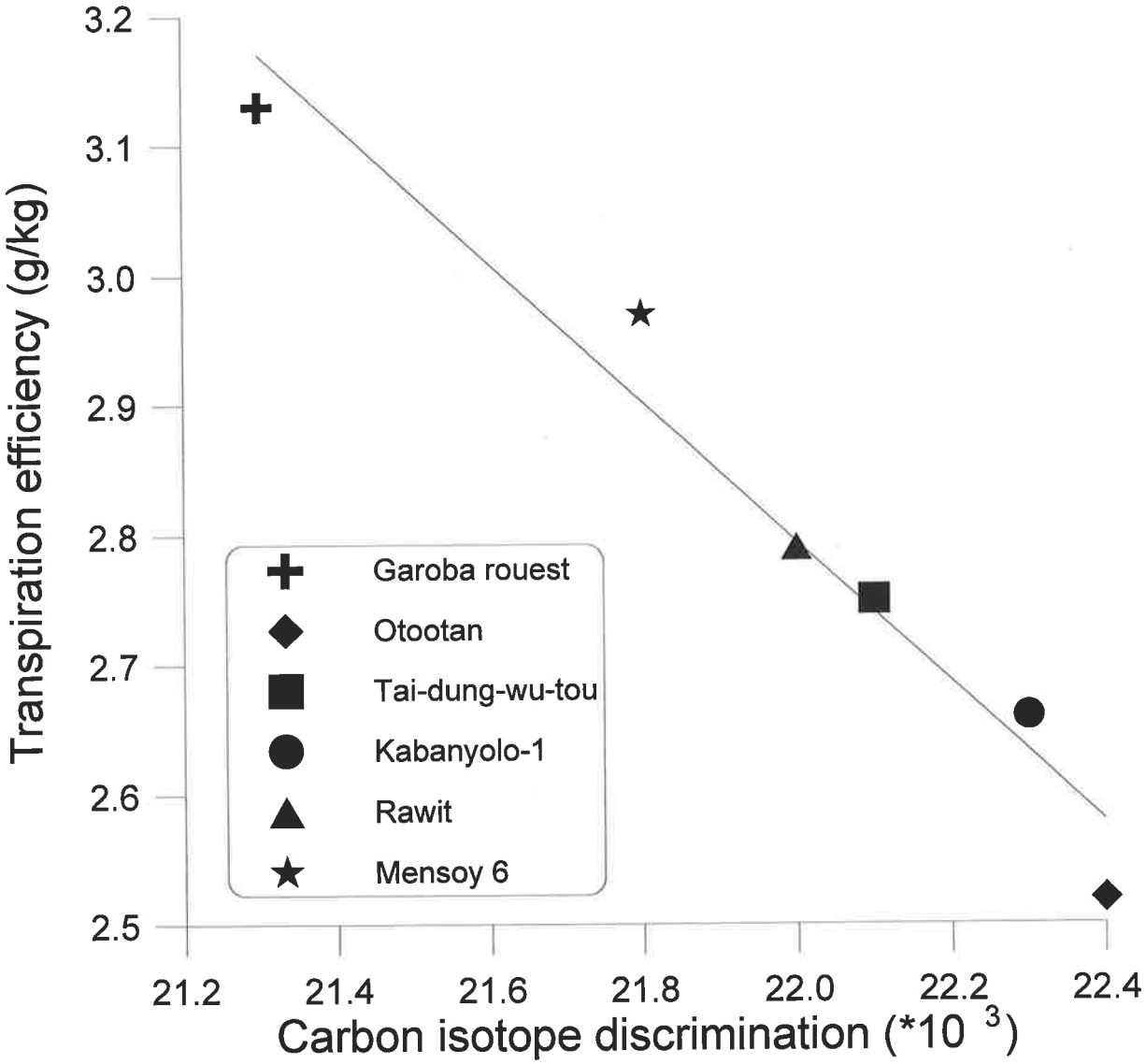


Figure 7 - Relationship between TE and Δ measured from six soybean genotypes grown in the glasshouse under well-watered conditions. The regression equation for this relationship is described by $y = -0.52x + 14.31$ ($r = -0.98$; $P<0.01$).

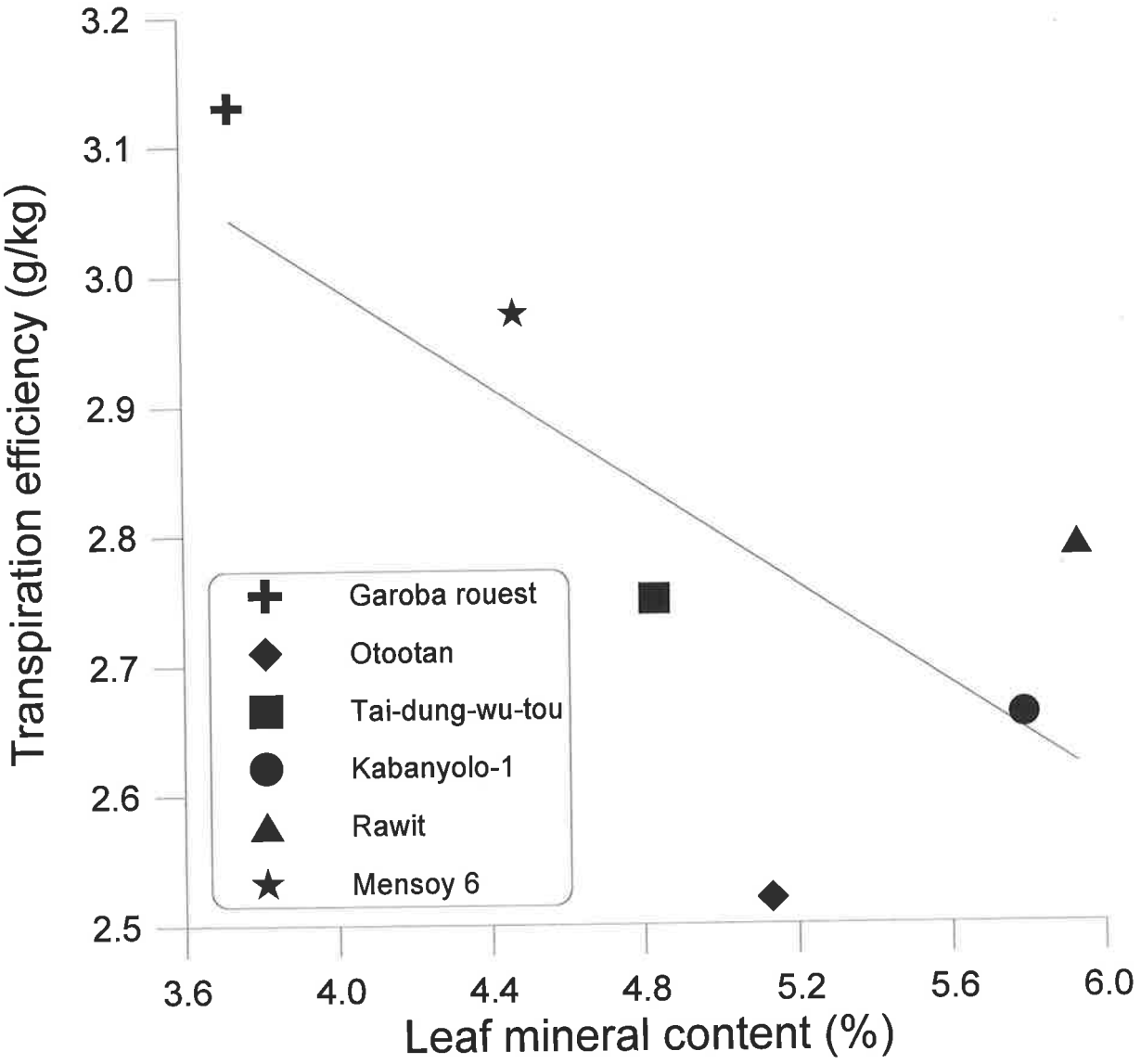


Figure 8 - Relationship between TE and leaf mineral content measured from six soybean genotypes grown in the glasshouse under well-watered conditions. The regression equation for this relationship is described $y = -0.19x + 3.76$ ($r = -0.73$; $P<0.05$).

4.3.3.3 Instantaneous measurements of photosynthesis (P_n) and transpiration (T_i)

The genotype means for net photosynthesis (P_n), transpiration (T_i) and instantaneous TE (TE_i), pooled over three sampling times, are presented in Table 11. Significant ($P<0.05$) differences among genotypes were recorded for P_n and TE_i, but not for T_i. The range in P_n was 16.8 - 20.7 μmol m^{-2} s^{-1} and TE_i from 2.23 - 3.08 μmol mol^{-1}. The range in P_n and TE_i corresponded to genotypic variations of 23% and 38%, respectively. Garoba Rouest and Mensoy 6 had the highest TE_i and Otootan the lowest.

Table 11 - Photosynthesis, transpiration and instantaneous TE (TE_i) averaged from three samplings taken from soybeans grown under natural light conditions in the glasshouse.

Genotype	Photosynthesis (μmol m^{-2} s^{-1})	Transpiration (mol m^{-2} s^{-1})	TE_i (μmol mol^{-1})
Garoba Rouest	19.71	7.67	2.71
Otootan	16.80	7.83	2.23
Tai-Dung-Wu Tou	18.34	7.73	2.50
Kabanyolo-1	17.48	7.21	2.63
Rawit	17.33	7.92	2.25
Mensoy 6	20.73	7.30	3.08
LSD (P<0.05)	*1.90*	*n/s*	*0.52*
LSD (P<0.01)	*2.60*		*n/s*

The genotypic rankings in TE_i were relatively consistent with those of TE ($r = 0.67$ in Figure 9), with Garoba Rouest and Mensoy 6 having the highest TE_i and TE. The relative contribution of P_n and T_i to the TE_i of each genotype are shown in Figure 10. The high TE_i in Garoba Rouest was due almost entirely to higher than average photosynthesis, while for Mensoy 6 it was because of relatively high rates of P_n in conjunction with relatively low rates of T_i. Clearly there was a range of combinations of both P_n and T_i within the genotypes examined, resulting in wide TE_i variations.

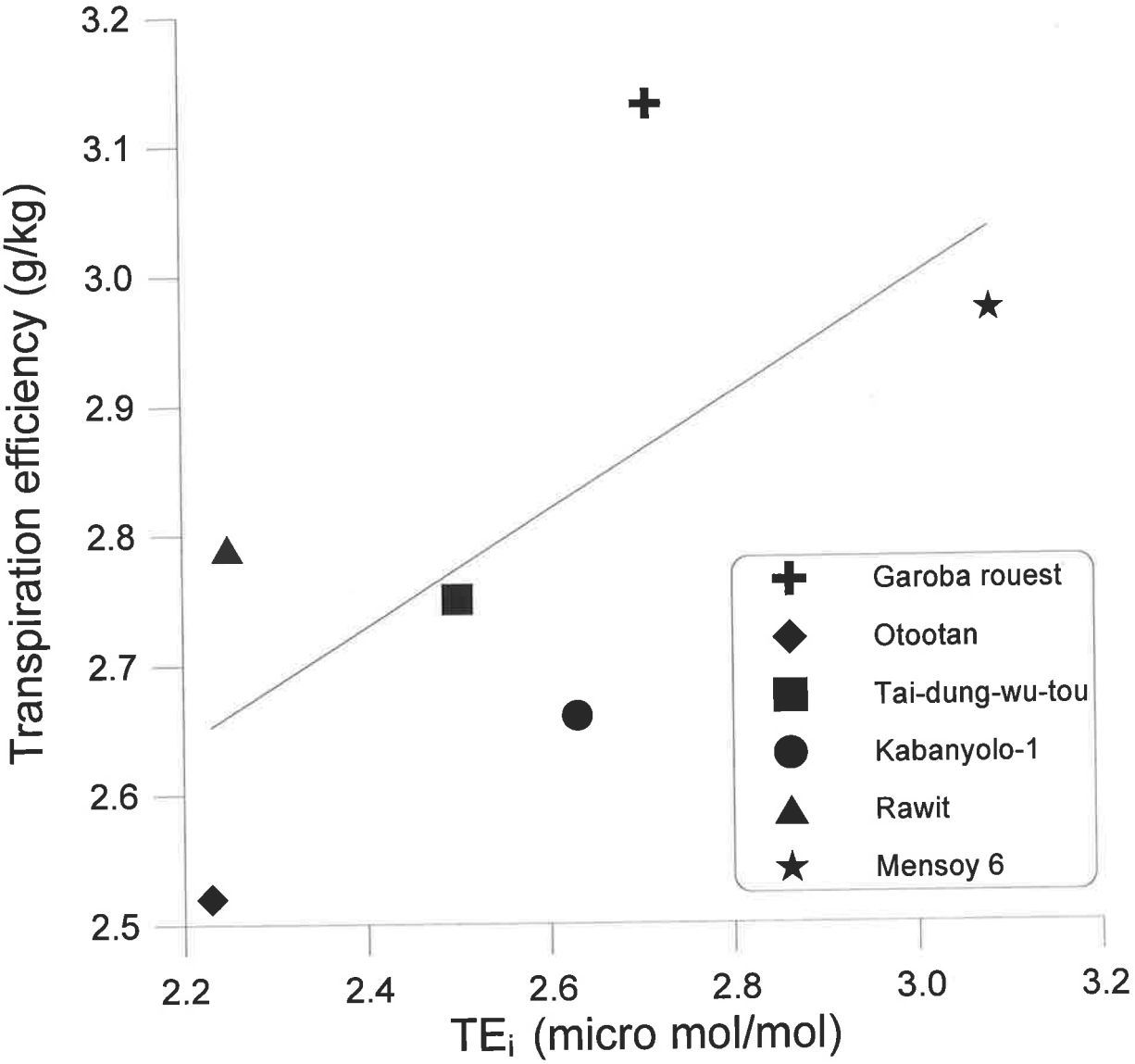


Figure 9 - Relationship between TE (calculated as whole-plant dry matter per unit of water transpired over 30 days) and instantaneous TE (TE_i; calculated as the ratio of the rate of photosynthesis to the rate of transpiration). The regression equation for this relationship is described by : $y = 0.45x + 1.65$ ($r = 0.67$; n/s).

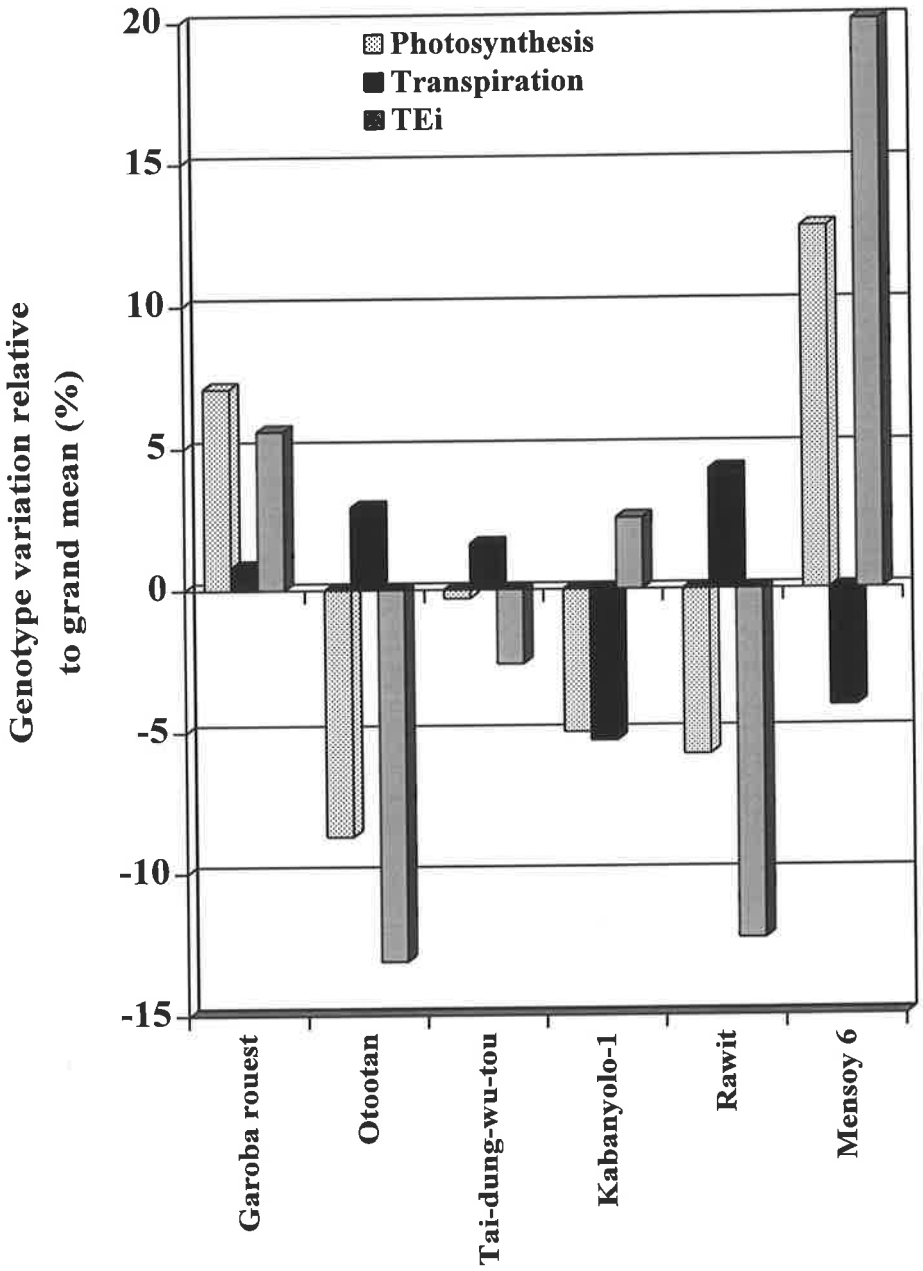


Figure 10 - Photosynthesis, instantaneous transpiration (T_i) and TE_i, expressed as the percentage deviation relative to the grand mean across genotypes, for each individual genotype.

4.4 Discussion

4.4.1 Pot experiment 1

There were significant ($P < 0.05$) differences among genotypes in both T and TDM during the treatment period (9 - 36 dae). The highly significant relationship between these parameters (Figure 4) has been found in many other studies (see review by Tanner and Sinclair, 1983) and is consistent with a reduction in transpiration via stomatal closure causing a decrease in the influx of CO_2 available for photosynthesis. However, the rate of TDM accumulation is not solely a function of transpiration and the atmospheric demand for water, as suggested by the models of Hanks (1983) and Tanner and Sinclair (1983). Rather, it is the product of T and TE (Richards, 1991) and consequently, the variation about the fitted regression line (Figure 4), is likely to be a reflection of experimental error as well as genotypic differences in TE. The variation in TE among genotypes in this study was of the order of 40%.

The variation recorded for TDM and T were similar (74 and 56% respectively), suggesting that a combination of mechanisms controlling water-loss control and CO_2 assimilation contributed to the observed variation in TE. Further insight into the mechanisms responsible for variation in TE can be gained by examining the relationships between TDM or T with Δ - an independent measure of TE. Ehleringer (1990) showed that in common bean, leaf conductance (and therefore T) was highly correlated with Δ ($r = 0.86$), suggesting that stomatal control of water loss dominated the variation in Δ, and presumably TE. In contrast, strong correlations between TDM and Δ in peanut (Wright *et al.*, 1988) and sunflower (Virgona *et al.*, 1990) provided evidence that differences in photosynthetic capacity were largely responsible for the genotypic variation in TE in these species (Wright *et al.*, 1993; Subbarao *et al.*, 1994). In the current experiment there were no significant relationships

between TDM or T with Δ in the soybean genotypes tested, suggesting that variations in both leaf conductance and photosynthetic capacity may have been responsible for the variation in TE among soybean genotypes. The weak, although significant relationship between TE and SLA (Figure 6) would tend to support this conclusion, indicating that variation in photosynthetic capacity effects were not dominating the TE variation. These data also support the considerably stronger relationships between TE and SLA that have been observed in other species (eg. peanut; Nageswara Rao and Wright, 1994), where photosynthetic capacity effects have been more dominant.

The significant ($P<0.05$) negative correlations between TE and Δ (Figure 5, $r = -0.58$) were consistent with the theory proposed by Farquhar *et al.* (1982), and support an ever increasing database reported for a range of C_3 species (Turner, 1993). The theoretically predicted range in TE of *c.* 60% based on the germplasm survey (Chapter 3) was somewhat greater than the large range in TE (41%) measured experimentally in this pot study. These findings support the use of Δ as a tool to indirectly survey the TE variation of large germplasm collections, as used by Hall *et al.* (1992) in cowpea. This data also supports the use of the > 1‰ benchmark Δ value as an indication of significant variation in TE within a given germplasm collection (Ehleringer *et al.*, 1993).

The results from this preliminary experiment were significant because they represent the first published report of TE variation among soybean genotypes. To further examine the stability of TE and its relationship with Δ and other potential surrogate measures, the results of subsequent glasshouse experiments are examined.

4.4.2 Pot Experiment 2

This pot experiment investigated 5 genotypes representing the full range in TE measured from pot experiment 1. Plant characters of interest were measured over a common vegetative growth period from 41-63 dae. Large genotypic variation was recorded for TDM (111 %) and T (92 %) in this study, with both parameters again being highly correlated ($r = 0.97$; $P<0.01$). In this study the genotypic variation in TE was c. 61%, compared with *c.* 41 % variation measured in TE for the same varieties during pot experiment 1.

In contrast to the TE data, the significant ($P<0.01$) genotypic variation in Δ was of similar magnitude to that of experiment 1 (1.5 ‰ and 1.7 ‰ for pot experiments 1 and 2, respectively), although in this case the association between TE and Δ was not significant. Large genotypic variation was recorded for the leaf traits SLA (105 %) and SLN (64 %), but neither were correlated with TE ($r = 0.36$ and $r = 0.45$, respectively) in this experiment. These results contrast with those from pot experiment 1, where reasonable correlations were found to exist between TE and both Δ and SLA.

The relationship between TE measured for common genotypes in pot experiment 1 and TE measured in this experiment was highly negative ($r = -0.95$; $P<0.02$), suggesting a re-ranking for TE among genotypes had occurred. This observation provides strong evidence that TE was strongly influenced by some as yet unknown environmental factors, which could also have been a contributing factor to the poor correlations observed between TE and both Δ and SLA.

An examination of the environmental factors that existed during the two experiments, indicated that while thermal regime and water supply were identical, the average daily incident solar radiation outside the glasshouse differed significantly between experiments (Figure 11). In pot experiment 1 (conducted in spring), mean daily radiation was increasing throughout the period of TE measurement, during which the cumulative radiation received was 546.8 MJ/m^2. In pot experiment 2, mean daily radiation was decreasing throughout the period of TE measurement, during which 461.4 MJ/m^2 of radiation was accumulated. Therefore, when growth rates were greatest in the latter half of the measurement period, differences in incident radiation (and hence growth potential) were at their greatest.

There is little published evidence of an effect of incident irradiance on TE, although Condon *et al.* (1990) advanced an hypothesis in this regard. This work was done with *Arabidopsis thaliana*, with germplasm screened for Δ variation in several experiments conducted under contrasting irradiance conditions. Narrower ranges in Δ were observed under lower irradiance conditions in a similar fashion to these findings for TE in soybean. They also found that genotypic ranking was 'usually maintained' across different irradiance environments, suggesting that irradiance may have caused some instability for Δ, and presumably TE. A similar effect may have occurred between the two pot experiments reported here. It is possible that variable incident irradiance between spring and autumn may have differentially affected TDM in genotypes that achieved high TE by having high unit leaf rates of photosynthesis. If this hypothesis is correct, it may be difficult to draw any further conclusions from the results of pot experiment 2. A third and subsequent experiment was undertaken during mid-late summer, when high irradiance conditions again prevailed.

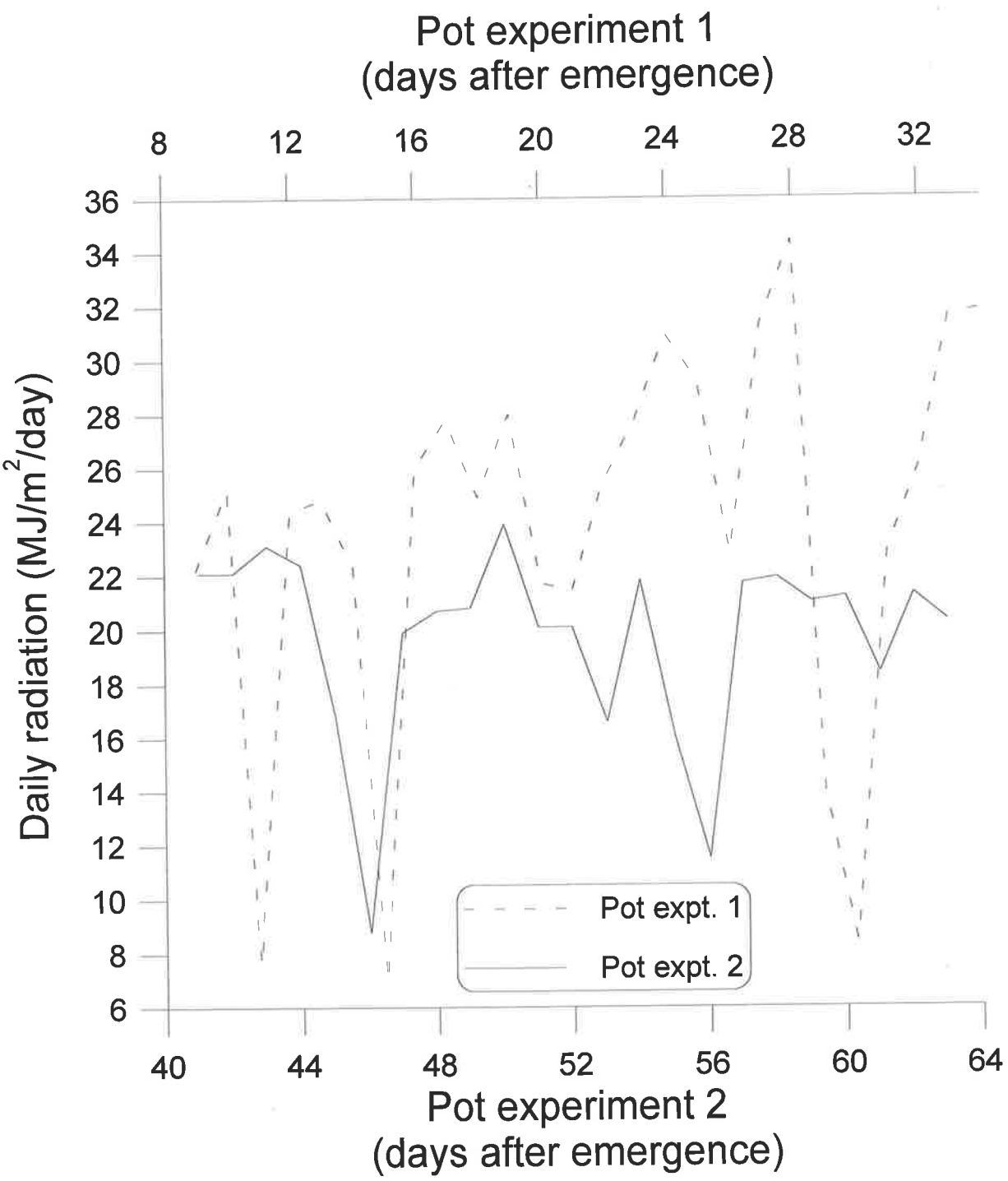


Figure 11 - Daily solar incident radiation recorded over the duration of TE measurement for pot experiments 1 and 2. These values have not been corrected for the glasshouse environment, where light reductions of between 40-55% have been recorded (Bell, *pers. comm.*)

4.2.3 Pot Experiment 3

During this study, highly significant ($P<0.05$) variation in TE (*c.* 25%) was observed from the six soybean genotypes examined. In addition, TE_{ag} was also measured in this study as it overcomes the difficulties in measuring the root dry matter component of TDM, and may therefore provide scientists with an easily measured approximation of TE (Wright *et al.*, 1993). However, TE_{ag} should only be used where TE and TE_{ag} are correlated, and for this to occur the r:s must remain constant over time and with variable water stress environments. The correlation between TE and TE_{ag} measured during this study was poor ($r = 0.53$). This was the result of re-ranking in r:s among genotypes between the two harvests, possibly as a result of differing phenology or partitioning characteristics. To fully explore the potential of TE_{ag} as a potential surrogate of TE, the genotypic ranking of TE_{ag} should also be evaluated under contrasting water stress environments. The data presented here suggest that under non-limiting water conditions genotypic differences in r:s over time will dictate that TE_{ag} may not be a reliable predictor of TE in soybean.

The importance of developing crop-specific strategies to select for high TE genotypes have recently been emphasised (Udaykumar *et al.*, 1996). Variation among genotypes for TE can be due to differences in photosynthetic capacity, and consequently TDM production, as in peanut (Wright *et al.*, 1988; Wright *et al.*, 1993); differences in stomatal control of water loss, and consequently T, as in common bean (Ehleringer *et al.*, 1993); or a combination of both mechanisms as in wheat (Condon *et al.*, 1990). Surrogate measures such as SLA and SLN can only reflect differences in photosynthetic capacity while others such as Δ provide a measure of both photosynthetic capacity and stomatal conductance effects. By knowing the

predominant mechanisms responsible for TE variation within a particular species, the most appropriate surrogate measure can then be selected.

In this experiment, investigations into the cause of TE variation were conducted at two levels of biological organisation; viz., the leaf level and the plant level. At the leaf level, genotypic differences in instantaneous TE (TE_i) were the combined result of differences in P_n and T_i, which in turn affect p_i/p_a (section 3.4). As p_i/p_a is independently correlated with TE_i (Farquhar *et al.*, 1982), differences in p_i/p_a indicate differences in TE_i. Condon *et al.* (1990) showed that in wheat, variation in p_i/p_a (and therefore TE_i) resulted from genotypic differences in P_n and T_i. In this experiment (Table 11 and Figure 10), we were able to confirm the earlier proposed hypothesis (section 3.2.3) that soybean behaved in a similar fashion to wheat, in that genotypic differences observed in both P_n and T_i were responsible for the observed variation in TE_i.

The higher than average TE_i in Garoba Rouest was due to average T_i but substantially higher than average P_n. This genotype therefore displayed similar characteristics to peanut, where there appears to be little variation in T (and presumably T_i) among genotypes, but quite significant variation in P_n (Wright *et al.*, 1988; Nageswara Rao and Wright, 1994; Subbarao *et al.*, 1994). Selecting for high P_n types in peanut therefore ensures automatic selection for high TE_i. In contrast, Kabanyolo-1 achieved relatively high TE_i through its relatively low T_i rates. This genotype displayed similar characteristics to cowpea, where strong correlations between T_i and p_i/p_a (and presumably TE_i) suggested that significant genotypic variation in T_i was responsible for TE_i variation (Udaykumar *et al.*, 1996). In contrast to both these genotypes, Mensoy 6 exhibited the highest TE_i because both mechanisms (ie. high P_n rates and low T_i)

were operating to produce high TE_i. It therefore seems that at the leaf level, TE_i variation in soybean is not exclusively the result of either P_n or T_i variation, but rather a combination of variation in both attributes.

To be useful in a plant breeding sense, potential differences in TE_i must also be reflected at the plant level. Genotypic differences in TDM and T were 13% and 25%, respectively after 30 days of measurement. Although not statistically significant, the variation was quite large for such a relatively short period of measurement. There were also non-significant correlations between TE and either TDM or T, indicating that at the plant scale of biological organisation, the mechanism of TE variation in soybean was also likely to be a combination of genotypic differences in both TDM production and T. Both measurement methods conducted at contrasting levels of biological organisation therefore produced consistent results. Together, they support the hypothesis that intra-specific differences in both TDM and T were responsible for genotypic variation in TE among soybean.

Given that exploitable variation for TE seems to exist in soybean, suitable surrogate measures to enable indirect selection for the difficult-to-measure TE trait would be required in large scale breeding programs. The most extensively studied surrogate measure of TE is carbon isotope discrimination (Δ). Among the six diverse genotypes examined, there was a range in Δ of *c.* 1.12‰. The negative correlation between TE and Δ ($r = -0.98$) was highly significant ($P<0.001$), confirming that the correlation between TE and Δ proposed by Farquhar and Richards (1984) was consistent among this subset of soybean genotypes. The degree of correlation between TE and Δ in soybean in the studies presented here were similar to findings in sunflower ($r = -0.97$; Virgona *et al.*, 1990 and peanut $r = -0.86$; Wright *et al.*, 1993), which

were also conducted under well-watered glasshouse conditions. From such evidence we conclude that Δ provides an accurate surrogate measure of TE for soybean - at least under non water-limiting conditions in the glasshouse.

If Δ is to be used to screen large numbers of genotypes, the stability of Δ across different plant parts becomes an important consideration. The results of this study showed that Δ measured from a small sample of upper canopy leaves and Δ measured from a bulk sample of total plant leaf (Table 10) were highly correlated (r = 0.94). This indicates that the necessary information on genotype TE can be gained from a relatively small (and potentially non-destructive) leaf sample, thus allowing the plant to continue growing to produce seed or other measurable attributes.

However, determination of Δ requires an expensive mass spectrometric facility, which limits its use as a tool for large scale screening of germplasm. The correlation of TE with cheap and easily measured attributes like SLA (Wright *et al.*, 1994) and leaf mineral content (m_a; Masle *et al.*, 1992) in other crop species was, therefore, worthy of investigation for soybean. Whilst SLA has been a useful surrogate measure for TE in species such as peanut (Rao and Wright, 1994) and sunflower (Virgona *et al.*, 1990), data obtained in this experiment (Table 10) showed it to be only weakly correlated with TE in soybean (r = 0.60). This was not surprising, given the variation in the contribution to TE_i made by P_n in the genotypes shown in Figure 10. Any variation in TE caused by genetic differences in T would be unaccounted for by SLA (or SLN) and hence the value of SLA as a surrogate trait for TE in soybean remains questionable.

Leaf mineral content (m_a) has been shown to be well correlated with TE in a range of species (Masle *et al.*, 1992) suggesting that m_a may also be an expedient surrogate measure for TE in early generation screening programs. Results from this experiment show that m_a was better correlated with TE (r = -0.73) than was SLA (r = -0.60). Other negative correlations between m_a and TE have been observed in sunflower (r = -0.81) and wheat (r = -0.62), but it was also found that the association was weakened with water stress (Masle *et al.*, 1992). In crested wheatgrass, m_a was negatively correlated with TE under well-watered field conditions (r = -0.60; P<0.01) but the correlation was not consistent across all environments (Mayland *et al.*, 1993). Thus, while the correlation between m_a and TE has been shown here to have promise in soybean, further evaluation under conditions of variable water supply is needed before it could be recommended as a reliable surrogate measure of TE. Results so far do indicate that it shows more potential than SLA as a predictor of TE in soybean. To summarise, it is suggested that potential surrogate measures of TE showing the most promise in soybean are Δ and m_a. Of these, m_a accounted for only 53% of the variation in TE, while Δ accounted for 96% of the variation. Additionally, m_a may be more susceptible to G x E influences than Δ, although this has yet to be confirmed.

The following chapter summarises the important findings from the four experiments presented to date. It also highlights those areas, related to the development of an efficient system for improving TE of soybean, which have not yet been addressed.

CHAPTER FIVE

Interim Discussion

5.1 Introduction

Many reviews have advocated that TE should be an important trait contributing to yield under water stress (Briggs and Shantz, 1916; Farquhar *et al.*, 1982; Fischer *et al.*, 1982; Tanner and Sinclair, 1983; Hubick *et al.*, 1986; Ludlow and Muchow, 1990). TE can be increased by modifying the environment (Tanner and Sinclair, 1983), crop agronomy (Fischer, 1979) or via genetic manipulation (Hubick *et al.*, 1986). Of these approaches, breeding for increased TE is the longer term approach for increased yield under water limited environments (Ehleringer *et al.*, 1993). However, for TE to be a useful selection tool in breeding programs, it is essential that the following criteria are met:-

- there is significant variation for TE within existing germplasm,
- TE is independent of the other determinants of yield (T and HI) under water limited environments,
- TE is relatively stable when measured on plants grown at different times and under different environments (ie. TE has low genotype x environment (G x E) interaction), and
- there are easily measured traits which are highly correlated with TE, to enable indirect selection for TE .

This chapter aims to integrate the experimental results presented to date, in order to determine the potential of using TE as an indirect selection criterion in breeding programs aimed at selecting for higher soybean yield under water limited conditions. To aid the review, key results have been summarised in Tables 12 and 13.

Table 12 - TE and Δ measured from 3 different experiments conducted under well watered conditions in the glasshouse at Kingaroy, Queensland.

Genotype	TE (g/kg)			Δ (‰)		
	1	2	3	1	2	3
Garoba Rouest	2.84	2.72	3.13	20.3	21.0	21.3
Ototan	2.46	3.33	2.52	21.2	21.5	22.4
Tai-Dung-Wu-Tou	2.15	-	2.75	21.7	-	22.1
Kabanyolo-1	2.56	3.12	2.66	21.8	21.7	22.3
Rawit	-	3.47	2.79	-	21.4	22.0
Mensoy 6	2.93	2.15	2.97	20.9	22.9	21.8
LSD (5%)	*0.32*	*0.36*	*0.35*	*0.50*	*0.79*	*n/s*

1= Selection experiment examining 20 genotypes during summer
2= Detailed physiological experiment examining 5 genotypes during autumn
3= Detailed physiological experiment examining 6 genotypes during summer
n.s. - not significant at $P<0.05$

Table 13 - The genotypic range in TE and Δ, and the correlation of TE with Δ, SLA and m_a measured from six soybean genotypes grown under well-watered conditions in the glasshouse.

Pot Experiment	Range in TE (g/kg)	Range in Δ ($*10^{-3}$)	Correlation (r) with TE		
			Δ (‰)	SLA (cm^2/g)	m_a (%)
1	0.78*	1.5**	-0.58	0.75	n.a.
2	0.76*	1.7**	-0.57	0.19	n.a.
3	0.61*	1.1**	-0.98**	0.60	-0.53

* denotes significance at the 0.05 level
** denotes significance at the 0.01 level

5.2 Quantifying TE variation among soybean genotypes

In order to successfully employ TE as an indirect selection criterion in soybean breeding programs, large variation in TE must be present among the available soybean germplasm. In each of the three glasshouse experiments significant variation in TE ($P<0.05$) was observed (Table 12), suggesting that the selected subset of cultivars exhibited exploitable variation in TE when grown under non-water limiting conditions in small pots. This expression of genetic variability for TE in pot studies is similar to findings for cowpea (Ismail and Hall, 1992) and wheat (Farquhar and Richards, 1984). Additionally, from the ranges in TE found in other crop species (eg. 0.88g/kg for peanut; Hubick *et al.*, 1986, 0.42 g/kg for cotton; Hubick and Farquhar, 1987, 0.52 g/kg for tomato; Martin and Thorstenson, 1988, 0.65 g/kg for barley; Hubick and Farquhar, 1989 and 0.56 g/kg for wheat; Condon *et al.*, 1993), it is clear that the available range in TE (Table 13) measured among the selection of soybean genotypes is relatively large.

These results provide strong evidence that there is substantial genotypic variation for TE in soybean, and refutes the hypothesis that intra-specific variation is likely to be small (Tanner and Sinclair, 1983) and that TE can only be improved by agronomic or environmental modifications (Ludlow and Muchow, 1990). Indeed, it is suggested that there is substantial scope to exploit this TE variation via selection in breeding programs.

5.3 Mechanisms underlying TE variation in soybean

As TE is derived from both TDM and T, there can be three causes of genotypic differences in TE. These are (i) genotypic differences in TDM; (ii) genotypic differences in T; or (iii) genotypic differences in both TDM and T. However, it must be noted that selection of

genotypes on the basis of TE alone could cause undesirable outcomes. High T is equally critical to biomass production and yield, as is high TE (Tanner and Sinclair, 1983). Therefore, in a species where high TE and low T are linked (eg. cowpea; Udaykumar *et al.*, 1996), selection for high TE could be at the cost of high T and consequently, higher yield. A fundamental understanding of the nature of the variation in TE is therefore required to enable development of a crop specific strategy to select genotypes with high levels of TE, while at the same time ensuring selection for high TE does not compromise the contribution of high T to yield.

In the three pot experiments reported to date, the cause of TE variation in soybean has been investigated at both the leaf and the whole plant levels of biological organisation. At the leaf scale, results have indicated that genotypic variation in both the rate of CO_2 assimilation (approximately 23%) and the rate of water vapour diffusion (approximately 10%) contributed to variation in instantaneous TE (approximately 38%) of the six soybean genotypes examined. At the whole-plant scale, TDM and T were the equivalent measures of the rates of CO_2 assimilation and water vapour diffusion, respectively. Significant ($P<0.05$) genotypic variation was observed for both TDM and T for all three pot experiments, which would also suggest that variation in plant-scale TE was driven by genotypic differences in both TDM and T.

These findings provide strong evidence that, for this subset of soybean cultivars grown in pots, there was no physiological link between high TE and small plant size (ie. low T), as observed in cowpea (Udaykumar *et al.*, 1996). Consequently, selection for high TE could be a beneficial strategy for yield improvement in soybean under water-stress environments.

5.4 Stability of TE as a potential selection trait

To be a useful trait for indirect varietal selection, the trait should be expressed consistently across different environments (Byth, 1981). Comparison of the genotypic ranking for TE among the soybean cultivars in the glasshouse trials was therefore used to investigate this stability.

The genotypic ranking in TE between experiment 1 and experiment 2 was inconsistent with evidence of a complete re-ranking in TE among genotypes ($r = -0.94$). Similarly, a weak negative correlation between the TE of experiment 2 and 3 indicated further instability for TE, again with a degree of re-ranking observed. However, when the TE of experiments 1 and 3 were compared, a positive correlation ($r = 0.66$) was observed. This data, therefore, suggests there was a significant G x E interaction influencing TE measured in the pot experiment 2.

We have presented a tentative hypothesis as to the nature of this G x E interaction, based on the possible influence of irradiance on TE. The cumulative irradiance during experiment 2 (conducted in autumn), was relatively lower (453 MJ/m^2) than that of experiment 1 (620 MJ/m^2) (Figure 11). In particular, the average daily radiation during the time when most dry matter was accumulated in experiment 1 was *c.* 26 MJ/m^2/day compared with an average of *c.* 20 MJ/m^2/day in pot experiment 2 (Figure 11). Differences in irradiance have been suggested to influence TE (Condon *et al.*, 1990) and in cowpea it has been demonstrated that lower light conditions contributed to instability of TE values (Udaykumar *et al.*, 1996). These observations support our hypothesis of irradiance being a significant environmental factor affecting TE stability - presumably through an effect on the relationship between TDM and T. While more detailed work is needed, these preliminary findings indicate that TE screening or

selection experiments conducted in the glasshouse should be limited to high radiation environments. Due to the inconsistent TE values recorded in experiment 2, it is suggested these results be treated with caution, particularly with consideration of the stability of TE, or the stability of correlations between TE and other leaf traits.

5.5 Relationship between TE and Δin soybean

The genotypic variation in Δ measured from each of the three experiments was highly significant ($P<0.01$, Table 13). While the relationship between TE and Δ in the first pot experiment was only significant at the 10% level, this was likely an artefact of the small number of replicates of each cultivar (2) and the resulting large errors associated with measurement of TE. In the third experiment a highly significant ($P<0.01$) and negative relationship ($r = -0.98$) between TE and Δ was observed. This strong correlation was in agreement with both theoretical predictions (Farquhar and Richards, 1984) and experimental findings from other species (Hall *et al.*, 1993; Turner, 1993).

The genotypic ranking in Δ was similar between experiment 1 and experiment 3 ($r = 0.88$), but inconsistent between each of these and the second experiment conducted in autumn ($r = 0.69$ and 0.61, respectively). Additionally, there was no significant relationship observed between TE and Δ in the second pot experiment (Table 13). These observations are consistent with the instability of TE ranking from experiment 2 relative to that in experiments 1 and 3, as observed for TE (section 5.4) and on this evidence, data from experiment 2 was not included in further analysis. However it does suggest that the relationship between TE and Δ may break down in unseasonal experiments subjected to low irradiance conditions.

The regression coefficients from correlations between TE and Δ measured in pot experiments 1 and 3 were tested for homogeneity and were found to be the same ($P<0.01$). This finding, in conjunction with the stable genotypic ranking in TE, suggests that the relationship between TE and Δ was relatively stable across the two glasshouse conditions. The fact that the y intercepts were different between both experiments is of little consequence to the reliability of Δ for predicting relative genotypic performance in TE, and was possibly due to differences in VPD between experiments.

5.6 Techniques to sample for Δ in soybean

It is not always convenient to measure Δ of the whole plant. This is a destructive technique which requires that all plant parts are harvested, then dried and ground into a homogenous mixture from which a small sub-sample is taken for analysis (Hubick and Farquhar, 1989). It would be more convenient if leaf Δ could be used to estimate whole-plant Δ. This was evaluated by comparing Δ values measured from different plant parts. We observed a strong correlation ($r = 0.95$) between Δ measured from a homogenous mix of bulk leaf and Δ measured from a small sample of newly expanded leaf. More extensive studies into variation of Δ between plant organs in peanut (Hubick *et al*, 1986), barley (Hubick and Farquhar, 1989) and wheat (Condon *et al*., 1992) also showed consistency in the ranking of Δ measured on different plant parts.

The strong correlations between TE and Δ, and between Δ values measured on different leaf samples suggested that use of Δ measured from leaf dry matter could be used as an indirect surrogate measure of TE in soybean. This procedure would also overcome the practical limitations involved with direct measurement of TE (Wright *et al*., 1993).

5.7 Relationship between TE and other potential surrogate measures

The specialised equipment, expertise and cost associated with routine measurement of Δ may limit the application of this technique, both in initial germplasm screening and in research conducted in developing countries (Turner, 1993). Cheaper surrogate measures of TE, for example SLA and m_a, would facilitate more widespread adoption of indirect selection for TE. Such leaf traits, which have shown promise in other crop species, were evaluated for soybean.

5.7.1 Specific leaf area (SLA)

Results of all three pot experiments showed that SLA was poorly correlated with TE (Table 13). This was not surprising, given that SLA is a surrogate measure of photosynthetic capacity, and ultimately TDM (Nageswara Rao and Wright, 1994). As variation in TDM was not closely associated with variation in TE among the germplasm tested, it follows that SLA variation should not be well correlated with TE. From these theoretical principles and the consistently poor correlations observed, it is suggested that SLA shows little promise as an indirect measure for TE in soybeans grown in the glasshouse.

However, further evaluation of SLA was undertaken in the field experiment (Chapter 6), as potentially large differences in T expressed by isolated plants may be suppressed under a field canopy environment due to the effects of differing boundary layers (Hubick and Farquhar, 1989). The greater uncoupling of plants in crop canopies from the surrounding atmosphere may mask the extent of genotypic variation in T, such that variation in photosynthetic capacity, and hence TDM, becomes the dominant cause of variation in TE. Water stress treatments should also be examined, as Condon *et al.* (1990) have shown that significant variation in T in wheat observed under well-watered conditions was not reflected under water

stress conditions. In this instance, restricting water supply effectively normalised the T differences between genotypes, such that variation in TDM may dominate genotypic differences in TE in the field. If this occurs, the field correlation between TE and SLA may be greater than in the glasshouse (or under irrigation).

5.7.2 Leaf mineral content (m_a)

The correlations between TE and m_a reported in the literature (Masle *et al.*, 1992; Mayland *et al.*, 1993; Febrero *et al.*, 1994; Main *et al.*, 1996;) have not been as strong or consistent as those reported for TE and Δ. However, requiring only a muffle furnace for determination, m_a is cheaper and easier to measure than Δ. Consequently, m_a may be a potentially useful tool in early generation screening for TE, where less precision can be tolerated (Masle *et al.*, 1992). We found that m_a was well correlated with TE (r = 0.73) in the second summer experiment (pot experiment 3), although the relationship was considerably weaker than that observed between TE and Δ (r = 0.98). The relationship between TE and m_a is not as well understood as the relationship between TE and Δ, but it is believed that measurements of m_a can integrate differences in both TDM and T (Masle *et al.*, 1992). This may also explain why m_a was a better TE surrogate than SLA in pot experiment 3. These preliminary findings suggest that m_a may be a useful surrogate measure of TE for use in screening or early generation evaluation of soybean germplasm.

5.8 Summary

Significant variation in TE among soybean genotypes has been demonstrated for isolated plants grown in the glasshouse under well-watered conditions. TE was highly correlated with Δ, and reasonably well correlated with m_a, suggesting that established techniques for indirect selection for TE might overcome the prohibitive methods associated with direct TE measurement. In contrast, SLA was shown to be an inferior surrogate measure of TE in soybean. No negative associations were observed between TE and T. This suggests that selection for high TE in soybean could be undertaken without compromising high levels of biomass production, and ultimately yield. Based on these findings, the possibilities of indirect selection for TE to improve yield performance of soybean under non-irrigated conditions are promising. However, results have all been obtained under well-watered, glasshouse conditions and it would be inappropriate to extrapolate these results directly from pots to crops in the field (Hubick and Farquhar, 1989). The ultimate application of surrogate measures for use as indirect selection criterion for TE will require further investigation under field conditions, where influences such as boundary layers (Subbarao *et al.*, 1994) and rooting characteristics (Hall *et al.*, 1992) can be quantified under a range of contrasting water stress conditions. The relationship between TE and other plant attributes, such as T, HI and yield, also need to be investigated to determine whether negative associations exist (Virgona *et al.*, 1990). A field experiment was conducted to address these issues, using the same six genotypes as in experiment 3, and grown in mini-lysimeters under a rainout shelter. Results from this experiment are reported in Chapter 6.

CHAPTER SIX

Mini-lysimeter field experiment

6.1 Introduction

There is a scarcity of data on field-measured TE for any crop species (Hubick *et al.*, 1986). This is predominantly because the technology used to measure T of field-grown plants is imprecise (Turner, 1986) and it is difficult to measure root dry matter in the field (Hall *et al.*, 1993). These problems can be partially overcome using a combination of mini-lysimeters and rainout shelters (Hall *et al.*, 1993). Although only a limited number of plants can be examined in this fashion, Wright *et al.* (1988) demonstrated that such technology can accurately measure TE of plants embedded within small canopies in the field.

Three pot experiments have been conducted to measure the variation in TE among soybean genotypes and to determine the relationship between TE and the leaf trait Δ (Chapter 4). Significant variation in TE was observed in two experiments (*c.* 36% and 24%, respectively) and the strong correlations between TE and Δ ($r = -0.58$ and -0.98, respectively) raised the possibility of indirect selection for TE via Δ. These results were, however, measured under controlled conditions in the glasshouse. In some instances, TE determined in studies with isolated plants may not show similar genotypic rankings when conducted in field-scale plant breeding nurseries (Subbarao *et al.*, 1994). Therefore, for a trait such as TE (or Δ) to be useful in breeding programs, it is essential that the same variation and correlative responses observed in the glasshouse experiments can also be expressed in the field. The problems associated with scaling up from isolated plants in pots to a community of plants in the field are mainly due to differences in the aerial environments of both systems (dePury, 1995).

At the plant level, leaf TE is the ratio of rates of photosynthesis (P_n) to transpiration (T_i) (P_n/T_i) (Hsiao and Acevedo, 1974), where P_n is the photosynthetic rate defined by equation 10.

$$P_n = ([CO_2]_{atm.} - [CO_2]_{leaf})/(r_{bndy.} + r_{epid.} + r_{mes.}) \quad \text{.....[equation 10]}$$

where $[CO_2]_{atm.}$ = CO_2 concentration of the atmosphere,

$[CO_2]_{leaf.}$ = CO_2 concentration inside the leaf, and

$r_{bndy.}$, $r_{epid.}$, $r_{mes.}$ = resistances to CO_2 diffusion from the atmosphere to the leaf (boundary layer resistance), into the leaf (epidermal resistance), and to the site of carboxylation in the mesophyll (mesophyll resistance), respectively.

T_i is the rate of evaporation from the surface ~~of~~ the leaf defined by equation 11.

$$T_i = ([H_2O]_{atm.} - [H_2O]_{leaf}) / (r_{epid.} + r_{bndy.}) \quad \text{.....[equation 11]}$$

where $[H_2O]_{atm.}$ = H_2O concentration of the atmosphere,

$[H_2O]_{leaf.}$ = H_2O concentration inside the leaf, and

$r_{epid.}$, $r_{bndy.}$ = resistances to H_2O diffusion out of the leaf (epidermal resistance), and from the leaf into the atmosphere (boundary layer resistance), respectively.

An isolated plant has a much smaller boundary layer than a plant embedded within a canopy (Oke, 1990). As such, a leaf on an isolated plant has a smaller boundary layer resistance, which couple its stomates more closely to the atmosphere (Wright *et al.*, 1988). This makes the stomata more responsive to changes in the atmospheric conditions than stomata shielded within the moist boundary layer of a plant canopy. Genotypic differences in stomatal control of water-loss will therefore be exacerbated under isolated plant conditions, having a large effect on T and ultimately TE. Consequently, in crops where T_i dominates variation in TE (eg. cowpea; Udaykumar *et al.*, 1996), large genotypic differences in TE measured from

plants grown in pots may be smaller and/or inconsistent when measured under field conditions.

On the contrary, coupling effects do not impact as greatly upon P_n because the largest resistance component within the CO_2 diffusion pathway, $r_{mes.}$(equation 10; Hsiao and Acevedo, 1974) is similar in both isolated plants and canopies. A change in the boundary layer resistance ($r_{bndy.}$) arising from coupling effects would be small in comparison to $r_{mes.}$ and would have negligible impact on the total resistance to CO_2 diffusion, and hence on P_n or TE. In peanut, where differences in P_n are predominantly responsible for variation in TE (Hall *et al.*, 1993; Subbarao *et al.*, 1994), it has been shown that the TE of single plants (Hubick *et al.*, 1986) was well correlated with the TE measured on plants growing within a canopy in the field (Wright *et al.*, 1988). Therefore, it would appear that coupling effects have little impact on TE in crop species where TE variation is dominated by genotypic differences in P_n. Work reported in this chapter seeks to investigate the effects of an increased boundary layer resistance, achieved by growing plants in a crop canopy, on TE of soybean cultivars known to vary in TE when grown as isolated plants (Chapter 4).

6.2 Materials and Methods

The experiment was conducted at the J. Bjelke-Petersen Research Station, during the 1994 - 95 summer growing season. The six soybean genotypes used (Garoba Rouest, Otootan, Tai-Dung-Wu-Tou, Kabanyolo-1, Rawit and Mensoy 6) were common to the previous pot experiments and have been shown to express contrasting TE. The maturity of these genotypes ranged from 116-156 dae in the Kingaroy environment. The experimental layout was a split plot design consisting of three replicates split into two irrigation treatments (main plots) with genotypes (sub plots) randomised within irrigation treatments. Each sub-plot consisted of four rows of 6.5 m length and spaced 0.30 m apart. Two mini-lysimeters were used to measure evapo-transpiration (ET) in each plot. One additional plot (with two mini-lysimeters) was established within each replicate to measure soil evaporation (E). This pot was maintained as an undisturbed bare-soil core for the duration of the experiment. A large rain-out shelter (Wright, 1995b) covering the entire lysimeter system was used to exclude rainfall while irrigation was applied using trickle irrigation (T-tape).

6.2.1 Lysimeter pot system

The weighing lysimeters consisted of a large volume of encased soil, embedded in the ground and weighed regularly to measure water loss equating to ET (Figure 12). Intact soil cores were excavated (0.80m deep x 0.30m diameter), and cased with large diameter PVC pipe using a soil coring machine (Evans Deakin Proline, South Australia). A tight fitting PVC cap was secured to the bottom of the core by two self-tapping screws and a silicon adhesive to ensure that the lysimeter casing was watertight. A metal handle was attached to the top of the pot to provide a firm support for lifting and weighing procedures. A watering tube was buried 0.20 m deep into the pot to enable watering below the soil surface and hence minimise soil

evaporation (E_s). The tube was manufactured using a perforated 25 mm hose joined into a circle using a T-connector, with a 0.5m length feeder tube. The lysimeters (hereafter referred to as pots) were then placed into holes (which had been dug to slightly larger dimensions than the pots), so that the height of the soil surface in the pot was at ground level. The holes had been lined with cylindrical, galvanised iron sleeves to prevent the surrounding soil from back-filling into the hole. A basal fertilizer of P (25 kg ha^{-1}) and K (25 kg ha^{-1}) was applied pre-plant to the field and post-plant to the pots. The rainout shelter and lysimeter systems are shown in Plate 2a.

6.2.2 Planting

The trial was planted on 12 December 1994, with 6 seeds pot^{-1} sown at a depth of 30 mm. Rows of the field plots were sown at an intra-row spacing of *c.* 50 mm, and an inter-row spacing of 30 cm. Emergence occurred after 6 days. The rows were thinned at 14 dae to a plant population of 33 plants m^{-2}. All pots were thinned to two plants pot^{-1} at harvest 1 (26 dae), to achieve the same plant population as the surrounding field.

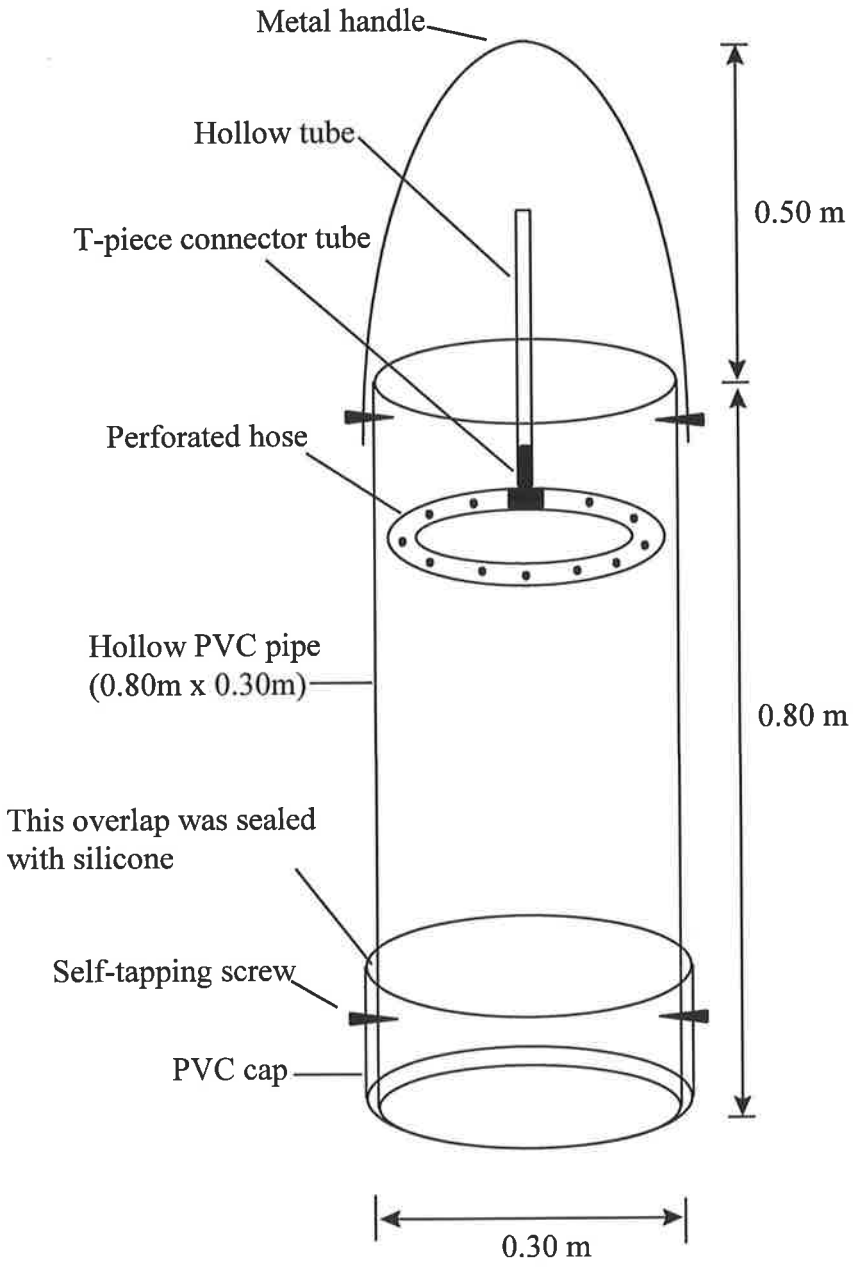


Figure 12 - Schematic illustration of the lysimeter pot system.

6.2.3 Watering Regime

Irrigation was applied to the field plots using 'T-tape' trickle irrigation, with tubes laid out along each row (0.3m spacing). The application rate was *c.* 10 mm h^{-1} and water meters were connected to each irrigation treatment to measure water application. At planting, both the field plots and pots were given 40 mm of irrigation water for emergence. From emergence until the commencement of the water stress treatments, both field plots and pots were kept fully irrigated. On 16 January (30 dae) when genotypes had achieved *c.* ≥ 60% light interception, the two water stress treatments were imposed (see section 6.2.4 below).

6.2.4 Irrigation Treatments - I_1 and I_2

At 30 dae, all pots were weighed and subsequently watered up to a pre-determined field capacity (Plate 2b). The field capacity weight was determined from the volumetric moisture content calculated from bulk density data and measurements of gravimetric moisture content taken at field capacity on soil surrounding the lysimeter area. Pot weighing was conducted using a gantry-mounted load cell (precision ± 0.1 kg) attached to a battery-operated winch. Water stressed (I_1) and fully irrigated (I_2) treatments were then imposed.

In the I_1 treatment two drought cycles were simulated. After beginning at field capacity, a drought was imposed until visual observations of early morning wilting indicated that the plants were unable to recover full leaf turgidity. At this point (51 dae) the pots were re-watered to the theoretical field capacity weight. A second drying cycle was then imposed up until the final harvest. Pots in the I_2 treatment were irrigated to the field capacity weight at each weighing event (twice weekly) to achieve non-limiting moisture conditions.

Plate 2 a (above) : Weighing lysimeters (foreground) and rainout shelter (background) measuring TE of soybeans grown in the field.

Plate 2 b (left) : Gantry-mounted weighing system used to measure water loss from the lysimeter pots.

Field plots surrounding the lysimeters were maintained at similar soil water conditions as the corresponding lysimeter pots, by calculating the average pot water use values and applying this amount to the bulk plots. This procedure meant the water stress conditions of the pot were simulated in the surrounding bulk crop.

6.2.5 Calculating transpiration (T)

T was estimated from evapo-transpiration (ET) using the following identity (Wright *et al.*, 1994).

$$T = (Wt_{fc} - Wt_{act}) - E_s \quad \text{.....[equation 12]}$$

where :- T = transpiration in kilograms,

Wt_{fc} = theoretical weight of the pot at field capacity,

Wt_{act} = the weight of the pot at the measurement event and

E_s = the soil evaporation during the period of water use.

E_s was estimated from the identity -

$$E_s = e\,(1-f) \quad \text{.....[equation 13]}$$

where :- e = the potential soil evaporation determined from the reduction in weight (water loss) of the bare evaporation pots, and

f = the fractional radiation interception by the foliage (Wright *et al.*, 1994).

Fractional photosynthetically active radiation (PAR) intercepted by the crop (f) was measured in the bulk crop of each plot every alternate week with a line quantum sensor (LI-COR, LI-191SB) and solar monitor.

6.2.6 Agronomic Practices

Manual weed control was undertaken at five weeks after planting. Insect inspections were conducted twice-weekly and it was necessary to apply a Thiodicarb/Methomyl insecticide at two, four, five and seven weeks after emergence to control insect pests.

6.2.7 Sequential Harvests

Harvest 1

Harvest 1 was conducted at 26 dae to roughly coincide with the beginning of the water stress treatment. The extra plants sown in the lysimeter pots were used for the initial dry matter harvest. DM and Δ were determined following the procedure outlined in Chapter 4.

Bulk Crop

Commencing at harvest 1 and continuing until maturity, above-ground dry matter samples were taken at fortnightly intervals from the bulk crop surrounding the pots. Samples consisted of 1m of row per plot and were separated into leaf, stem or pod components. The samples were dried at 70°C for 48h and weighed. The leaf sample from the third harvest was analysed for Δ as outlined in Chapter 4. HI was estimated as the ratio of final grain weight to the weight of the total dry matter measured at the harvest when the maximum vegetative dry matter weight occurred.

Final Harvest

The final harvest was conducted at 84 dae and coincided with the end of the second drought episode of the I_1 treatment. The pot-grown plants were harvested and partitioned into leaf, stem, and pod components. A leaf sub-sample was measured for leaf area (*c.* 1000 cm^2) and

dried at 70°C for 48 hours then weighed to allow calculation of SLA (cm^2/g), then ground for determination of Δ, leaf N% and m_a as outlined in Chapter 4. The remaining above-ground plant parts were dried and weighed using the same procedure. The pots were transported to a washing station where they were allowed to soak in water for approximately 2 hours, then agitated with a high pressure (1500 kPa) water hose. The resulting slurry was then passed through a sieve (0.8 cm) to recover the roots which were then hand-washed to remove foreign particles before final drying and weighing.

In this experiment, TE was calculated as the ratio of whole-plant DM (including roots) to water transpired and TE_{ag} was calculated as above-ground DM per unit of water transpired. The TE coefficient (k) was calculated as the product of TE and VPD. VPD was estimated from mean daily maximum and minimum temperatures from the empirical relationships described by Tanner and Sinclair (1983), as detailed in appendix 2.

6.2.8 Meteorological Conditions

Meteorological conditions are summarised in Table 14. These data were recorded from a meteorological station located 300 m from the experimental site. Average VPD calculated during the experimental period was 2.06 kPa.

Table 14 - Summary of environmental conditions during the lysimeter experiment.

MONTH	*Minimum Temperature (C)*	*Maximum Temperature (C)*	*Pan Evaporation (mm/day)*	*Solar Radiation ($MJ/m^2/day$)*
December 1994	15.9	32.1	5.0	22.6
January 1995	17.6	31.4	6.4	21.7
February 1995	18.2	28.3	4.4	18.0
March 1995	15.1	29.8	5.3	21.0

6.3 Results

Total pot dry matter components determined at 26 and 84 dae are shown in appendices 5 and 6. It is the accumulation of TDM, T, and the average of several plant traits measured over the treatment period which are of interest to this study.

6.3.1 Dry matter production and transpiration

During the measurement period (26 - 84 dae), the DM accumulated among genotypes varied significantly ($P<0.05$), and ranged from 21.4 - 30.0 g pot^{-1} and 32.0 - 59.7 g pot^{-1} for the I_1 and I_2 treatments, respectively (Table 15). Despite the large variation in DM values (*c.* 65%) between irrigation treatments, there was no significant ($P<0.05$) irrigation effect on DM. There was no significant correlation for accumulated DM of individual genotypes between irrigation treatments ($r = 0.50$), suggesting that water stress may have had different effects on the ability of genotypes to produce dry matter.

T measured over the treatment period ranged from 9.2 - 15.3 kg pot^{-1} (*c.* 65% variation) and 12.9 - 28.2 kg pot^{-1} (*c.*120% variation) for genotypes in the I_1 and I_2 treatments, respectively. The amount of T was significantly ($P<0.01$) different between genotypes, and water stress was observed to decrease the T of all genotypes by an average of 88% (Table 15). From this observation, it was clear that plants grown in the water-stressed treatment had in fact suffered significant water stress relative to the plants grown in the fully irrigated treatment. T values were affected by a significant genotype x irrigation treatment interaction ($P<0.05$) with the result that mean genotype T values were not significantly ($P<0.05$) correlated between irrigation treatments ($r = 0.58$).

Under both irrigation treatments, TDM among genotypes was significantly ($P<0.05$) correlated with T ($r = 0.84$ and $r = 0.83$ for I_1 and I_2, respectively), indicating that water stress affected the expression of genotypic differences in both TDM production and T to a similar degree. This finding has important implications for the stability of the TE trait among genotypes, when measured under contrasting water stress conditions.

6.3.2 Harvest Index

Using grain yield and fortnightly growth harvests, the harvest index (HI; appendix 6) of each genotype was approximated (refer to section 6.2). Owing to the nature of HI estimation, HI was not included in the statistical analysis. Despite this, HI ranged from 0.52-0.53 in the I_1 treatment (4% variation) and from 0.48-0.59 in the I_2 treatment (25% variation).

6.3.3 Transpiration Efficiency

In both irrigation treatments Garoba Rouest and Mensoy 6 had the highest TE (2.78 g/kg and 2.48 g/kg, respectively for I_1 and 2.23 g/kg and 2.44 g/kg, respectively for I_2) (Table 15). The large genotypic variation in TE was maintained across both water stress treatments, while the significant correlation for TE between watering treatments ($r = 0.80$, $P<0.01$; Figure 13), indicated there was low G x E for this trait. TE_{ag} was significantly correlated with whole-plant TE ($r = 0.85$, $P<0.01$; Figure 14), which was expected as a result of the close relationship in root:shoot ratios between water stress treatments (Figure 15). The absence of a significant genotype x water stress interaction for TE is a significant finding which implies TE is a stable trait across contrasting water stress environments.

Table 15 - Accumulated DM and T, and derived values of TE, TE_{ag} and *k* (TE corrected for VPD) measured on six soybean genotypes from 30-84 dae under two contrasting water-stress regimes in large lysimeter pots in the field.

Genotype	**DM** (g)	**T** (l)	**TE** (g/kg)	**TE_{ag}** (g/kg)	***k***
Water stressed					
Garoba Rouest	24.7	9.2	2.78	2.48	5.73
Otootan	26.5	13.5	2.04	1.84	4.20
Tai-Dung-Wu-Tou	30.0	13.4	2.27	1.88	4.68
Kabanyolo-1	21.4	10.9	2.03	1.75	4.18
Rawit	32.6	15.3	2.14	1.84	4.41
Mensoy 6	23.2	9.6	2.48	2.07	5.11
Non-water stressed					
Garoba Rouest	39.1	18.5	2.23	2.25	4.64
Otootan	43.1	24.5	1.88	1.69	3.48
Tai-Dung-Wu-Tou	59.7	28.2	2.04	1.75	4.20
Kabanyolo-1	44.7	28.2	1.66	1.48	3.42
Rawit	43.4	23.6	1.95	1.71	3.52
Mensoy 6	32.0	12.9	2.44	2.67	5.05
LSD (P<0.05)					
Genotype	*13.6*	*5.4*	*0.66*	*n/s*	*1.73*
Irrigation	*n/s*	*10.3*	*n/s*	*n/s*	*n/s*

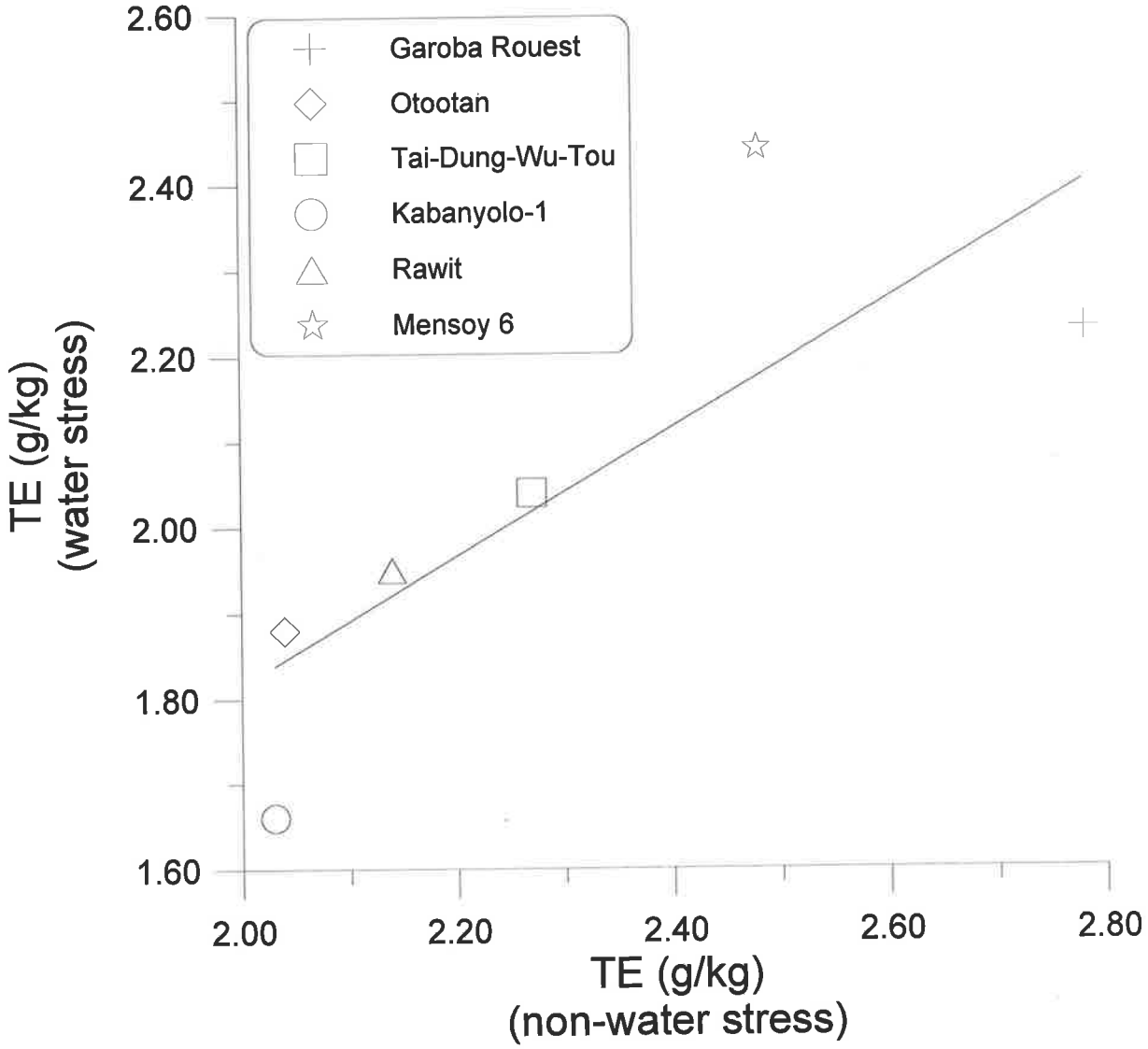


Figure 13 - The relationship between TE measured under contrasting water-stress treatments. The regression equation for this relationship is described by $y = 0.75x + 0.32$ ($r = 0.80$; $P<0.05$).

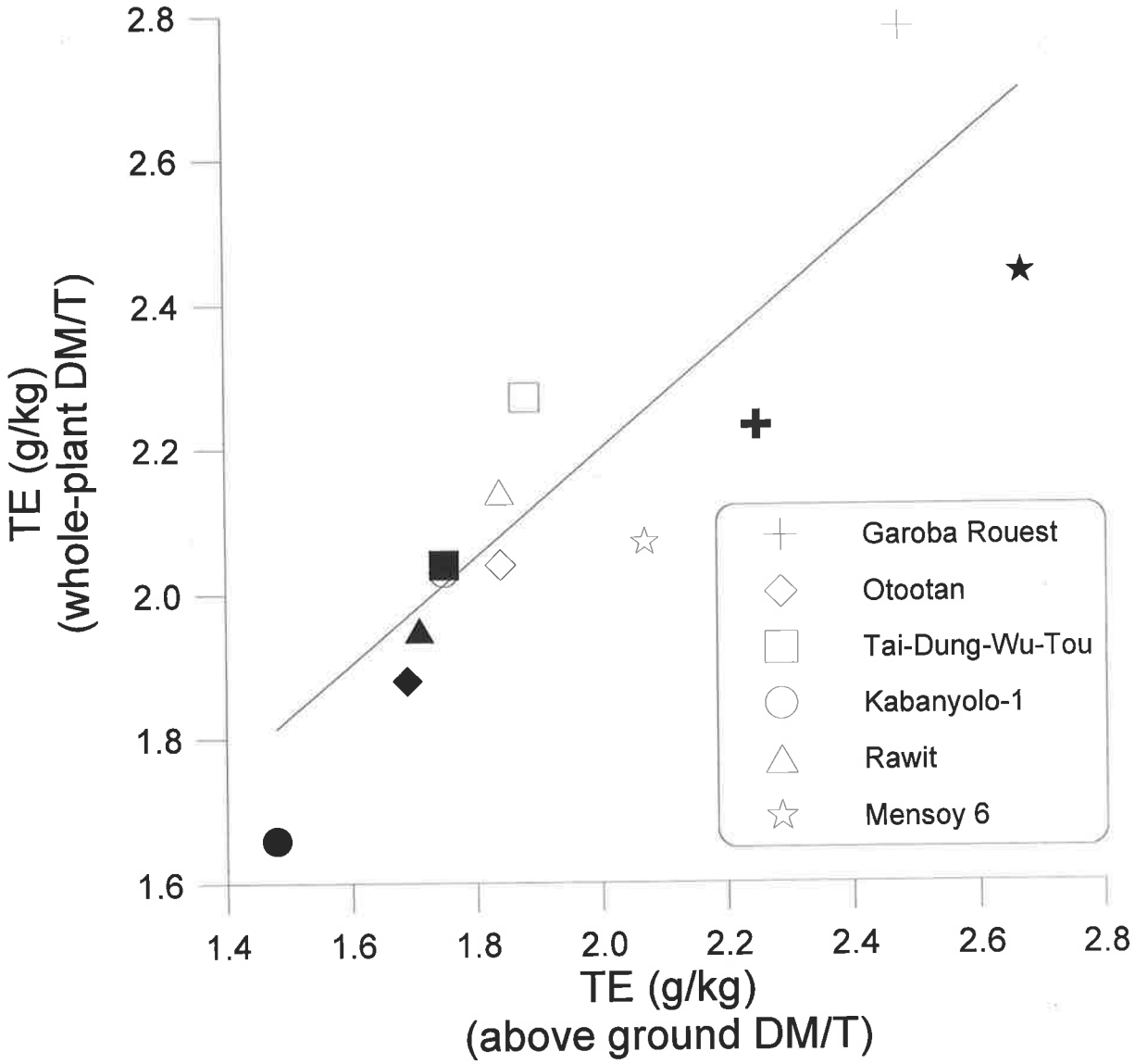


Figure 14 - Comparison of TE with TE_{ag} measured from six soybean genotypes under water-stressed (open symbols) and well-watered (closed symbols) conditions in the field. The regression equation for this relationship is described by $y = 0.74x + 0.72$ ($r = 0.85$; $P<0.01$).

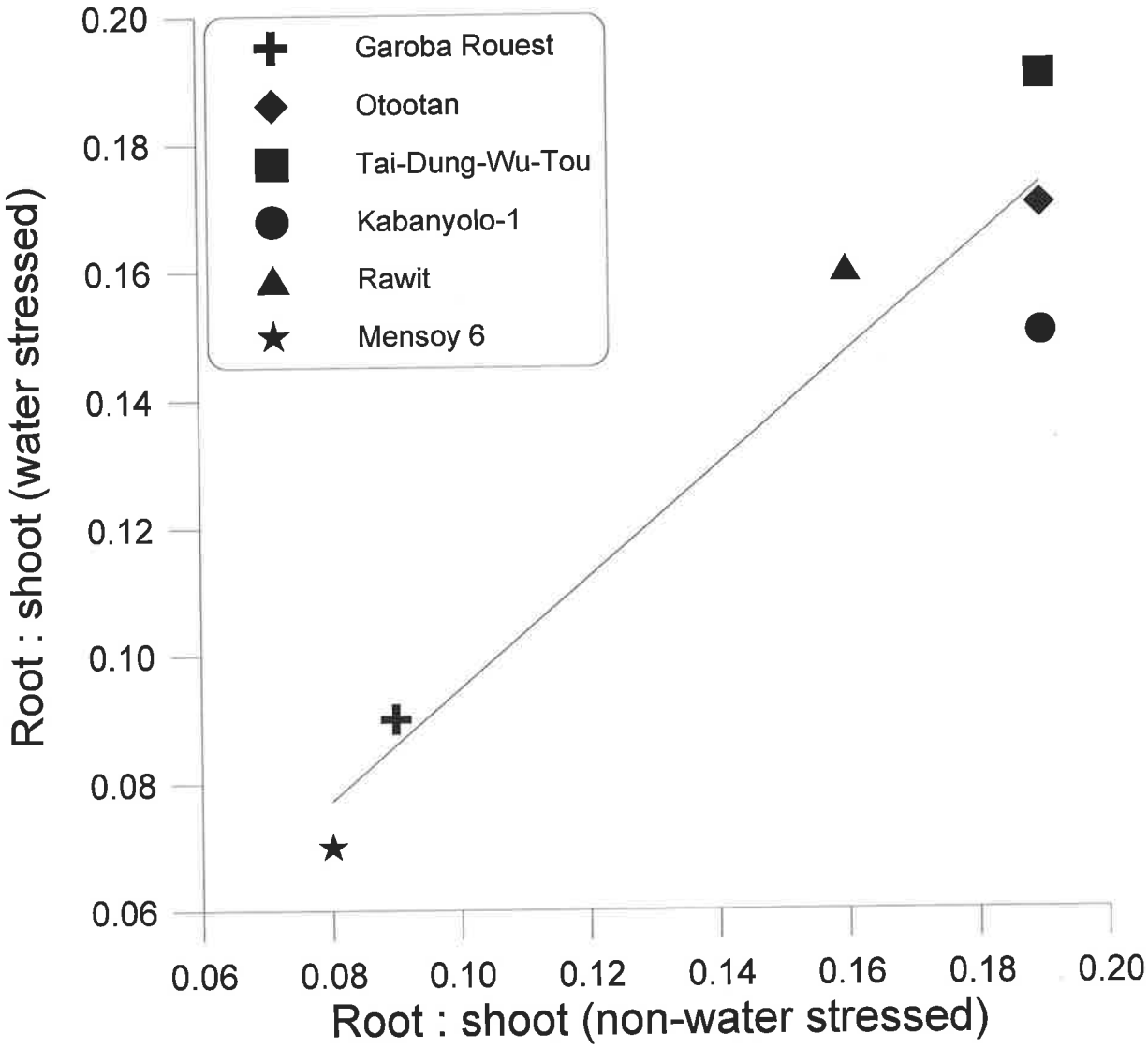


Figure 15 - Relationship between root:shoot ratios of six soybean genotypes observed under contrasting water stress conditions from lysimeter pots in the field. The regression equation for this relationship is described by $y = 0.87x + -0.007$ ($r = 0.95$; $P<0.01$).

6.3.4 Relationship between TE and Δ

The range in Δ observed among genotypes was 0.7 ‰ under I_1 and from 0.9 ‰ under I_2 (Table 16). There was a highly significant ($P<0.01$) irrigation effect on Δ with values being reduced under the influence of water stress. Despite this irrigation effect there was no genotype x irrigation effect, which indicated the genotypic ranking in Δ was maintained across irrigation treatments. Highly significant ($P<0.05$) correlations between TE and Δ were recorded in both water stress treatments (Figure 16). Carbon isotope discrimination (Δ) was also well correlated with T under fully irrigated ($r = 0.81$, significant at $P<0.05$) and water stress ($r = 0.69$, not significant) conditions, however its correlation with TDM under both water stress treatments was poor ($r = 0.20$ and 0.46, respectively). This finding suggests that genetic variation in T contributed more to genotypic variation in TE than did variation in TDM production under field conditions. This contrasts with findings under glasshouse conditions where neither component dominated TE variation and with boundary layer theory which predicts that differences in T should be reduced under field conditions (Jarvis and McNaughton, 1985).

6.3.5 Validating the use of lysimeter pots to simulate the field environment

Leaf DM of field-grown plants surrounding the pots were sampled and analysed for Δ. By comparing these Δ values to those of plants grown within the pots, it can be determined whether the leaf gas exchange characteristics and rooting environment of field-grown plants were accurately simulated by the pots. Pot Δ values from I_2 were well correlated with field Δ values ($r = 0.87$, $P<0.05$), suggesting that field and pot environments were similar under well watered conditions. In contrast, there was a poor correlation between Δ values of the field and pots in I_1. This suggests that either the pot system does not accurately represent field

conditions or some factor affected the field environment differentially to that experienced in the pots.

Table 16 - Δ, SLA, SLN, leaf N % and m_a measured at 84 dae from six soybean genotypes grown under two contrasting water-stress regimes within mini-plant-canopies in the field.

Genotype	Δ (‰)	SLA ($cm^2\ g^{-1}$)	SLN (mg N m^{-2})	N (%)	m_a (%)
Water stressed					
Garoba Rouest	20.4	260.7	1.2	3.21	7.68
Otootan	21.1	342.0	0.5	1.70	8.12
Tai-Dung-Wu-Tou	20.7	330.8	1.0	3.59	7.77
Kabanyolo-1	20.8	335.0	0.8	2.88	8.45
Rawit	20.8	257.9	1.2	3.22	8.15
Mensoy 6	20.6	296.7	1.1	3.31	8.87
Non-water stressed					
Garoba Rouest	21.3	257.6	1.1	2.82	7.72
Otootan	21.9	337.5	0.8	2.98	8.68
Tai-Dung-Wu-Tou	21.9	362.7	0.9	3.33	7.98
Kabanyolo-1	22.0	378.5	0.7	2.64	9.30
Rawit	22.2	291.8	1.0	2.97	8.38
Mensoy 6	21.4	266.7	1.2	3.28	7.97
Genotype LSD (P<0.05)	*0.6*	*71.1*	*0.4*	*1.43*	*2.32*

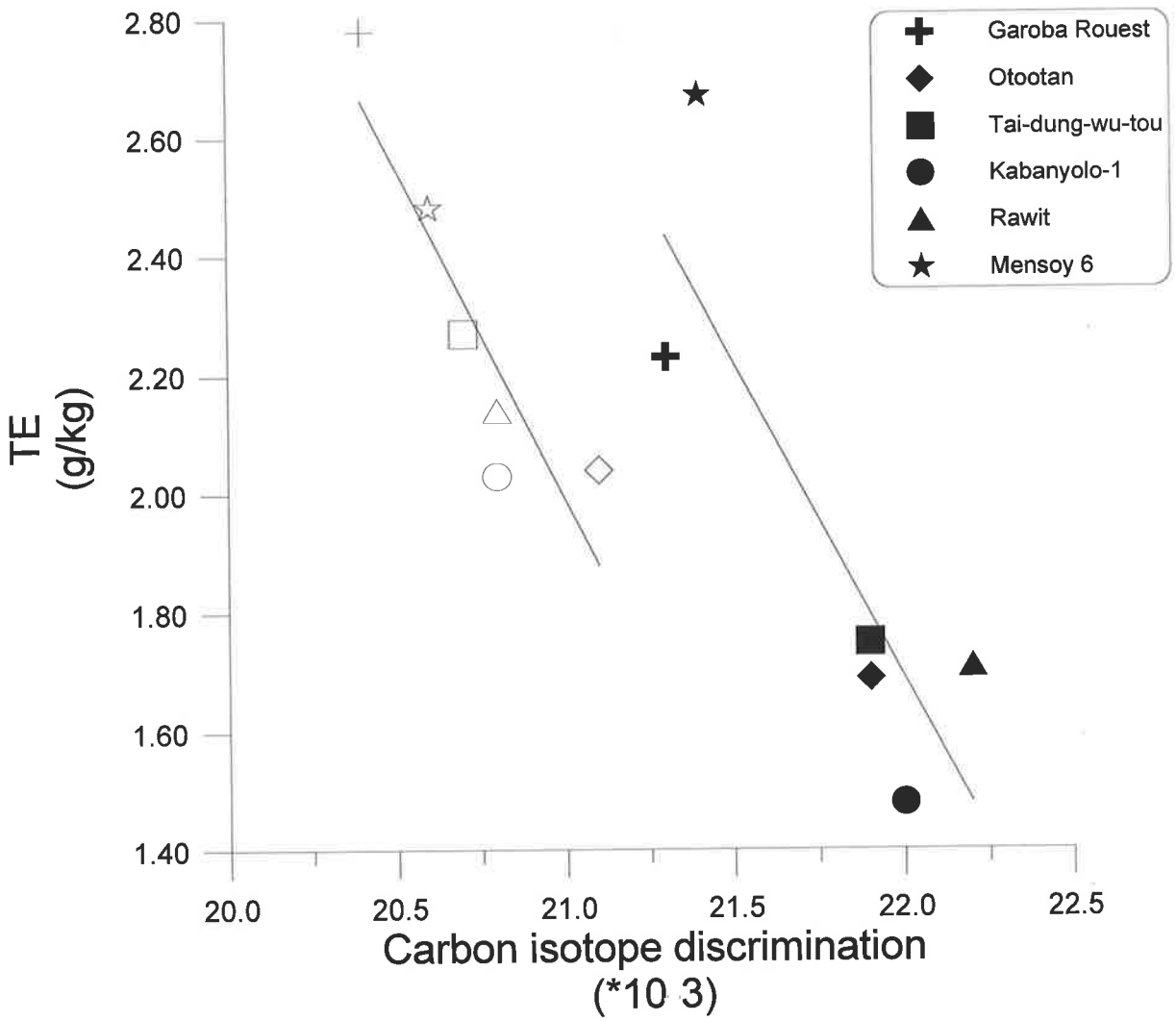


Figure 16 - Relationship between TE and Δ under water-stressed (open symbols) and well-watered (closed symbols) conditions in the field. The regression equations are described by the equations $y = -1.12x + 25.58$ ($r = 0.89$; $P<0.05$) and $y = -1.06x + 25.03$ ($r = 0.79$; $P<0.05$) for water stress and non-water stress conditions, respectively.

6.3.6 Other potential surrogate measures of TE

There was significant ($P<0.05$) genotypic variation observed for N%, SLN and m_a (Table 16). SLA was well correlated with SLN in both irrigation treatments ($r = 0.85$; $P<0.02$ and $r = 0.91$; $P<0.01$ for I_1 and I_2 treatments, respectively), which was consistent with findings from earlier glasshouse studies. This supports the use of SLA to approximate SLN as a potential indicator of photosynthetic capacity among soybean genotypes. SLA was poorly correlated with TE in the water stressed treatment ($r = 0.61$, n/s) but was reasonably well correlated under the non-water stressed treatment ($r = 0.80$; $P<0.05$). The other potential TE surrogate measures, N% and m_a were not significantly correlated with TE under either of the water stress treatments.

6.4 Discussion

This experiment has demonstrated large and consistent genetic variability in canopy TE among soybean genotypes under both water stressed and non-water stressed conditions. Furthermore, canopy TE was well correlated with leaf Δ, although its relationship with other potential TE surrogates was poor or inconsistent. The key issues for the application of such findings to drought resistance breeding in soybean are summarised under the following sections.

Harvest Index

The germplasm examined in this study was very diverse in origin. This raises the possibility that any high TE donor lines identified may be relatively 'undomesticated' meaning that they have not undergone selection for plant traits such as HI, which are vital to grain yield. Therefore it is important to investigate whether the high TE soybean lines have reasonably

high HI. The approximate HI of the six genotypes used in this study (Appendix 6) appear to be consistent with harvest indices recorded for other Australian soybean varieties at Katherine, Northern Territory (*c*. 0.56) and Lawes, Queensland (*c*. 0.47) (Muchow *et al*., 1993). This observation indicates that the high TE germplasm identified in this study has acceptable HI characteristics combined with reasonable yields (appendix 6).

The possible influence of boundary layer effects on field-measured versus pot-measured TE in soybean

Significant ($P<0.05$) genotypic variation in canopy TE of soybean was measured (Table 15) in this study, with the *c*. 40% range in canopy TE among genotypes being similar in magnitude to that observed from isolated plant studies (Chapter 4). These results indicate that canopy boundary layer effects had minimal influence on the range of TE expressed by the soybean genotypes used in this study. This supports the hypothesis proposed by dePury (1995) that the boundary layer effect on TE has been overstated in the literature. He concluded that despite the presence of large boundary layers, low stomatal conductance can translate to high levels of TE under canopy conditions and thus the coupling effect on TE has been overstated in the literature. The significant implications of these findings for screening and selection methodologies suggest that TE studies could be conducted under the more convenient conditions of the glasshouse and would be reasonably representative of field performance.

The influence of genotype x water stress interaction for TE

Results from this field study showed that TE increased by 13% under water stress (Table 15). This observation is consistent with findings from studies on tomato (Martin and Thorstenson, 1988) and wheat (Condon *et al.*, 1990), where TE was observed to increase under water stress conditions. Significantly, the genotypic ranking in TE was maintained across the two contrasting water stress environments (Figure 13). Studies on other crop species such as peanut (Hubick *et al.*, 1986; Hubick *et al.*, 1988; Wright *et al.*, 1988), barley (Hubick and Farquhar, 1989), tomato (Martin and Thorstenson, 1988), and sunflower (Virgona *et al.*, 1990) also showed stable genotypic ranking in TE across different water stress environments. In soybean, it also appears TE is under strong genetic control, which would facilitate rapid progress in increasing TE through selection by breeding.

Using TE_{ag} (above-ground dry matter/unit transpiration), as an estimate of actual TE (whole-plant dry matter/unit water transpired)

In other species such as wheat (Condon *et al.*, 1990; Condon *et al.*, 1993; Farquhar and Richards, 1984), workers have used TE_{ag} as an easy-to-measure approximation of TE. Results from this experiment have shown that TE and TE_{ag} are also reasonably well correlated in soybean ($r = 0.85$ combining data from both water stress and non-water stress conditions, Figure 14). A further independent assessment of how well TE_{ag} can estimate TE, can be made by comparing TE_{ag} with Δ. Studies reported here show that the correlation between TE and Δ ($r = 0.89$) was stronger than that between TE_{ag} and Δ ($r = 0.81$) under water stress conditions. Similar responses have been observed in peanut under field (Wright *et al.*, 1988), and pot (Hubick *et al.*, 1986) conditions.

It was expected that the correlation between TE and Δ would have been much stronger than between TE_{ag} and Δ. This observation may have been due to the consistent genotypic ranking in r:s across both water stress treatments (Figure 15), which is a surprising result considering the well documented genotypic variation in preferential re-distribution of assimilate from shoots to roots under the influence of drought (Hsiao and Acevedo, 1974; Passioura, 1983). However, in support of our finding, Martin and Thorstenson (1988) observed constant r:s in tomato grown over a large range of soil moistures.

In summary, TE_{ag} was sufficiently well correlated with TE in our study to suggest it could be used as a surrogate in general applications, such as genotypic surveys for TE variation. The high correlation between TE and TE_{ag} may however be a fortuitous one, based on the narrow range of germplasm studied. It is likely that other soybean genotypes may exhibit large variation for assimilate partitioning under water stress conditions. If this were the case, TE_{ag} would not correlate well with TE. Clearly more research on a wider range of germplasm is required before it could be recommended that TE_{ag} might be used as a routine selection protocol.

Validation of the use of mini-lysimeter pots to represent field conditions

The mini-lysimeter and rainout shelter facility allowed the accurate measurement of TE under simulated field conditions. To validate whether plants grown in the large 56 L lysimeter pots had similar leaf gas exchange and root environment conditions to field-grown plants, Δ values were measured on field-grown plants adjacent to the lysimeter pots and compared with Δ values from plants grown within the lysimeter pots. Under the well-watered treatment it was found that field Δ was highly correlated with pot Δ ($r = 0.87$), while under water stress

conditions the correlation broke down. The reasons for the breakdown are unknown, however there could possibly have been some sub-surface lateral flow of water into the bulk area which meant the bulk crop may have been better hydrated than the 'sealed' pots. Such sub-surface flows are known to occur on this soil type (Smith and Kent 1993). The finding that Δ values between lysimeter-grown and field-grown plants were well-correlated under non-water stressed conditions does however suggest that the mini-lysimeters and rainout shelter facility provided an accurate simulation of TE under a canopy environment in the field.

Evaluation of cheaper and non-destructive surrogate measures of TE in soybean

This study has confirmed that Δ was a highly correlated surrogate measure of TE, providing an effective method for indirect selection of TE among soybean genotypes. However, Δ has the disadvantage of being expensive to analyse (*c*. $20AUD per sample), so this experiment has also explored whether cheaper surrogate measures of TE could be developed for soybean. The pot studies reported earlier showed only weak correlations between TE and SLA in soybean, and this was reflected in the lysimeter field experiment (r = 0.60 and 0.80 under water stressed and well watered conditions, respectively). Clearly, SLA was not as robust a measure of TE as was Δ.

The correlation between leaf mineral content (m_a) and TE observed in the field experiment (r = 0.52 and 0.61 for water stressed and well watered treatments, respectively) was of similar magnitude to the previously reported pot experiment (Chapter 4). However, m_a only accounted for 25 - 35% of the variation in TE on each of these occasions which suggests that m_a is not able to predict TE accurately enough to be useful as a tool in screening germplasm for TE variation in soybean. In contrast to SLA and m_a, Δ provided a very close indirect

measure of TE in soybean; and accordingly is recommended as the preferred surrogate measure of TE in future selection programs.

Summary

The results from this study confirm that variation in TE observed among soybean genotypes in pot experiments was consistent under field conditions where contrasting water stress treatments were applied. The strong and negative correlations between TE and Δ under water stress and well watered conditions indicate it should be possible to improve TE in soybean by selection for low Δ in conventional breeding programs. However, TE is just one of three functional components (equation 1) which determine grain yield under water limited environments, so the relationship between TE and other yield determinants must also be considered during any future selection program. These issues are discussed further in Chapter 7.

CHAPTER SEVEN

Concluding Discussion

The work reported in this thesis has focused on physiological studies into the variation in TE and its correlation with Δ among a range of soybean genotypes in the glasshouse and field. These studies have demonstrated there is good potential for using Δ to indirectly select for high TE in soybean. However, before advocating selection on the basis of Δ, several issues relating to TE at the community scale of biological organisation need to be investigated. These are summarised in the following sections.

7.1 Consistency in genotypic ranking for TE and the correlation between TE and Δ

Most breeding programs conduct their experimental work in both the glasshouse and the field. Therefore, it is important that genotypic variation for a particular trait (eg. TE) is expressed consistently under each of these environments. TE values of a range of soybean genotypes have been measured in three glasshouse experiments, as well as under contrasting water stress conditions in the field. The stability of TE for soybean genotypes was determined by correlating the TE values measured from each of the environments (Table 17). The TE values measured in pot experiment 2 were not significantly ($P<0.05$) positively correlated with either pot experiment 1 ($r = -0.94$) or pot experiment 3 ($r = 0.66$). This was probably a result of the prevailing low radiation environment (see Chapter 5) and consequently, these data were not included in the following analysis.

Although the relationships between genotype TE values measured during pot experiment 1 and those measured during each of the other experiments were not statistically significant ($P<0.05$; Table 17), the magnitudes of correlations were moderately high and consistent. The

fact that only 5 genotypes were examined, and that the procedures for measuring TE were undergoing refinement during this experiment, may have contributed to the relatively poor correlations observed.

Table 17 - Correlation matrix for genotype TE values measured during a series of glasshouse (pot) and field experiments.

	Pot1	**Pot3**	**Fdry**	**Fwet**
Pot1	1			
Pot3	0.67	1		
Fdry	0.60	**0.95	1	
Fwet	0.57	*0.77	**0.80	1

Pot1 - five genotypes grown in the glasshouse under fully irrigated conditions.
Pot3 - six genotypes grown in the glasshouse under fully irrigated conditions.
Fdry - six genotypes grown in the field under water stressed conditions.
Fwet - six genotypes grown in the field under fully irrigated water conditions.
* - denotes significance at the $P<0.05$ level of significance.
** - denotes significance at the $P<0.01$ level of significance.

In contrast to pot experiment 1, the genotypic ranking in TE was consistent between pot experiment 3 and both sets of field data (Table 17). These highly significant ($P<0.01$) correlations demonstrate that TE in soybean is a relatively stable trait, and that it may be under strong genetic control. Similar stability for TE has also been demonstrated for peanut (Wright *et al.*, 1993). However, the Δ leaf trait would be measured as an indirect estimate of TE in practical breeding programs. Therefore to determine whether the observed stability in TE has potential utility in breeding programs, the stability of the relationship between TE and Δ under a range of environmental conditions was also investigated.

Figure 17a shows the relationship between TE and Δ, measured on a common set of genotypes, over the 2 glasshouse and 2 field experiments. A test for homogeneity of

regression coefficients (data not shown) indicated that the slopes of the regression lines from both glasshouse experiments were not significantly different ($P<0.05$). In a similar fashion, the slopes of both field experiments were not significantly different ($P<0.05$). These responses demonstrate that the relationship between TE and Δ was relatively consistent when measured in different experiments within similar environments. However, Figure 17a also shows that the slopes of these relationships were quite different when comparing glasshouse and field environments.

Variation in TE, and hence Δ, can be caused by differences in the prevailing VPD (Wright *et al.*, 1991), so the observed differences between environments could have been the result of differences in the prevailing VPD. This hypothesis was tested by adjusting the TE values measured in each experiment for VPD (Figure 17b). The two independently measured relationships between k and Δ measured under glasshouse conditions came closer together after the VPD correction, such that the pooled data gave a highly significantly relationship ($P<0.01$; Figure 17b). However, this correction did not account for the differences between glasshouse and field experiments, or between the two field-measured relationships (Figure 17b). Indeed, statistical analysis of the average of slopes of the regressions between k and TE in the glasshouse versus the field environments indicated they remained significantly ($P<0.05$) different.

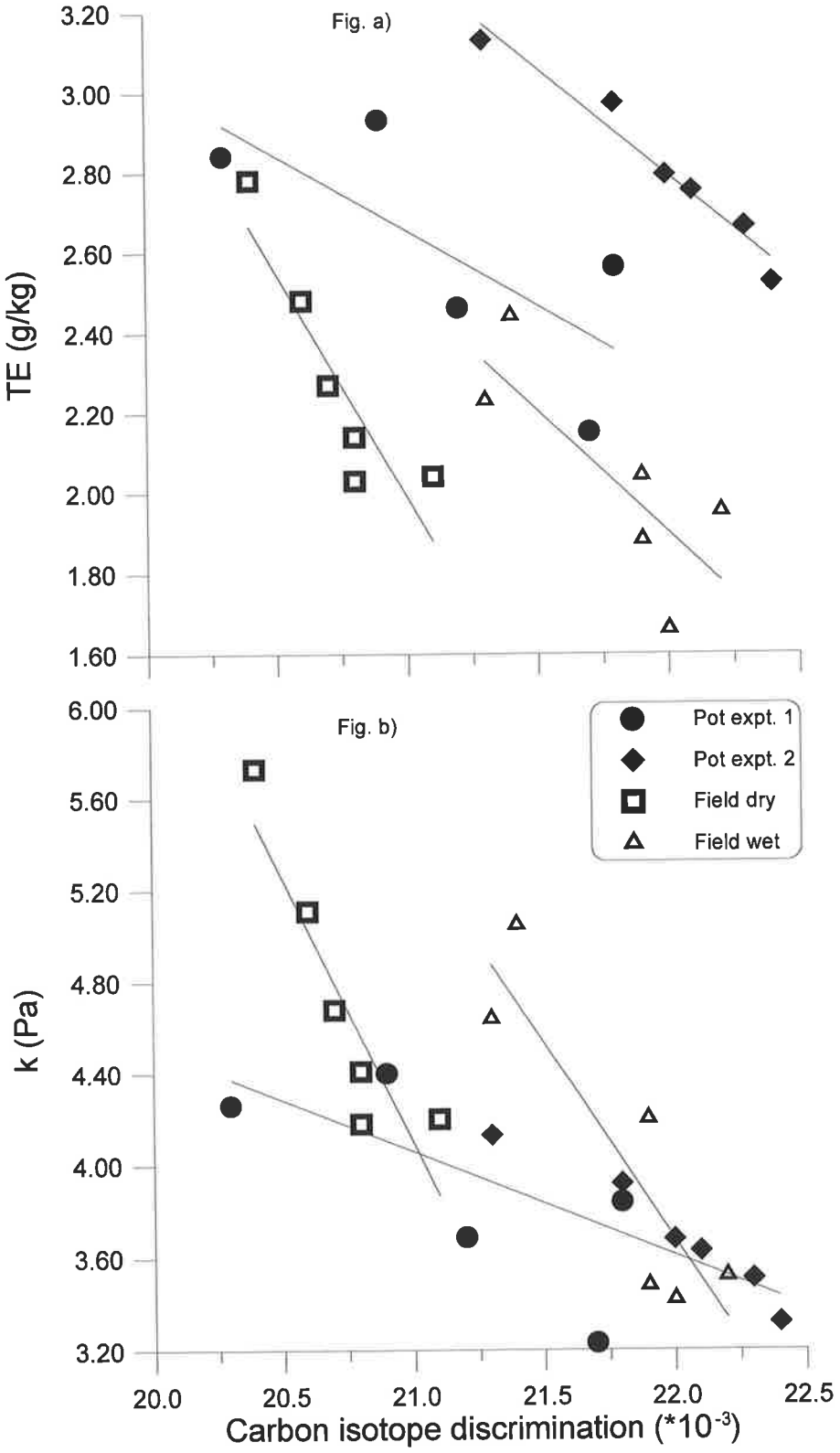


Figure 17 - Relationships between TE and Δ (a) and *k* and Δ (b), measured under four contrasting environments. The regression line in (b) was fitted through the pooled pot experiment data and is described by the equation y = -0.447x+13.45 (r = -0.76; P<0.01).

The inability to homogenise glasshouse and field data sets using a VPD correction may have been due to a number of factors. The most likely is that the method used to calculate VPD was based on an empirical relationship between VPD and maximum and minimum temperatures derived in field environments (Sinclair, 1986), and this may not have been valid for glasshouse experiments with artificial heating or cooling. As well, it has been assumed that leaf and ambient temperatures are similar for the purposes of the average VPD calculation. This assumption may hold true for isolated plant growing in the glasshouse, but it may be invalid for a field-grown canopy where substantial boundary layers exist (Oke, 1990). For example, in the field the calculated VPD was the same for both well-watered and water-stressed treatments owing to the fact that ambient temperatures of both treatments were also the same (Chapter 6). Measurements of canopy temperature differentials between treatments using an infra-red thermometer were shown to be as large as 7°C (data not shown), thus highlighting a potential source of error in the calculation of VPD.

From a surrogate selection tool standpoint, the most significant finding from this analysis is that consistency in genotypic ranking for TE and Δ was maintained. This finding suggested that Δ might be a useful tool for indirect selection for TE in programs aiming to increase soybean yield under drought-prone environments such as the Australian sub-tropics.

7.2 Interaction between TE and both T and HI

When studying options to improve the grain yield of crops grown under water limited environments, yield can be viewed as the product of three factors - the amount of water transpired (T), the ratio of biomass production to T (TE) and the ratio of grain yield to total dry matter or harvest index (HI; Passioura, 1977). From this simple analytical model,

improvement in any one of the three factors can potentially increase grain yield if there are *no significant negative interactions between any of the three factors*. Therefore, while the work reported in this thesis indicates large potential for indirect selection to improve soybean TE, the relationship between TE and both T and HI needs to be explored to assess the nature of any potential negative associations between traits.

Transpiration

The seasonal crop T, which is an indication of the ability of a genotype to extract soil moisture via root water uptake, was unable to be measured in this study. An analysis of the relationship between TE and T cannot therefore be performed. Further detailed water use studies are needed in the field to confirm the results obtained from pot and gas exchange studies, which showed that TE and T were not negatively associated.

Harvest Index

Most of the significant yield improvements in soybean (Gay *et al.*, 1980), and indeed most major crop species (Donald and Hamblin, 1976; Evans, 1980; Mozingo *et al.*, 1987), have come about through dramatic increases in HI. To ensure that the decades of such breeding progress are retained, it is important that there are no negative associations between TE and HI and that high TE soybean genotypes with reasonably high HI can be identified. The sources of high TE reported for soybean in this study were not found among commercial or advanced breeding germplasm collections, which all possess high HI. Rather, they were found in a collection of exotic lines randomly selected from a germplasm collection which contained many undomesticated genotypes. This raises the possibility that the high TE lines

identified in this study may be relatively undomesticated and consequently exhibit relatively low HI.

The HI of all six genotypes examined in the field experiment was estimated using the procedure described in section 6.2. Results presented in Appendix 6 show that the HI's of the highest TE genotypes Garoba Rouest (*c*. 0.50) and Mensoy 6 (*c*. 0.50) were similar to other HI values reported for commercial Australian soybean varieties measured by Muchow *et al.*, (1993)at Katherine, NT (0.56) and Lawes, Qld. (0.47). Furthermore, there were no negative relationships between TE and HI (r = 0.10 and 0.32 under water stressed and non-water limited conditions, respectively) suggesting TE and HI were independent traits among the six soybean genotypes examined in the field lysimeter study. If these genotypes are representative of the wider germplasm, it would appear that high TE soybean genotypes with relatively high HI may be available for use in breeding programs.

7.3 Summary

The Australian soybean industry is concentrated in the sub-tropical zone of Australia (Lawn *et al.*, 1986) and has enjoyed the benefits of production under mainly high rainfall or fully irrigated regions. However, as higher value crops move into these areas, soybean production is migrating to the more marginal dryland environments which are predisposed to erratic droughts (Wright, 1997). This has prompted breeding programs to focus on developing soybean cultivars with enhanced drought tolerance which are better suited to these newly emerging environments.

Several recent attempts (Rose *et al.*, 1983; Rose *et al.*, 1992; James *et al.*, 1993) to develop physiological drought tolerance traits as selection criteria for soybean breeding programs have resulted in only limited success. Consequently, there are no commercial soybean varieties specifically released for production in the dryland cropping regions of the sub-tropics (Lawn and Imrie, 1991). In fact, very few examples exist where a drought tolerance trait has been successfully used in breeding programs to increase grain yield under drought-prone environments for any of the major crop species (Passioura, 1977). The work reported in this thesis has demonstrated that a relatively unexplored drought tolerance trait (TE), may be potentially incorporated into existing varieties to improve yield performance under droughted conditions.

The physiological mechanisms responsible for high TE among high biomass producing soybean genotypes suggest that selection for increased TE may be a method of improving grain yield of soybean under rainfall-limited environments. This finding was consistently demonstrated at several levels of biological organisation, including the community level where breeding efforts are mainly targeted. Carbon isotope discrimination (Δ), an easily measured surrogate measure of TE, was consistently shown to accurately predict TE among genotypes under a range of contrasting environments including glasshouse and field, isolated plant and canopy, and water stressed and fully irrigated. Thus, Δ was demonstrated to be an attractive selection tool for plant breeders.

In conclusion, this thesis has developed a protocol suited to indirect selection for high TE soybean genotypes under a range of environments. This protocol has immediate application to the several Australian soybean breeding programs which have had limited success to date in developing soybean varieties specifically adapted to the dryland production areas of the Australian sub-tropics.

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APPENDIX 1: Entry number, name, country of import and Δ values of a diverse collection of 98 soybean genotypes from the QDPI Genetic Rsources Centre, Biloela, Queensland.

Entry Number	Name	Country of Import	Delta (Δ) ($*10^3$)
1 060352	Shih-shih	Thailand	21.8
2 060447	Taichung 3	Taiwan	21.6
3 060440	Sukhothai 1	Thailand	20.8
4 060396	Coc chum	Vietnam	21.1
5 060201	Pingtung pearl	Taiwan	20.8
6 060435	Sudan No 13	Sudan	21.2
7 060207	Caloria	Austria	20.5
8 060353	Chung hsien 1	China	21.1
9 060003	Skynteja	USSR	21.1
10 060943	Kujbysevskaja	E. Germany	20.2
11 060053	Amurskaia 42+B28	Mongolia	20.6
12 060256	Rawit	Indonesia	21.0
13 060511	Dornburger weiss	W. Germany	20.4
14 060494	Disoy	Poland	20.2
15 061008	Mamloxi	Nigeria	21.0
16 060436	Sudan No 124	Sudan	20.7
17 061037	Mis 28 EB3901	Brazil	21.4
18 060704	PF 72-317	Brazil	21.0
19 060085	Avrdc	N. Zealand	20.3
20 060622	Garoba Rouest	Morocco	19.0
21 060889	Nagyzemu feher	Hungary	20.7
22 060924	Oribi	Zimbabwe	21.4
23 060490	Dieckmanova	Czech	20.9
24 060553	Ecuador 2	Ecuador	21.6
25 061659	PI290125	Canada	20.5
26 061335	G230	Taiwan	19.6
27 061148	C 8012	Angola	20.7
28 060054	Amurskaja	Germany	20.4
29 060712	SR-30	Brazil	20.7
30 061147	C 8010	Angola	21.1
31 060165	Bogatic soya	Yugoslavia	21.0
32 060152	Black forage	Nigeria	21.2
33 060640	Genja Rewok	Indonesia	21.0
34 061799	Otootan	Zaire	21.3
35 060030	AGA 1/73	Samoa	20.8
36 060450	Tai-Dung-Wu-Tou	Japan	21.3
37 060488	Bidgoska 057	Poland	20.7
38 060044	Amurskaja 21	E. Germany	20.9
39 060411	Con khuong	Vietnam	20.9

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	Entry Number	Name	Country of Origin	Delta (Δ) (*10^3)
40	060444	Szurkebarat	Romania	20.1
41	060989	Lincoln	Argentina	21.5
42	060553	Tsurunoko	N. Zealand	21.1
43	061757	PR143-23	Puerto Rico	21.3
44	060120	Beljska Kasna	Yugoslavia	19.7
45	061393	IPB 12-75	Brazil	21.0
46	060137	Bilofield	Philippines	20.9
47	060094	Bacatete	Mexico	20.8
48	061782	No 9	Thailand	20.6
49	060077	Apache	USA	20.5
50	060627	Gaterslebener	W. Germany	19.5
51	060694	Yellow kedele	Tanzania	20.9
52	060263	Rhosa	Zimbabwe	20.9
53	060546	Tro-pagana	Myanmar	20.8
54	060953	Kwangdu	Korea	20.2
55	061986	A2 H55 F4/195/1	Tanzania	20.3
59	060163	Blyvoor	S Africa	20.7
60	061149	C 8014	Angola	20.2
61	060596	Vansoy	Canada	20.5
62	060637	Vniimk-6	USSR	21.7
63	060274	Roanoke 49376	Philippines	21.3
64	060291	Saliut 216	Mongolia	19.4
65	060219	Planalto	Brazil	20.8
66	060109	Bau 1/4	Bangladesh	20.8
67	060168	Bongeni	S. Korea	20.5
68	060814	ISRA-IRAT 44a/73	Senegal	20.7
69	060856	Kabanyolo-1	Uganda	21.2
70	061366	IAC-2	Brazil	20.3
71	060821	Java	Myanmar	20.8
72	060914	Ocepar 2 Iapo	Brazil	21.1
73	061610	PI 165989	Canada	20.9
74	060537	Tetabiate	Mexico	21.2
75	061350	G869	Taiwan	20.2
76	060886	KG 20	Canada	19.9
77	060320	Seminole	Israel	20.8
78	061029	Mensoy 6	Argentina	19.9
79	060955	Kwangkyo	S. Korea	20.3
80	060863	Karangduen 1	Indonesia	19.6
81	061126	BR 78-23403	Brazil	20.7
82	061505	LO 55 2089	Brazil	21.0
83	060605	Fruhe Gelbe	Romania	19.2

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	Entry Number	Name	Country of Origin	Delta (Δ) ($*10^3$)
84	061352	G2261	Taiwan	20.2
85	062786	CP127086	Congo	20.8
86	061695	PR8-1-4-B-2	Puerto Rico	20.8
87	062889	CPAC 904-76	Brazil	20.7
88	061608	PI 159926	Peru	20.4
89		Not surveyed		0.0
90		Not surveyed		0.0
91	061162	CPAC X3-76	Brazil	20.2
92	060110	Bau 1/4	Bangladesh	21.3
93	060532	Ecuador 1	Ecuador	21.1
94	060113	Bayano	Panama	21.0
95	060221	CEP 7138	Zambia	20.2
96	060229	Chabarovskaja	E. Germany	19.6
97	060981	Len Sin Pin din	China	20.8
98	060862	Kaoshung E-32	Thailand	20.2
99	060813	ISRA/IRAT 26/72	Senegal	20.3
100	060142	Biloxi	China	20.6

APPENDIX 2: Accounting for the influences of vapour pressure deficit (VPD) differences on TE

Differences in VPD can have a profound effect on TE (Tanner and Sinclair, 1983). A rise in air temperature increases the saturation vapour pressure gradient and hence the vapour pressure deficit. This causes transpiration (T) to increase while dry matter production remains static. This phenomenon has been observed for soybean (Jones, 1976) and imposes restrictions on comparing TE from multi-locational or multi-seasonal experiments which are subjected to different VPD conditions. (Tanner and Sinclair, 1983) proposed a concept to allow comparison of TE among species across different environments independently of VPD, using the proportionality constant *k*, from equation 3 below.

$$TE = k/(e_i - e_a)$$

where -TE is transpiration efficiency
- *k* is the proportionality constant
- and, $(e_i - e_a)$ is the vapour pressure gradient leaf interstice to the atmosphere

Average VPD during the experimental period was calculated from measurements of maximum and minimum temperature (recorded for the duration of the experiment with an automatic temperature logger) using the empirical relationship (Sinclair, 1986) of equation 9.

$e_i - e_a$ (VPD) = 0.7*(vpd Corrected Min - vpd Corrected Max)/10

where:

Transformed Max = 6.107*EXP(17.269*max temp/[237.3+max temp])
Transformed Min = 6.107*EXP(17.269*min temp/[237.3+min temp])

Thus, using the calculated $e_i - e_a$ (vpd) and measured TE in equation 8, the proportionality constant *k* (Pa mol C/mol H_2O) was calculated to allow genotypic differences in TE to be examined without the confounding effects caused by environments differing in VPD as previously reported (Condon and Richards, 1992a; Condon *et al.*, 1992).

APPENDIX 3 : Summary of results from pot experiment 2. Measurements of TDM, T, leaf area pot^{-1}, leaf N (%), SLA and SLN measured on fully-irrigated soybean genotypes at harvest 1 (41 dae) and harvest 2 (63 dae).

Genotype	TDM ($g.\ pot^{-1}$)	T ($kg.\ pot^{-1}$)	Δ (‰)	Leaf Area ($cm^2\ pot^{-1}$)	N (%)	SLA (cm^2/g)	SLN ($g\text{-}N/m^2$)
HARVEST 1							
Garoba Rouest	33.4	11.6	21.6	6065.5	5.05	482.1	1.05
Otootan	40.9	14.4	22.3	6962.0	5.00	485.5	1.03
Kabanyolo-1	45.3	15.7	22.4	7913.5	5.30	522.4	1.02
Rawit	44.0	14.2	21.7	6588.5	4.90	409.8	1.20
Mensoy 6	40.3	14.3	21.7	6461.8	4.65	421.7	1.10
LSD							
* P<0.05 level	7.3	2.3		1614.4	0.49		
** P<0.01 level	n/s	n/s	0.62	n/s	n/s	76.7	0.11
HARVEST 2							
Garoba Rouest	122.2	40.0	21.2	7 173	3.91	315.0	1.28
Otootan	160.5	50.5	21.6	20 474	2.82	375.7	0.79
Kabanyolo-1	170.7	54.5	21.9	24 903	3.13	464.3	0.67
Rawit	132.2	38.8	21.5	9 686	4.27	269.4	1.58
Mensoy 6	99.8	34.7	22.3	5 971	3.84	378.9	1.03
LSD							
** P<0.01 level	11.5	3.0	0.54	2026.7	0.44	63.8	0.26

APPENDIX 4 : Summary of results from pot experiment 3 at harvest 1 (36 dae) and harvest 2 (44 dae) from lysimeter pots grown in the field.

Genotype	Leaf DM (g)	Stem DM (g)	Root DM (g)	Leaf Area (cm^2)	R:S	TDM (g)	T (kg)	TE (g/kg)	TE_{ag} (g/kg)	Δ (‰)	Leaf N (%)	SLA (cm^2/g)	SLN ($g.N/m^2$)
Harvest 1													
Garoba Rouest	12.4	15.2	5.9	6066	0.17	33.5	11.8	2.91	2.41	21.6	5.1	482.3	1.1
Otootan	14.3	19.2	7.5	6962	0.19	40.9	14.3	2.86	2.33	22.1	5.0	485.8	1.0
Kabanyolo-1	15.1	20.9	9.3	7914	0.20	45.3	14.8	3.05	2.42	22.4	5.3	522.5	1.0
Rawit	16.2	18.5	9.4	6589	0.21	44.0	13.8	3.19	2.51	21.7	4.9	394.8	1.2
Mensoy 6	15.5	18.0	6.9	6462	0.17	40.4	13.2	3.05	2.53	21.8	4.7	422.0	1.1
LSD. (5%)	*n/s*	*n/s*	*2.4*	*n/s*	*n/s*	*n/s*	*n/s*	*n/s*	*n/s*	*n/s*	*n/s*	*n/s*	*0.1*
Harvest 2													
Garoba Rouest	23.2	30.6	15.4	7173	0.14	110.0	40.0	2.75	1.73	21.2	3.9	315.0	1.28
Otootan	50.3	73.5	36.7	20474	0.23	160.5	50.5	3.19	3.19	21.7	2.8	375.7	0.75
Kabanyolo-1	53.9	78.5	38.3	24903	0.22	170.7	54.5	3.15	3.15	21.9	3.1	464.2	0.92
Rawit	34.2	53.3	33.0	9686	0.26	129.5	38.8	3.36	3.13	21.5	4.3	269.4	1.56
Mensoy 6	16.0	15..6	12.4	5971	0.14	86.9	34.7	2.51	1.27	22.3	3.8	378.9	1.03
LSD (5%)	*4.7*	*6.7*	*9.4*	*2091*	*0.05*	*14.1*	*4.3*	*0.25*	*0.27*	*0.51*	*0.4*	*55.4*	*0.44*

APPENDIX 5 : Dried weight of plant components measured at 14 dae from plants grown within mini-lysimeters grown in the field.

Genotype	**Leaf DM** (g pot^{-1})	**Stem DM** (g pot^{-1})	**Root DM** (g pot^{-1})	**TDM** (g pot^{-1})
Garoba Rouest	2.6	1.9	0.6	5.1
Otootan	1.5	0.9	0.3	2.7
Tai-Dung-Wu-Tou	1.9	1.2	0.4	3.5
Kabanyolo-1	2.4	1.6	0.5	4.4
Rawit	2.8	2.0	0.7	5.5
Mensoy 6	3.5	1.8	0.7	5.9
Genotype LSD ($P<0.05$)	*0.7*	*0.4*	*0.2*	*1.1*

APPENDIX 6 : Summary of results from the mini-lysimeter field experiment harvested at 83 dae.

Genotype	Leaf DM (g pot^{-1})	Stem DM (g pot^{-1})	Pod DM (g pot^{-1})	Root DM (g pot^{-1})	TDM (g pot^{-1})	r:s	Grain Yield (t/ha)	Harvest index
Water stressed								
Garoba Rouest	3.2	5.0	17.6	2.8	28.9	0.09	1.77	0.52
Ototan	10.9	13.7	0.0	5.7	30.3	0.19	2.75	0.53
Tai-Dung-Wu-Tou	13.2	14.9	0.0	6.7	34.8	0.19	2.48	0.52
Kabanyolo-1	10.1	11.2	0.4	5.0	26.7	0.19	2.05	0.53
Rawit	11.1	13.0	7.9	6.1	38.1	0.16	2.33	0.52
Mensoy 6	3.0	4.1	17.3	2.2	26.4	0.08	1.50	0.54
Non-water stressed								
Garoba Rouest	8.1	8.4	24.2	3.9	44.5	0.09	2.14	0.53
Ototan	17.3	21.2	0.0	8.2	46.6	0.17	3.87	0.59
Tai-Dung-Wu-Tou	22.7	29.0	0.0	12.9	64.5	0.19	1.79	0.47
Kabanyolo-1	16.5	20.4	4.2	7.5	48.6	0.15	2.48	0.50
Rawit	12.1	16.1	12.9	7.4	48.4	0.16	3.58	0.53
Mensoy 6	5.7	5.5	23.2	2.6	37.0	0.07	1.93	0.48
Genotype LSD (P<0.05)	*4.2*	*5.1*	*6.1*	*2.7*	*13.66*	*0.04*	*0.64*	*n.a.*
Irrigation LSD (P<0.05)	n/s	n/s	n/s	n/s	n/s	n/s	n/s	n/a