# WATER USE EFFICIENCY (WUE) AND GAS EXCHANGE CHARACTERISTICS IN SELECTED $C_3$ AND $C_4$ SPECIES - AN ASSESSMENT UNDER SIMILAR WATER LIMITED CONDITIONS

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# CERTIFICATE

This is to certify that the thesis entitled "WATER USE EFFICIENCY (WUE) AND GAS EXCHANGE CHARACTERISTICS IN SELECTED $C_3$ AND $C_4$ SPECIES - AN ASSESSMENT UNDER SIMILAR WATER LIMITED CONDITIONS" submitted by Mr. RAVISHANKAR, H.M. in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE (AGRICULTURE) in CROP PHYSIOLOGY to the University of Agricultural Sciences, Bangalore, is a record of the research work carried out by him under my guidance and supervision and that no part of the thesis has been submitted for the award of any other degree, diploma, associateship, fellowship or other similar titles.

Bangalore
December 30th, 1988

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# ABBREVIATIONS

A - Assimilation rate

$A/g_s$ - Assimilation to conductance ratio

CWT - Cumulative water transpired

CWA - Cumulative water added

Ci - Calculated intercellular $CO_2$ concentration

$\Delta$ DM - Dry matter produced during the experimental period

DAS - Days after sowing

E - Evaporation

ET - Evapotranspiration

FC - Field capacity

$g_s$ - Stomatal conductance

LAD - Leaf area duration

LWP/$\psi$ - Leaf water potential

NAR - Net assimilation rate

RLER - Relative leaf expansion rate

$r_s$ - Stomatal resistance

SDM - Shoot dry matter

TDM - Total dry matter

TQ - Transpiration quotient

VPD - Vapour pressure difference

WUE - Water use efficiency

# INTRODUCTION

# I. INTRODUCTION

Water is the major production constraint in rainfed agriculture. Rainfed cultivation however, is a major component of our agriculture system where the transient water deficit is an inevitable and recurring feature. Although drought causes more yield reduction than the combined effect of all other abiotic stresses the progress made in enhancing productivity under water limited environment is not significant. This is because of the complexity of the problem; the magnitude, duration and time of occurence of drought stress is unpredictable and often compounded by the variations in other environmental conditions and soil factors.

The concept of drought resistance is viewed differently by molecular biologists, biochemists, physiologists and agronomists. The major concern (objective) of agriculture scientists is to enhance biomass returns under limited input of water which is characteristic of our rainfed agriculture. There are three important approaches to enhance productivity of rainfed crops viz., which (i) enhance soil moisture conservation by agronomic practices, (ii) or minimize the transpiration losses by usage of antitranspirants and (iii) are concerned with the identification of suitable crop species or genotype resistant to moisture stress and thus more productive under water limited conditions.

The concept of developing a drought resistant variety has been there for quite some time. But the progress made is not encouraging. In nature the crops which survive under extreme drought have developed certain strategies which favour their survival. Often there is an inverse relationship between the extent of resistance to drought stress and the productivity of a genotype. But our major objective is to maximize biomass production and not to ensure mere survival.

However, there are certain adaptive mechanisms of plants which do not decrease productivity to a greater extent when the stress is not very severe, which is true of many rainfed areas in India. But appropriate research programme needs to be evolved to identify plant characteristics which favour the maintenance of growth rates with limited input of water.

The important adaptive mechanisms when the moisture stress is not very severe are water harvesting and its utilization efficiency. The two plant characteristics associated with this strategy are root factors, and morphological, physiological and biochemical traits associated with high water use efficiency (WUE). Though total evapotranspiration (ET) is related to TDM produced it has been shown that, at a given level of ET difference in WUE alone is the major factor contributing for differences in productivity among the species or genotypes.

WUE refers to the amount of dry matter produced per unit amount of water used and the reciprocal of it is often referred to as transpiration quotient (TQ) or transpiration ratio ($R_T$). From the physiological point of view it is the amount of carbon fixed per unit amount of water transpired. Hence, at a leaf level, it is a balance between carbon uptake and water loss. However, at the canopy level WUE is determined by canopy transpiration and canopy carbon exchange rates.

Though this parameter is important it is very difficult to study WUE differences in field conditions because of inherent problems associated with determination and delineation of E and T and problems associated with root extraction. The problem is further accentuated because WUE, though an intrinsic character, has been shown to be affected by several environmental factors like relative humidity, temperature and moisture stress. Therefore it is more relevant to study differences in WUE amongst and within a species at a given input of water. Unlike the other environmental variables drought stress has been shown to affect both the important plant processes which reflect WUE. The relative effect of tissue water status on the rate of water loss and carbon gain determines differences in WUE. Several physiological and biochemical processes associated with carbon assimilation and the stomatal behaviour will therefore determine the WUE of a species or a genotype at a given input of water. It is therefore necessary to study the variation in WUE and identify the physiological traits which favour higher WUE when plants are grown at a given input of water. Keeping this in view, experiments were conducted in this study with the following objectives.

i) To assess the differences in WUE in sunflower and amaranthus at two different moisture regimes (100 per cent field capacity and 60 per cent field capacity).

ii) To assess the differences in water use efficiency in six species under adequate and inadequate input of water similar to all the species.

iii) To identify the physiological and gas exchange characteristics associated with WUE.

# REVIEW OF LITERATURE

# II. REVIEW OF LITERATURE

Droughts are an inevitable and recurring feature of world agriculture and despite our improved ability to predict their onset and modify their impact, drought remains the single most important factor affecting world food security and the condition and stability of the land resource from which that food is derived. Transient water deficit is a normal and universal experience for crop plants and most have evolved appropriate responses to cope with this stress.

Turner (1986) suggested that the mechanisms of adaptations of water deficits can be divided into three categories:

1. Drought escape: The ability of a plant to complete its life cycle before serious soil and plant water deficits develop (Turner, 1979).

2. Drought tolerance with high plant water potential: The ability to endure periods without significant rainfall whilst maintaining a high plant water status i.e., dehydration postponement (Kramer, 1980).

3. Drought tolerance with low plant water potential: The ability of a plant to endure periods without significant rainfall and endure low tissue water status, i.e., dehydration tolerance (Kramer, 1980).

Of the several adaptive mechanisms for drought tolerance, one of the important avoidance mechanism under rainfed conditions is increasing the water harvesting and its utilization efficiency. The total productivity of any crop depends on the evapotranspiration, water use efficiency and harvest index (Passioura, 1983). The important physiological processes associated with water harvesting and water utilization are root factors and plant processes associated with water use efficiency. Therefore it is important to identify physiological processes associated with water harvesting and water utilization efficiency.

Given below is a brief review of a discussion on the concept of WUE, its various definitions, techniques used for determinations and important controlling factors.

## Definitions

WUE in general terms is defined as the amount of dry matter produced per unit amount of water expanded.

Monteith (1984) has pointed out, the word 'efficiency' (in WUE) is inappropriate in that a maximum established by theory or observation does not exist for reference. So the most convenient expression of WUE is transpiration quotient (TQ) or transpiration ratio (Stanhill, 1986) which is determined as;

$$\text{Transpiration ratio}(R_T) = \frac{\text{Amount of water used in transpiration(ml)}}{\text{Amount of dry matter produced(g)}}$$

Fischer (1979) used the term transpiration efficiency rather than WUE for DM production per unit of transpiration, retaining the term WUE for dry matter production per unit of ET. Accordingly,

$$\text{Transpiration efficiency} = \frac{\text{Dry matter produced(mg)}}{\text{Water used in transpiration(g)}}$$

$$\text{WUE} = \frac{\text{DM produced (mg)}}{\text{Water lost in ET(g)}}$$

Further, WUE is defined differently in different contexts.

## Hydrological

In a purely hydrological context WUE has been defined as the ratio of the volume of water used productively, i.e., transpired and in some cases also evaporated from the area under study, to the volume of water potentially available for that purpose that is, that reaching the crop growing region via rainfall and irrigation plus that available from the soil (Stanhill, 1986).

## Agronomical

Agronomists compare WUE of crops on the basis of above ground biomass or only the economic yield per unit rainfal received in the growing season (French and Schultz, 1984).

$$\text{WUE} = \frac{\text{Grain yield or above ground biomass(kg)}}{\text{Precipitation during the growing season(mm)}}$$

Use of ET in the above context results in more variability in WUE because E is affected by leaf cover and frequency of soil wetting, independently of T (Tanner, 1981).

Physiological

Here, WUE can be defined at the level of single leaf or at the whole plant or crop level.

At the level of the leaf, it is the ratio of carbon assimilated to water lost in transpiration and is expressed as mg $CO_2\ g^{-1}\ H_2O$ or m mol $CO_2\ mol^{-1}\ H_2O$.

At the level of the crop WUE is the ratio of the total dry matter per unit of water used. But roots are difficult to harvest. So WUE is calculated based on above ground biomass. Hence, more the assimilates a plant transfers to its roots the lower will be its WUE (Passioura, 1983). Second complication is difficulty in delineating evaporation from total evapotranspiration (Turner, 1986).

## Determination of WUE

By gas exchange technique: At the single leaf level, the WUE is determined by the following equation.

$$\text{WUE} = \frac{\Delta c}{\Delta e} \times \frac{Dc\ (r_a + r_s)}{De\ (r_a + r_s + r_i)}$$

Where, $\Delta c$ and $\Delta e$ are the leaf to air concentration differences for $CO_2$ and water vapour respectively; Dc and De are the diffusivities of $CO_2$ and water vapour; and $r_a$, $r_s$ and $r_i$ are the boundary layer, stomatal and internal resistances to diffusion (Fischer and Turner, 1978). If $CO_2$ concentration at the chloroplast is assumed as zero, c equals the concentration of $CO_2$ in the atmosphere, 0.59 mg $l^{-1}$ at 25°C. Assuming Dc/De is 0.6, the equation can be simplified to, WUE = (360 x 0.6)/ $_e$ X $(r_a + r_s)/(r_a + r_s + r_i)$.

This gas exchange theory has been extended to horizontally uniform plant communities such as crops and forests and canopy gas exchange models have been used to predict canopy WUE (Sinclair *et al*., 1975).

## Based on DM accumulation

Much information is available on WUE from determination of dry matter accumulation and transpiration of plants in containers, crops or natural communities, and sampled at intervals of one week to several months. WUE based on dry matter in field grown plants suffers from two complications. First, transpiration and soil evaporation may not be separated so that WUE is calculated using ET. Second, roots are commonly neglected in measuring dry matter accumulation. Studies where the above complications were absent or of minimal significance were for obvious reasons usually container experiments (Fischer and Turner, 1978).

### WUE in container or pot studies

The first out door study of transpiration ratio of crop plants was conducted by Lawes (1850). The transpiration of wheat, barley, bean, pea and clover plants was measured by gravimetric method. The containers held 19 kgs of soil whose surfaces were sealed against evaporation and which were protected from wetting by rain, were weighed at weekly intervals to arive at cumulative water transpired. On both yield and total dry matter basis, transpiration ratio was found to vary widely. The minimum values of transpiration ratio, 148 was for clover and the maximum, 272 was for barley when plants were grown at highest fertilizer level.

Farquhar, and Richards (1984) determined the WUE of wheat genotypes on the basis of the dry matter accumulation and water transpired in containers. They observed that the range in WUE among the wheat genotypes was from 4.0 to 6.5 g DM $kg^{-1}$ $H_2O$.

This technique of container studies for determinations of WUE has also been extended to different $C_3$ and $C_4$ crops by Briggs and Shantz (1914) and Shantz and Piemeisel (1927).

In cowpea (Nagamani, 1985), soybean (Arun, 1985), minor millets (Sashidhar, 1987) and in selected $C_3$ and $C_4$ species (Uma, 1987) WUE determinations have been done based on container studies.

Thus, this is a convenient technique to assess WUE at whole plant level.

## Field studies on WUE by the ET method

There are very few field studies where WUE was determined based on dry matter accumulation. In one such field study, Teare et al. (1973) compared WUE of sorghum, a $C_4$ species and soybean, a $C_3$ species. WUE of sorghum was approximately three times greater than soybean with a peak WUE of 7.0 mg DM g $H_2O^{-1}$.

Garrity et al. (1982) conducted a field experiment using a modified line source sprinkler system to study the

ET-yield relationships in grain sorghum. WUE consistently decreased as ET declined below the maximum. It appeared that water stress, regardless of timing, tended to reduce WUE. The reductions were smallest when stress increased gradually throughout the growing season. The relationship between ET and grain yield and dry matter was linear. Reduction in ET under all conditions tested incurred some yield reduction.

French and Schultz (1984) studied the relationship between yield and water use under mediterranean type of environment in wheat. The highest DM and grain productions were 37 and 12.7 kg ha$^{-1}$ respectively for each mm of water use.

Similar field studies were conducted for soybean (Singh and Whitson, 1976) and for sorghum, corn and pearl millet (Eastin _et al_., 1982). However, there were no studies to delineate E to assess WUE based on transpiration alone.

## Genotypic differences in WUE

There exists a considerable variation in TQ among species and also among genotypes within a species. Furthermore experimental observations suggest the WUE of $C_4$ crops to be twice that of $C_3$ crops.

Sashidhar (1987) in container studies reported a considerable range in WUE amongst different genotypes of finger millet. Similarly, Arun (1985) has reported a considerable variation in WUE among different soybean genotypes.Nagamani (1985) found significant variations in WUE amongst different cowpea genotypes. Similarly Hubick et al. (1986) reported a range of 1.41 to 2.29 g DM $kg^{-1}$ $H_2O$ among diverse peanut genotypes. Blake et al. (1984) observed significant differences in WUE among 17 popular clones and hybrids. Farquhar and Richards (1984), in container studies, have reported a range of 4.0 to 6.5 g DM $kg^{-1}$ $H_2O$ as WUE among wheat cultivars.

Teare et al. (1973) reported a three fold greater WUE for sorghum, a $C_4$ crop when compared to soybean, a $C_3$ crop. Similarly Caldwell et al. (1977) determined WUE of 4.3 mg DM g $H_2O^{-1}$ for a $C_4$ shrub when compared to 2.9 mg DM g $H_2O^{-1}$ for a $C_3$ shrub community. Shantz and Piemeisel (1927) reported a range of 415 to 912 ($R_T$) for 29 $C_3$ species with a mean of 628 whereas, $C_4$ plants like maize, proso millet, grain sorghum had $R_T$ values of 349, 267 and 305 respectively.

Uma (1987), in container studies estimated the transpiration quotient for two $C_3$ species (sunflower and groundnut) and two $C_4$ species (ragi and amaranthus).

TQs for sunflower and groundnut were found to be 552 and 490 whereas it was 292 and 263 for amaranthus and ragi under nonstressed conditions.

Sashidhar (1987) studied WUE differences six minor millet species and found a TQ range of 201.9 in little millet to 392.4 in kodo millet.

Ku and Edward (1978) also found distinct difference in WUE between $C_3$ and $C_4$ species. $C_4$ plants had a higher WUE than $C_3$ plants. Mabrouk and Jones (1984) while studying the species differences in sensitivity of stomata to air humidity in cassava and other warm climate species, found WUE of $C_4$ species to be higher than those of $C_3$ species. He attributed this to the higher rates of photosynthesis of $C_4$ species rather than lower transpiration rate.

Goudriaan and Van (1978) studied the relations between leaf resistance and $CO_2$ assimilation at different $CO_2$ concentrations in maize, beans, lalang grass and sunflower. They reported that though the $CO_2$ assimilation in sunflower responded better to external $CO_2$ than in maize and beans, it was less water use efficient than the other species, maize was more water use efficient than beans.

Hattendorf *et al.* (1988) studied the water use characteristics of $C_3$ and $C_4$ crops. They found a mean dry matter use efficiency of 17.5 Mg $ha^{-1}$ $M^{-1}$ for the group of $C_3$ crops (Pintobean, soybean and sunflower) and 33.3 Mg $ha^{-1}$ $M^{-1}$ for the group of $C_4$ crops (maize, sorghum and pearl millet).

A study by Hitoshi Oguro *et al.* (1985) supported the difference in WUE in $C_3$ and $C_4$ plants and reported that the transpiration rate was high in $C_3$ and $C_3$-$C_4$ intermediate species but low in $C_4$ species. The value for WUE of $C_3$ and intermediate species varied from 10 to 15 mg $CO_2$ g $H_2O^{-1}$ while those of $C_4$ were 30 to 50 mg $CO_2$ g $H_2O^{-1}$.

## Intrinsic water use efficiency

Osmond *et al.* (1980) suggested the term intrinsic water use efficiency which is given by $A/g_s$ i.e., the rate of photosynthesis obtained for any given stomatal conductance. This $A/g_s$ ratio as a reflection of intrinsic WUE can be conveniently used in comparing WUE of different species at the single leaf or whole plant level. The relative differences in the $A/g_s$ ratios determined at the leaf level are also reflected at the plant stand level. This also reflects in a similar difference in

WUE based on dry matter production over the growing season (Ludlow and Wilson, 1972).

Osmond _et_ _al_. (1980) also reported that this ratio is higher in $C_4$, undoubtedly a direct result of the $C_4$ pathway serving as an internal $CO_2$ concentrating mechanism. They observed that $C_4$ plants have $A/g_s$ ratio two and half to three times those of $C_3$ plants when compared under similar environmental conditions.

Slatyer (1970) observed that a $C_4$ species (_Atriplex_ _spongiosa_) consistently had 2.5 to 3.0 times higher $A/g_s$ ratios than a $C_3$ species (_A_. _triangularis_) during the experimental period.

Osmond _et_ _al_. (1980) also observed that high $A/g_s$ ratios were particularly important under moisture stress when partial closure of stomata occurs.

Farquhar and Richards (1984) observed that differences in WUE in wheat genotypes under moisture stress was related to the internal $CO_2$ concentration(Ci). They suggested that an increase in WUE was associated with higher $A/g_s$ ratios and low Ci. These findings were confirmed by Guy and Reid (1986) in a $C_3$ halophyte (_Puccinellia_ _nuttaliana_) subjected to different levels of salinity.

## Effect of atmospheric factors on WUE

### Effect of relative humidity and temperature

The major environmental factor to influence water use efficiency is air saturation deficit through its influence on vapour concentration in the air, and hence $\Delta_e$ (saturation vapour pressure deficit). Other things remaining equal, WUE is linearly related to the reciprocal of $\Delta_e$ and thus decreases as the saturation deficit increases (Fischer and Turner, 1978). The major influence of air temperature on WUE operates via its effects on $\Delta_e$; $\Delta_e$ is usually closely coupled to air temperature in the field (Schulze *et al*., 1973). Thus, increased air temperatures reduce WUE. An increase in the vapour pressure deficit of the atmosphere around a leaf increases transpiration without a commensurate increase in photosynthesis, leading to a decrease in transpiration efficiency (Turner, 1986).

Mabrouk and Jones (1984) studied the effect of varying VPD and water stress on photosynthesis, transpiration, stomatal conductance and WUE of two cassava cultivars. Apparent photosynthesis and transpiration were sharply reduced by increase in VPD above 1.8 to 2.0 KPa in both stressed and nonstressed plants. This trend coincided with changes in leaf conductance over similar ranges of VPD. The WUE of single leaves, estimated as the ratio of $CO_2$ uptake to $H_2O$ loss, decreased with increasing VPD over the range 1 to 4 KPa. The unwatered plants were more efficient at any given VPD. The rapid closure of stomata in dry air was independent of bulk leaf water potential.

Rawson and Begg (1977) compared the effect of varying VPD on photosynthesis,transpiration and WUE of several crop species with $C_3$ and $C_4$ pathways. WUE decreased in response to a VPD change from 5 to 10 milli bars in nonstressed plants of barley. In stressed plants, the WUE increased from 10 ng $CO_2$ $ng^{-1}$ $H_2O$ X $10^2$ to about 17 ng $CO_2$ $ng^{-1}$ $H_2O$ X $10^2$ in response to a VPD change from 5 to 15 milli bars in whole barley plants.

Khairi and Hall (1976) studied the effect of temperature and humidity on WUE of cultivars of orange (Citrus sinensis) and one cultivar of marsh grape fruit orange (Citrus paradisi). They observed that in all the cultivars the actual WUE decreased at higher temperature (38°C) and greater VPD (34 m bars). However, they observed that the intrinsic WUE calculated for a constant VPD increased with increase in VPD difference, indicating a possible adaptive mechanism for conditions of high evaporative demand.

Turner et al.(1984) determined the WUE in five species; sunflower, cowpea, cotton, almond and pistachio, in response to a change in vapour concentration differences (VCD) from 10 mPa $Pa^{-1}$ to 25 mPa $Pa^{-1}$. WUE decreased in all species at the greater VCD. Differences in WUE at the highest VCD amongst the species was significant and ranged from 2.3 to 3.3 m mol $CO_2$ $mol^{-1}$ $H_2O$.

Jones et al. (1985) studied the effect of temperature on soybean CER and transpiration at 28, 31, 33 and 35°C respectively and observed no significant effect on CER. Transpiration increased with increasing temperature and thus WUE decreased with increase in temperature.

Similar observations were made by Downes (1970) in wheat and sorghum. So, apart from the intrinsic assimilation and conductance characteristics, WUE thus seems to be affected considerably due to $\Delta_e$ which in turn is affected by T and RH.

## Effect of light

The importance of another important environmental variable, incident irradiance derives from the fact that transpiration is always positive, showing a relationship which is linear or curvilinear upward with increasing irradiance, because of rising leaf temperature and falling $r_s$, while net photosynthesis, especially of $C_3$ species, shows downward curvilinearity with increased irradiance after an optimum. Thus, there is often an optimum irradiance for maximum water use efficiency (Fischer and Turner, 1978); this optimum is usually less than the irradiance incident upon a leaf oriented normally to the sun's rays.

Supporting the above view were Mabrouk and Jones (1984) who studied the influence of light intensities on WUE of cassava using gas exchange techniques at two different leaf to air VPD. At a VPD range of 1.0 to 1.5 KPa the optimum light intensity for maximum WUE was 1100 µEi $m^{-2}s^{-1}$ whereas at greater VPDs of 2.0 to 2.5 KPa the optimum light intensity decreased to 600 µEi $m^{-2}s^{-1}$.

Downes (1970) investigated the effect of variation in light intensity and leaf temperature on WUE based on gas exchange measurements in wheat and sorghum. In both species, WUE decreased as temperature increased at all light intensities. He also observed that WUE was greater at the moderate light intensity (0.26 cal $cm^{-2}$ $min^{-1}$) and the highest light intensity but not at the lowest light intensity of 0.06 cal $cm^{-2}$ $min^{-1}$. In sorghum WUE was highest at the medium light intensity.

Ludlow and Wilson (1972) determined the effect of several environmental factors, oxygen concentration, light and vapour pressure difference (VPD) on the transpiration ratio ($R_T$) of a $C_4$ grass (Panicum maximum) and a $C_3$ legume (Phaseolus atropurpureus). The $R_T$ did not change with decrease in oxygen concentration from 21 per cent to 0.2 per cent in the $C_4$ grass while it decreased in the $C_3$ legume. With regard to the effect of light, they observed an increase in the $R_T$ in both species with decrease in light intensity. However, the $R_T$ was always two fold higher in the $C_3$ legume at all light intensities. This study suggested that the high WUE of $C_4$ species was maintained at all light intensities. An increase in VPD from 0 to 30 mm Hg increased $R_T$ in both the species but the increase was significantly more in the $C_4$ grass than in the $C_3$ legume.

## Nutritional factors

The nutrient status of the soil can also influence the WUE. When plants are not well supplied with nutrients, TQs tend to be higher. This means that when nutrition is inadequate, water is less efficiently utilized for crop production. This relationship results from the fact that under nutrient stress conditions dry matter production is more affected than water consumption.

Nitrogen seems to have a beneficial effect in increasing WUE. Morgan (1985) in a growth chamber experiment on wheat, showed that WUE of leaves calculated as $CO_2$ exchange rate/transpiration increased from 9.1 to 13.0 milli moles $CO_2$ per mole $H_2O$ for plants grown with higher N fertilization as water became limiting as compared to an increase from 7.9 to 9.1 milli moles $CO_2$ per mole $H_2O$ for low N plants.

Pandey et al. (1977) showed that higher N application substantially increased the WUE of wheat. However, higher P application was not found to influence WUE. But, Rathore and Singh (1977) reported that WUE of wheat increased with increasing levels of both N and P.

Olson et al. (1960) reported that nitrogen fertilization increased WUE of four non-irrigated cereal crops grown in different agroclimatic regions. They concluded from 29 such experiments that even under very dry conditions, WUE was increased by fertilization and that it was higher under dry conditions than when moisture supply was more plentiful.

A container experiment specifically designed to examine the effects of K on $R_T$ was conducted by Walker and Richards (1985). They studied the effect of ninteen different concentrations of plant available K in four soil types on WUE of alfalfa and found no effect of nutrient status in any of the soil.

## Effect of carbon dioxide

Probable effects of increasing global atmospheric $CO_2$ concentration have been extensively reviewed by Kimball and Idso (1983). About 430 observations of the yields of 37 plant species grown with $CO_2$ enrichment were extracted from the literature and analysed. $CO_2$ enrichment increased the biomass yields by 36 per cent. Additional analysis of 81 experiments which had controlled $CO_2$ concentrations showed that yields will probably increase by 33 per cent with a doubling of atmospheric $CO_2$ concentration. Doubling of $CO_2$ would reduce transpiration by 34 per cent, which combined with the yield increase, indicates that WUE may double.

Jones *et al*. (1985) have shown that the WUE of soybean canopies was significantly higher in a $CO_2$ enriched atmosphere. In this study a two fold higher photosynthetic rate of the canopy was observed at higher $CO_2$ levels associated with a significant reduction in canopy transpiration due to partial stomatal closure. But WUE, at any given level of $CO_2$ was found to decrease with increased temperature.

Under well watered conditions, $CO_2$ enrichment increases WUE of both $C_4$ and $C_3$ crops but only $C_3$ crops stand to perform better in relation to total dry matter production. Under moisture stress conditions, both $C_3$ as well as $C_4$ perform better. Patterson (1986) studied effect of $CO_2$ enrichment on the total biomass production of three $C_4$ grass weeds and a $C_3$ soybean. Under nonstress conditions the total biomass of $C_4$ grass weeds did not increase (when $CO_2$ concentration was increased from 350 to 675 ppm) but T per unit leaf area decreased and hence WUE increased. Under moisture stress conditions $CO_2$ enrichment increased leaf area and total dry weight of both the $C_4$ grasses and soybean. Growth enhancement in response to $CO_2$ enrichment was greater in soybean than in the $C_4$ grasses.

Gifford (1979) has shown that $R_T$ was significantly less for two wheat cultivars grown in a $CO_2$ enriched atmosphere. The relative difference between control (ambient $CO_2$) and $CO_2$ enriched environment increased as the water supply became more restrictive. There was a small positive effect of $CO_2$ enrichment on the crops harvest index and thus $R_T$ expressed on a yield basis showed a some-what greater response to $CO_2$ enrichment.

Similar results were obtained by Gifford and Morison (1985) in a $C_4$ forage grass (Paspalum plicatum), and Morison and Gifford (1984) in plants of 18 species.

Wong and Dunin (1987) reported in Eucalyptus, a 50 per cent increase in CER and a 30 per cent reduction in T rates resulting in a reduction in transpiration quotient by about 80 per cent when $CO_2$ concentration was doubled from 340 ppm to 680 ppm.

From the above it is clear that primary effects of enriched $CO_2$ on agriculture are likely to be the beneficial increases in crop yields and WUE.

## Plant factors associated with WUE

### Effect of leaf characteristics on WUE

Leaf movements which orient the leaf parallel to the sun's rays, leaf rolling and flagging, erect leaves and needle like leaves, all common features of dry situations especially once leaf water potential beings to fall, are considered adaptations to increase WUE (Fischer and Turner, 1978).

Shackel and Hall (1979) observed that diurnal changes in leaf orientation and leaf rolling under moisture stress can also improve WUE by reducing the effective incident irradiance.

Increased reflection of incident radiation would tend to increase WUE. Sorghum leaves with normal wax bloom which increases reflectivity had slightly higher WUE values than bloomless leaves in a study of isogenic lines (Chatterson _et_ _al_., 1973).

Johnson _et_ _al_. (1983) observed differences in WUE in isogenic lines differing in wax deposition in leaves of sorghum. WUE was 43 per cent higher in the glaucous lines than in the non-glaucous selections under field conditions.

Leaf structure is of greater significance and $R_T$ can be expected to decrease as the ratio of the leaf's internal assimilating surfaces to its external transpiring surface increases. Nobel (1980) has reported a range of two orders of magnitude in this ratio between an extremely xerophytic and mesophytic species. He also observed a range between 17 and 30 for cotton leaves grown under different levels of irradiation and soil water potential.

## Leaf gas exchange characteristics and its relation to WUE

Transpiration and photosynthesis are the two important physiological processes associated with WUE. The plant carbon and water relations are linked by the diffusion pathway of $CO_2$ and water through the stomata which in turn are controlled by stomatal, boundary layer and mesophyll resistances.

Schulze et al. (1975) showed that humidity induced stomatal closure altered the T/P ratio in apricot (Prunus armeniaca). Low T/P ratios were recorded in the dry season at a time when stomata respond strongly to high VPD by increase in resistance ($r_s$).

Slatyer (1970) studied the comparative photosynthesis, growth and transpiration of two species of Atriplex. Transpiration rates were consistently lower in A. spongiosa ($C_4$) than in A. hastata ($C_3$). As a consequence, WUE both on a single leaf and whole plant basis was much greater in $C_4$ species. The $C_4$ species had a consistently higher stomatal and lower mesophyll resistances than $C_3$ species which provides the explanation for the substantially higher WUE in $C_4$ species.

Similarly very high correlation between photosynthesis and transpiration was found in $C_3$ ($r = 0.901$) but not in $C_4$ ($r = 0.100$) by Hitoshi Oguro et al. (1985) and thus WUE was high in $C_4$ when compared to $C_3$. Das and Shantakumari (1977) showed that lower stomatal conductance was associated with higher WUE in $C_4$ compared to $C_3$ species. Similarly Brown and Simmons (1979) reported higher mesophyll conductances contributing to greater WUE for $C_4$ species than $C_3$

species where stomatal and boundary layer conductances were greater.

Rawson et al. (1980) observed that though the net photosynthesis of sunflower was extremely high under all conditions, similar to $C_4$ species, the WUE was lower and equal to other $C_3$ species because of very high transpiration rates. It was found that sunflower does not close stomata readily under high VPD which lead to very low WUE.

Similarly Uma (1987) also found that sunflower had a very high rate of water loss per unit leaf area when compared to amaranthus ($C_4$) and thus had a very high TQ values in spite having an almost similar net assimilation rate.

Rawson and Begg (1977) observed that the boundary layer + stomatal resistance values were 4.17 and 3.67 sec $cm^{-1}$ respectively in sorghum and barnyard millet as compared to 1.53, 2.09 and 1.68 in wheat, soybean and sunflower respectively.

Blake et al. (1984) observed lower transpiration rates associated with higher stomatal resistances, smaller stomata,earlier partial opening in the morning and lower stomatal frequency on the adaxial leaf surface, in water use efficient clones of poplar.

Rawson (1979) studied the effect of vertical wilting on WUE of sunflower leaves. He observed that WUE improved when leaves change from a horizontal to a vertical orientation apparently through changes in leaf temperature. Wilting induced a much lower reduction in photosynthesis than in heat load and transpiration demand and thus a large improvement in WUE during wilting. Initial improvements in WUE during the drying cycle were associated with small increases in residual conductance (gm) and relatively higher reduction in leaf conductance ($g_s$). Further increase in WUE when stress progressed resulted from further reductions in leaf conductance with no change in residual conductance.

Hasegawa (1977) studied the different transpiration rates of $C_3$ and $C_4$ plants. The T rates of $C_3$ and $C_4$ species were 5.17 - 3.09 and 2.89 - 0.94 g $H_2O$ 100 $cm^{-2}$ $hr^{-1}$, respectively at 40 per cent RH and 3.08 - 2.09 and 2.21 - 0.64 g $H_2O$ 100 $cm^{-2}$ $hr^{-1}$ at 80 per cent RH. Under both wet and dry regimes leaf temperatures of $C_3$ plants were lower than $C_4$ plants. These temperature differences between leaf and air was negatively correlated with transpiration rate.

Constable and Rawson (1980) studied the effect of leaf position, expansion and age on photosynthesis, transpiration and WUE of cotton. They observed that photosynthesis and transpiration per unit leaf area were unaffected by leaf position and in all the leaves peak photosynthesis was attained 13 to 15 days after leaf unfolding, when the leaf had developed to 75-90 per cent of maximum area. Stomatal and internal conductances changed in parallel as leaves aged and as a consequence, internal $CO_2$ concentration and WUE essentially remained constant.

## Influence of moisture stress on leaf gas exchange characteristics and WUE

Moisture stress is an environmental variable. Its effect on WUE depends on its effect on two important physiological processes, transpiration and photosynthesis. Hence, the effect of stress is being reviewed here.

Decrease in tissue water status as a result of soil moisture stress has been shown to affect carbon exchange rate affecting both $g_s$ and also mesophyll factors. A reduction in apparent photosynthesis was observed by several workers under stress conditions. Baker and Musgrave (1964) observed nearly 40 to 50 per cent reduction in the rates of apparent photosynthesis in corn under stress.

Several workers have shown that decrease in assimilation due to stress is primarily due to increase in stomatal resistance ($r_s$). However, at severe stress levels decrease in leaf water potential has been shown to affect even the mesophyll factors.

In single leaf chamber studies in cotton by Troughton (1969), decreasing water content resulted in an increase in the calculated leaf diffusive resistance and a decrease in $CO_2$ exchange. Plant water status primarily affects $CO_2$ exchange by regulating stomatal aperture. Mesophyll resistance increased progressively as relative water content (RWC) dropped from 75 to 56 per cent.

Jones (1976) reported that when plants were subjected to a slower field drying, the increase in stomatal resistance was always accompanied by an increase in $r_m$ , which would result in a decrease in WUE under severe stress conditions. They concluded that in such a situation the photosynthetic machinary would also be affected.

In growth chamber and field studies with winter wheat, Zadi (1983) found a depletion in net $CO_2$ exchange and an increase in stomatal resistance under water stress. $CO_2$ exchange per unit increase in stomatal resistance indicated that some cultivars developed a higher stomatal resistance with a minimum reduction in their photosynthetic activity. Reduction in water loss through stomata as a result of higher stomatal resistance was at the expense of $CO_2$ exchange.

Slatyer (1973) studied the species differences in $r_m$ sensitivity to water stress and reported that with decrease in leaf water potential, wheat, pearl millet and salt bush showed a smaller increase in $r_m$ as compared to corn and cotton.

Under moisture stress depending on its relative effect on $g_s$ and $g_m$, the WUE may increase or decrease. In this regard there were reports which showed a significant increase in WUE but a few other authors also gave adequate experimental evidences to suggest that WUE decreases under stress.

Cavagnavo and Jain (1985) observed an increase in WUE of four amaranthus species subjected to stress. Significant differences in WUE were found among the three irrigation treatments but only slight differences among the four species.

Farquhar and Richards (1984) reported an increase in WUE amongst four wheat cultivars in response to a decline in water potential. In the severe stress regime, the range in WUE amongst cultivars was from 4.0 to 6.5 m mol $CO_2$ mol $H_2O^{-1}$. The increase in WUE under stress was associated with greater decrease in stomatal conductance than assimilation.

Blum and Sullivan (1986) studied the comparative WUE of sorghum and pearl millet races from dry and humid high rainfall regions. They observed that WUE increase under moisture stress in both species was associated with a greater reduction in transpiration under stress than in CER.

Steven et al. (1984) studied the effect of water stress on photosynthesis and carbon partitioning in soybean grown in the field at different $CO_2$ levels. Water stress reduced CER in non-enriched plants to a greater extent than in $CO_2$ enriched plants. As CER declined stomatal resistance increased but this was not the primary cause of the decline in assimilation since internal $CO_2$ concentrations remained relatively constant.

Uma (1987) determined the WUE of four species and found amaranthus and ragi to be high WUE types compared to sunflower and groundnut under nonstressed conditions. When the plants were stressed WUE increased in all the species and this was associated with a greater decrease in rate of water loss compared to net assimilation rate. Similar results were also obtained in select cowpea genotypes (Nagamani, 1985).

Rawson et al. (1978) studied the agronomic and physiological responses of soybean and sorghum crops to water deficits. The net carbon fixation per unit area of sorghum leaves was 2.3 times greater than that of soybean while transpiration losses were less. A ratio of gas phase and residual resistances remained relatively stable, over the range of leaf water potential measured. However, the WUE of single leaf was reduced with increasing soil water deficits because of changes in leaf temperature and leaf to air VPD.

Similarly, Jones (1976) observed a reduction in WUE in plants subjected to severe stress.

It can thus be concluded that moisture stress can either increase or decrease WUE depending upon the relative stability of mesophyll factors to moisture stress and the accompanying variations in the environmental factors like VPD and temperature. In general the effect of mild stress seems to be more on stomatal factors thus enhancing WUE. However, if a moisture stress is accompanied by high VPD and temperature it may result in a decrease in WUE.

## Moisture stress and root growth

It is well established that moisture stress enhances root to shoot ratios. Increase in root growth rates and a significant increase in root to shoot ratio have been shown by several workers.

Drought usually increases root to shoot ratio, which can exceed 0.3 in droughted crops. This has been noticed in maize (Struik and Bray, 1970) and wheat (Schultz, 1974).

Masle and Passioura (1987) reported an increase in root to shoot ratio with increasing soil strength in wheat, primarily because of a greater effect of stress on shoot. An absolute increase in root growth under stress has been reported in case of apricot (Evenari _et al_., 1977) and in few horticultural species (Brouwer, 1981).

Teare _et al_. (1973) studied the water use efficiency of sorghum and soybean in relation to crop canopy area, stomatal regulation and root distribution. Higher WUE in sorghum was associated with higher canopy stomatal resistances, higher net assimilation rates and a two fold higher root dry matter per unit soil volume than that found in soybean.

Irrespective of the method which is adopted to assess WUE,in general, root biomass is not taken into account (Passioura, 1983). Though the reports on the effect of moisture stress on WUE are conflicting it is possible that WUE increases under stress because often root biomass increases under stress which was not included for estimations of WUE. The information available on this aspect is not adequate, however, to conclude the relative advantage of enhanced root growth for achieving higher WUE.

## WUE V/s $^{13}C$ carbon isotope discrimination

Though there are several techniques for determining WUE, a precise measurement of TQ or WUE in large number of genotypes is still difficult. This problem gets accentuated due to changes in environmental factors like temperature and VPD. In view of this problem, several attempts are being made to assess the intrinsic WUE by indirect techniques. Carbon discrimination is one such method.

Plants with $C_3$, $C_4$ and CAM photosynthetic pathways show characteristically different discrimination against $^{13}C$ during photosynthesis. $C_4$ plants show lesser carbon isotope discrimination (isotope composition is reflected by $\delta^{13}C$ values) than $C_3$ plants. This difference in isotope composition has become one of the standard methods to distinguish $C_4$ plants from $C_3$ plants. O'Leary (1981) extensively reviewed the components affecting overall isotope discrimination during photosynthesis.

Troughton et al. (1974) found a mean $\delta^{13}C$ values for $C_4$ plants as -13.5 ± 1.5 ‰ (parts per milli) and for

$C_3$ plants as -28.1 ± 2.5 ‰ i.e., $C_3$ plants discriminated $^{13}C$ twice that by $C_4$ plants.

Also, $C_3$ plants have TQ values twice greater than $C_4$ plants. So, attempts are now underway to relate variation in carbon isotope composition with variation in water use efficiency among genotypes of a particular species and also among different species.

Farquhar and Richards (1984) reported a three-fold range in WUE amongst wheat cultivars under different moisture regimes. WUE increased in all the cultivars in response to a decline in water potential. In the severe stress regime the range in the WUE amongst cultivars was from 4.0 to 6.5 m mol $CO_2$ per mol $H_2O$. They showed a linear relationship between the carbon isotope composition as reflected by $\delta^{13}C$ values, and WUE. The increase in WUE under stress was associated with greater decrease in stomatal conductance than assimilation and with positive shifts in $\delta^{13}C$ values. They observed that with an increase in WUE under stress $\delta^{13}C$ values became less negative. They concluded with a suggestion that carbon isotope analysis may be a useful tool in selection for improved water use efficiency in breeding programme for $C_3$ species.

Masle and Farquhar (1988) studied the effects of soil strength on the relation of WUE and growth to carbon isotope discrimination in wheat seedlings. They noticed that the WUE of wheat seedlings increased with increasing soil strength and that WUE was negatively correlated with carbon isotope discrimination. Decrease in intercellular partial pressure of $CO_2$ accounted for increase in WUE with increasing soil strength.

Hubick _et_ _al_. (1986) studied the correlation between water use efficiency and carbon isotope discrimination in diverse peanut germplasm. Variation was found in WUE and also isotope discrimination, interspecific variation amongst _Arachis_ species and intraspecific variation amongst _A_. _hypogaea_ cultivars. Variations in WUE and discrimination were strongly correlated.

Working in this specific line Evans _et_ _al_. (1986) have devised a technique for simultaneous measurement of carbon isotope discrimination and gas exchange. They found that isotopic discrimination measured over the short term during gas exchange measurements correlated strongly with that determined from combusted leaf material.

Even in salinity stress the relationship between carbon isotope composition, stomatal conductance and WUE was studied by Guy and Reid (1986) in a $C_3$ halophyte, Puccinellia nuttalliana. The WUE increased from 2.7 to 5.7 mg $CO_2$ g $H_2O^{-1}$ as the solute potential of the growing media was altered from 0 to -1.6 MPa. This was associated with a greater decrease in $g_s$ than photosynthesis. $\delta\,^{13}C$ values of leaf tissues also showed a corresponding positive shift from -27 to -20 parts per milli.

Turner (1986) suggests that this technique has offered a new tool to explore the extent of genetic variation for WUE in dryland crops and assess the value of this trait in adaptation to conditions of limited water availability.

# MATERIAL AND METHODS

# III. MATERIAL AND METHODS

To assess the differences in water use efficiency among the species at different soil moisture regimes and to identify the physiological and gas exchange characteristics associated with water use efficiency, several pot culture experiments were conducted.

All the experiments were conducted at the botanical garden, Department of Crop Physiology, University of Agricultural Sciences, Bangalore. The place is situated at an altitude of 930 m from mean sea level and located at a latitude of 12° 58' north and longitude of 27° 35' east. The average annual rainfall of the area is about 890.9 mm.

## Experiment-I: Differences in water use efficiency in sunflower and amaranthus and the physiological characteristics associated with WUE

This pot culture experiment was conducted during kharif, 1987. The experiment was conducted using battery containers made of carbonised rubber measuring 28 x 14.4 x 12.5 $cm^3$ (L x B x H). The different treatments were;

Species : 1. Sunflower *Helianthus annus* (Hybrid BSH-1)
2. Amaranthus *Amaranthus* sps. (Var.R-104)

Moisture regimes : 1. Control - Pots were watered to field capacity twice a day.

2. Stress - Pots were watered to 60 per cent field capacity twice a day.

Replications : Five pots were maintained for each treatment.

Preparation of the pots

Each pot was filled with 8 kgs of air dry red loamy soil containing sufficient farm yard manure. Twice the recommended fertilizer dose was applied at the time of sowing and one more dose on 28th day after sowing. The recommended fertilizer dosage for both sunflower and amaranthus is 25:30:25 kg NPK acre$^{-1}$. The exact quantity of fertilizers applied was calculated based on soil weight considering that top 6" one acre furrow slice will weigh around 1 million kgs. Since, in a field experiment, the spacing given is wider and the soil volume is higher compared to that in a pot culture experiment, totally thrice the recommended fertilizer dosage was given.

Sowing

About 10 to 12 seeds were sown in each pot for sunflower and about 100 seeds were sown in each pot for amaranthus. Two thinnings were done on 12 DAS and 20 DAS respectively and ultimately two plants were maintained per pot. The pots were watered daily and maintained to field capacity upto 28 DAS.

### Treatment imposition

The experiment was initiated by imposing two stress regimes. The plants were grown at two moisture regimes, one in which pots were watered to FC twice a day and the other where pots were watered to 60 per cent FC twice a day. The treatments were imposed from 28th DAS and continued upto 56th DAS. Thus, the overall duration of the experiment was for 28 days. Before imposing the treatments the pots were brought to field capacity and the amount of water required to bring the soil to field capacity was determined. For red loamy soil, which was used in this experiment, the moisture content at FC is around 20 per cent. After bringing the pots to FC, the drainage holes of the pots were plugged with clay and cement. The soil surface was completely covered with plastic pieces (500 to 700 g) to prevent evaporation losses.

### Schedule of watering

The daily water loss through transpiration was determined in each pot by gravimetric method. The weights of the individual pots with plants were recorded with the help of Salter bench platform type of balance of 20 kg capacity and a resolution of 50 g. The pots were weighed daily to record the amount of water lost through evapotranspiration. After weighing, based on previous computations, water was added to bring back the soil to FC or 60 per cent FC. Though the objective was to maintain the pots at 100 per cent FC and 60 per cent FC it was not possible in the present set up. So, some random pots were weighed in the afternoon to know the depletion of water in the pots by mid day and based on approximations known amount of water was added to all the pots.

Determination of possible evaporation

The evaporation losses were determined (even after covering the soil surface with plastic pieces) by maintaining pots at FC and 60 per cent FC without plants and these losses were deducted from the cumulative water added to experimental pots with plants to arrive at cumulative water transpired.

The following primary observations were recorded during the experimental period.

1. Initial leaf area on 28 DAS and final leaf area on 56 DAS.
2. Initial DM and the final DM.
3. Cumulative water transpired (CWT).

4. Gas exchange parameters using Portable Photosynthesis system
5. Leaf area development and relative leaf expansion rates.
6. Soil moisture determination.

From the above primary values, the following parameters were computed.

1. Leaf area duration (functional leaf area available during the experimental period).
2. Dry matter accumulated during the experimental period.
3. Transpiration quotient (TQ)
4. Water use efficiency (WUE)
5. Rate of water loss (CWT/LAD)
6. Net assimilation rate (NAR)
7. DM/LAD ratios
8. $A/g_s$ ratios.

## Leaf area (LA)

The initial leaf area on the 28th day and the final leaf area on the 56th day after sowing were measured by a destructive method using a LICOR leaf area meter (Model LI-3000) and expressed as $cm^2$ per pot. For determining the initial LA five pots (each with two plants) for each crop were used. On the 56th DAS, the leaf area of all the 10 plants in the remaining five pots were determined and expressed as final leaf area.

### Initial and final DM

The initial and final DM was estimated using the same samples in which initial and final leaf area were estimated. The plant material was oven dried at 80°C for 48 hours. The weights were recorded and expressed as g dry weight per pot (each pot had two plants. Root dry matter was not accounted for in final DM).

### Cumulative water transpired (CWT)

Cumulative water transpired is the total amount of water transpired by the plants during the experimental growth period. The amount of water added both during morning and afternoon to bring it to the respective water regimes was summed up and this minus the estimated evaporation losses was the cumulative water transpired. CWT is expressed as ml/pot.

### Gas exchange parameters using portable photosynthetic system

Using LI-6000 Portable Photosynthesis System, photosynthetic rate (A), stomatal conductances ($g_s$), leaf temperature (LT), relative humidity (RH), photosynthetically active radiation (PAR) were determined for control and stress plants, once before watering and once after watering.

LI-6000 consists of a system console, a $CO_2$ analyser and a sensor housing that can be attached to one of the several different leaf chambers. The LI-6000 is designed to measure simultaneously and rapidly the instantaneous $CO_2$ and water vapour exchange rates of leaves.

The LI-6000 incorporates a transient measurement technique whereby an actively photosynthesising and transpiring leaf, when enclosed in a container, causes the humidity of air in the container to be increased and the $CO_2$ to decrease. The rate at which the humidity and $CO_2$ exchange depend directly upon the stomatal conductance of the leaf and its apparent photosynthetic rate, given the leaf size and the container volume. The rates of change of humidity and $CO_2$ are obtained by making measurements separated by intervals of time. The $CO_2$ measurement is made in a separate gas analyser.

The chamber volume used for both sunflower and amaranthus was of 1073 $cm^3$. Photosynthetic rate was determined in the topmost fully expanded leaf. Observations were recorded between 9.00 to 12.00 AM during cloud free days. Two sets of observations were made. In the first set, the pots were irrigated prior to making observations. In the second set, the observations were recorded prior to watering.

Photosynthetic rate (A) is expressed in $\mu$ mol $m^{-2}$ $s^{-1}$, conductances ($g_s$) in mol $m^{-2}s^{-1}$, LT in $^{o}C$ and PAR in $\mu$ Ein $m^{-2}s^{-1}$.

## Leaf area development and relative leaf expansion rates

To assess the effect of moisture stress on leaf expansion rate, the leaf area development of the plant in different treatments was monitored during the entire period of the experiment by measuring leaf area in situ. The leaf area of the individual leaves was measured once in two days by determining length and breadth of the leaf and computing LA using a factor which was 0.695 for sunflower and 0.730 for amaranthus.

From these primary values it was possible to arrive at the leaf expansion rates (LER) of different leaves of the plant. The LER of just emerging first, fourth and seventh leaves were computed later on.

Relative leaf expansion rate (RLER) is the ratio of the natural logarithm of the successive measurements of total leaf area divided by the time interval between the two measurements. This was computed using the formula given by Masle and Passioura (1987).

$$\text{RLER} = \frac{\text{Log LA}_2 - \text{Log LA}_1}{t_2 - t_1}$$

Where;

$LA_1$ - Leaf area at time $t_1$

$LA_2$ - Leaf area at time $t_2$

$t_2 - t_1$ - Time interval between two successive leaf area measurements

RLER is expressed as $cm^2\ cm^{-2}\ day^{-1}$.

## Soil moisture determinations

Though it was aimed to maintain plants at 100 per cent field capacity and 60 per cent field capacity, the scheduling of watering was such that the plants were subjected to a cycle of stress in a day between two successive waterings. Twice in a day the pots were brought back to the respective water regimes but were never maintained at it. However, the soil moisture content or soil water potential prior to bringing it back to the required moisture regimes may vary between species depending on their water use. The amount of water retained in the pots between successive irrigations may have a significant effect on leaf expansion rates. Keeping this in view the moisture content of the pots prior to early morning watering was computed either as per cent of field capacity or per cent of soil dry weight.

<u>Leaf area duration (LAD)</u>

The LAD is a reflection of the functional leaf area available for assimilation during the active growth period. LAD for container studies is calculated using the formula given by Hunt (1983).

$$\text{LAD} = \frac{L_1 + L_2}{2} \text{ X } t_2 - t_1$$

Where;

$L_1$ - Initial leaf area at time $t_1$

$L_2$ - Final leaf area at time $t_2$

$t_2$-$t_1$ - Duration in days between initial and final samplings (28 days).

<u>DM accumulation ($\Delta$ DM)</u>

Total dry matter accumulation during the experimental period was computed as the difference between the final DM and initial DM and expressed as g dry weight per pot.

## Water use efficiency (WUE)

Water use efficiency is defined as the amount of dry matter produced per unit amount of water transpired.

WUE was estimated as the ratio of the dry matter accumulated during the duration of the experiment (28 DAS to 56 DAS) to that of the cumulative water transpired during the same period and expressed as g DM $kg^{-1} H_2O$.

## Transpiration quotient (TQ)

Transpiration quotient is the ml of water required for one g DM production. The transpiration quotient was estimated as per the method of Ludlow (1972).

$$TQ = \frac{\text{Cumulative water transpired (ml/pot) during the experimental growth period}}{\text{Total dry matter produced during the same period (g per pot)}}$$

## Rate of water loss

The ratio of cumulative water transpired (CWT) and the functional leaf area (LAD) is a reflection of the rate of water loss or measures mean transpiration rate. This was calculated by the formula.

$$\text{Mean transpiration rate} = \frac{\text{Cumulative water transpired (CWT)}}{\text{Functional leaf area (LAD)}}$$

and expressed as ml $H_2O$ $dm^{-2}$ day.

## Net assimilation rate (NAR)

It is the rate of increase in dry weight per unit leaf area and is expressed as g $dm^{-2}$ $d^{-1}$. It was estimated by using the formula given by Gregory (1926).

$$NAR = \frac{\text{Log } LA_2 - \text{Log } LA_1}{LA_2 - LA_1} \times \frac{W_2 - W_1}{t_2 - t_1}$$

Where;

$LA_1$ - Leaf area at time $t_1$

$LA_2$ - Leaf area at time $t_2$

$W_1$ - Dry matter at time $t_1$

$W_2$ - Dry matter at time $t_2$

$t_2$-$t_1$ - Duration in days between the first and second sampling.

## DM/LAD

This ratio is an indication of the assimilation capacity per unit leaf area. In other words, it is an indirect reflection of net assimilation rate. This was computed using the dry matter accumulated during the experimental period and the functional leaf area of that period.

$$DM/LAD = \frac{\text{Final DM-Initial DM}}{LAD}$$

and expressed as g $dm^{-2}$ day.

## $A/g_s$ ratio

The $A/g_s$ ratio i.e., assimilation rate per unit conductance is an indication of intrinsic water use efficiency measured at the single leaf level. The $A/g_s$ ratio was determined based on the instantaneous measurements of assimilation rate and stomatal conductance.

## Experiment-II: Differences in water use efficiency in selected $C_3$ and $C_4$ species at a given level of moisture input:

The intrinsic differences in water use efficiency is generally assessed by maintaining the plants at a particular moisture regime. However, under the field conditions (rainfed situations) the input of water by precipitation will remain same for all the species. So, it is more appropriate to assess differences in water use efficiency amongst different species at a given input of water. Keeping this in view another pot culture experiment was conducted with three selected $C_3$ species and three selected $C_4$ species. All the crops were supplied with a similar input of water and differences in water use efficiency were estimated under two moisture regimes. The treatments used were;

Species : 1. Sunflower, Helianthus annus (Hybrid BSH-1)
2. Amaranthus, Amaranthus sps. (Var.R-104)

3. Cowpea, *Vigna unguiculata* (Var.C-152)
4. Ragi, *Eleusine coracana* (Var.JNR-852)
5. Sorghum, *Sorghum vulgare* (Var.CSH-5)
6. Soybean, *Glycine max*. (Var.Hardee)

Moisture: regimes
1. Moisture regime-1 - Pots were watered to near field capacity twice a day.
2. Moisture regime-2 - Watered with 60 per cent of the water applied under moisture regime-1.

Replications: Eight pots were maintained for each treatment.

The procedure adopted for preparation of the pots, raising of the plant material and fertilizer application was similar as in the first experiment.

Ragi was sown a week in advance of other crops to achieve similar leaf area and growth as that of other crops by the time of treatment imposition. Only one plant per pot was maintained in case of sunflower and amaranthus whereas two plants were maintained per pot in case of the remaining four crops. Here also, evaporation was minimised by mulching the soil surface with plastic pieces but no effort was made to delineate the possible evaporation losses from soil surface from that of actual transpiration as was done in the first experiment. The experimental growth period for sunflower and amaranthus was 26 days (31 DAS to 57 DAS), 27 days (31 DAS to 58 DAS) for cowpea, 26 days (38 DAS to 64 DAS) for ragi, 29 days (31 to 60 DAS) for soybean and 30 days for sorghum (31 to 60 DAS).

Moisture regimes

Based on the results obtained in the first experiment and also the experiments carried out earlier in the Department, the amount of water required to maintain plants at around near field capacity was approximated. Care was taken to see that there was no water logging especially in $C_4$ plants. The amount of water required for cowpea to maintain field capacity was given to all the species under moisture regime-1, 60 per cent of that was given for the second moisture regime. The plants were irrigated twice in a day to the respective moisture regimes.

In the initial stages the water required for sorghum, cowpea and soybean was less and hence to give the same total input of water the duration of the crop growth period was extended by a day for cowpea, three days for soybean and four days for sorghum.

The following observations were recorded during the experimental period.

1. Initial leaf area and the final leaf area
2. Initial and final shoot DM
3. Final root dry matter
4. Final total dry matter
5. Cumulative water added
6. Gas exchange parametefs using portable photosynthesis system.

From the above primary values the following parameters were computed.

1. Leaf area duration
2. Shoot dry matter accumulation ( Δ SDM) and total dry matter accumulation ( Δ TDM) during the experimental period
3. Transpiration quotient (TQ)
4. Water use efficiency (WUE)
5. Rate of water loss ($CW^A$/LAD)
6. Net assimilation rate (NAR)
7. DM/LAD ratios
8. Root to shoot ratios and root DM as a per cent of TDM
9. Root weight to leaf area ratio
10. Assimilation to conductance ratios ($A/g_s$ ratios).

### Root DM

Root system was extracted from the soil by forcing a jet of water into the pots to get rid of soil. Then the roots were dried at 80°C for 48 hours. Root DM was expressed as g per pot.

### Final total DM

Final TDM was estimated as the sum of the final above ground DM (shoot DM) and below ground DM (root DM) and expressed as g per pot.

### Cumulative water added (CWA)

This was estimated as the total amount of water added to the pots during the experimental growth period to maintain the pots either at near field capacity or 60 per cent of the water supplied to control regimes. Since the input of water was uniform for all crops, the CWA under a given moisture regime was same to all the crops used in this experiment.

### DM accumulation ( $\Delta$ DM)

Shoot DM ( $\Delta$ SDM) accumulation was calculated as the difference between the final SDM and initial SDM.

Total DM accumulation ( $\Delta$ TDM) was calculated as the difference between the final TDM and initial SDM. Here initial root DM has been neglected. DM accumulation is expressed as g per pot.

## Root to shoot ratios

This was calculated as the ratio of root DM to shoot DM and expressed as mg root DM per g SDM.

Root DM as a per cent of TDM was calculated using the relation,

$$\text{Per cent root DM} = \frac{\text{Root DM (g)}}{\text{TDM (g)}} \times 100$$

## Root weight to leaf area ratio

This ratio gives an indication of the amount of root DM put forth by a particular species to meet the transpirational demand of its unit leaf area. This was estimated as the ratio of the root DM to leaf area on the final day of the experimental period and expressed as mg root DM $dm^{-2}$ leaf area.

Remaining observations were recorded as in the first experiment. However, TQ, WUE, NAR and DM/LAD were determined separately based on $\Delta$ SDM alone and also based on $\Delta$ TDM.

## Experiment-III: Effect of moisture stress on gas exchange characteristics in sunflower and amaranthus:

Objective of this experiment was to see the effect of moisture stress on gas exchange characteristics like assimilation rate (A), stomatal conductance ($g_s$) and intercellular $CO_2$ concentration (Ci). It was also aimed at the possibility of arriving at the threshold leaf water potential for gas exchange characteristics.

Plant material was raised separately for this experiment just the same way as described in first experiment. When the plants were 40 days old, 12 pots (two plants per pot) each were selected for sunflower and amaranthus with uniform growth and development. The plants were subjected to the following four treatments. In each treatment there were three pots.

| | | |
|---|---|---|
| Control | - | Pots were watered to FC twice a day |
| Stress-1 | - | Pots were irrigated daily twice with 75 per cent of water given to control pots. |
| Stress-2 | - | Pots were irrigated twice daily with 50 per cent of water given to control pots. |
| Stress-3 | - | Pots were irrigated daily twice with 25 per cent of water given to control pots. |

Water lost by the control plants was estimated gravimetrically. The various moisture regimes were imposed for two days and on the third day the following two observations were made.

i) Photosynthetic rate and stomatal conductance.
ii) Leaf water potential (LWP).

## Photosynthetic rate and stomatal conductance

Top most fully expanded leaves were used to record the photosynthetic rate and stomatal conductance in all the treatments. 'A' and $g_s$ were recorded using LI-6000 portable photosynthesis system. In the same leaves water potential was also recorded using psychrometer (water potential data system HP-115).

## Leaf water potential (LWP)

Leaf water potential was estimated using a HP-115 water potential data system, in the dew point mode. Leaf punches were taken from the same leaves in which observations were made for 'A' and $g_s$. These leaf punches were brought to the laboratory in saturated petridishes to avoid any water loss during transit. LWP has been expressed in Megapascals.

## Intercellular $CO_2$ concentration (Ci)

Intercellular $CO_2$ concentration (Ci) was calculated using ambient $CO_2$ concentration (Ca), assimilation rate(A) and conductance ($g_s$).

$$Ci = Ca - 1.6 \frac{A}{g_s}$$

Where;

Ca and Ci are ambient and intercellular $CO_2$ concentrations expressed in parts per million.

A = Assimilation rate ($\mu$ mol $m^{-2}$ $s^{-1}$)

$g_s$ = Conductance (mol $m^{-2}$ $s^{-1}$)

1.6 = The ratio of diffusivities of water vaoupr and $CO_2$.

Experiment-IV: Shade effect on the extent of stomatal closure in three selected $C_3$ species and three selected $C_4$ species

The light, $CO_2$, VPD and tissue water potential affect directly or indirectly the stomatal conductance($g_s$). The threshold level for an environmental factor for decreasing stomatal conductance or closing the stomata may vary in different species. Often the species which show a higher threshold for a particular environmental factor for a decrease in $g_s$ are termed as sensitive crops for stomatal behaviour. The sensitivity of stomata can also be assessed by determining the rate of closure of stomata on transfer to low light or high $CO_2$ concentration. So an attempt was made in this study to assess the relative sensitivity of these species to light with regards to their stomatal behaviour.

## Imposition of light treatments

The experiment was conducted on a day when the plants were continuously exposed to full sunlight for a period of three hours; such plants were transferred to shade with a light intensity of 50 ± 25 $\mu$ Ein $m^{-2}s^{-1}$ and on transfer the stomatal conductance was monitored as a measure of sensitivity.

## Measurement of stomatal resistance ($r_s$)

Stomatal resistance ($r_s$) was measured in the topmost expanded leaf in all the treatments before transfering to shade and at regular intervals after transfering to shade (low light). The $r_s$ was measured 10 minutes and 40 minutes after transfering to low light. Stomatal resistances were determined using a portable steady state porometer (LICOR-1600 ).

The LI-1600 steady state porometer is a battery operated portable instrument designed for rapid and precise measurements of diffusive resistances. The LI-1600 consists of two parts, the readout control console and the sensor head (encloses the sampling cuvette). Several interchangeable aperture caps can be used to facilitate different sizes of leaves. A matching leaf clamp is attached to hold the sample in place over the cuvette.

A sample is placed onto the cuvette aperture and held in place by a clamping action. The relative humidity of the cuvette, in contact with the sample, is maintained at the ambient level. The diffusive resistance for water loss is determined under these conditions by the instrument.

# EXPERIMENTAL RESULTS

# IV. EXPERIMENTAL RESULTS

To assess the differences in water use efficiency (WUE) among and between selected $C_3$ and $C_4$ species at different soil moisture regimes and to identify the physiological and gas exchange parameters associated with water use efficiency, in all, four pot culture experiments were conducted in this study.

## Experiment-I: Differences in water use efficiency in sunflower and amaranthus and the physiological characteristics associated with WUE

The major objective of this experiment was to determine the relative differences in water use efficiency in sunflower and amaranthus under both stressed and nonstressed conditions. This was done using a gravimetric method in both control and stress plants. During the experimental period, each crop species was grown at two moisture regimes, one in which pots were watered to field capacity (FC) twice a day and in the other pots were watered to 60 per cent FC twice a day. In this experiment two plants were maintained per pot.

The experiment was carried out from 28 DAS to 56 DAS using plants grown in battery containers. During this period the dry matter produced ( $\Delta$ DM) and cumulative water transpired (CWT) were recorded for the purpose of computing transpiration quotient (TQ). Alongside, leaf area and leaf expansion rates were also recorded. From the above, several growth characteristics were computed. Gas exchange measurements were also made.

## Transpiration quotient and growth characteristics

### Leaf area, leaf area duration, dry matter accumulation and cumulative water transpired

The data on leaf area, LAD, DM and CWT are presented in Table-1. Leaf area and DM determinations were made on 28th DAS and also at 50th DAS.

### Leaf area (LA)

Initial leaf area was recorded on 28th DAS. It was found to be 793 $cm^2$ for sunflower and 571 $cm^2$ for amaranthus.

Final leaf area was recorded on 56th DAS. Amaranthus produced significantly higher leaf area than sunflower under both stressed and nonstressed conditions. Amaranthus recorded final leaf areas of 6985.55 $cm^2$ and 5990.06 $cm^2$ whereas sunflower recorded 5000.52 $cm^2$ and 3294.76 $cm^2$ under nonstressed and stressed conditions respectively. Thus, the per cent reduction in leaf area under stress was higher in sunflower (34.11%) compared to amaranthus (14.25%).

### Leaf area duration (LAD)

LAD is the total functional leaf area available for assimilation or transpiration during the experimental period.

**Table 1.** Leaf area, LAD, dry matter and **cumulative water transpired in sunflower and amaranthus** during the experimental period.

| Species | Moisture regime | Initial LA (cm$^2$) | Final LA (cm$^2$) | LAD (dm$^2$ days) | Initial DM (g) | Final DM (g) | $\Delta$DM (g) | CWT (ml) |
|---|---|---|---|---|---|---|---|---|
| Sunflower | Control | 793 | 5000.52 | 811.11 | 4.08 | 65.35 | 61.27 | 26920 |
| | Stress | 793 | 3294.76 | 577.34 | 4.08 | 43.70 | 39.62 | 16755 |
| | | | (34.11) | (28.82) | | (33.13) | (35.34) | (37.76) |
| Amaranthus | Control | 571 | 6985.55 | 1057.86 | 4.68 | 69.82 | 65.14 | 18370 |
| | Stress | 571 | 5990.06 | 904.02 | 4.68 | 53.41 | 48.73 | 12450 |
| | | | (14.25) | (14.55) | | (23.15) | (25.19) | (32.23) |
| CD (P=0.05) | S | - | 598.02 | 91.49 | - | 6.04 | 6.04 | 2375 |
| | M | - | 598.02 | 91.49 | - | 6.04 | 6.04 | 2375 |
| | S x M | - | 845.72 | 129.39 | - | 8.54 | 8.54 | 3359 |

Control - Pots watered to FC twice a day; Stress- Pots watered to 60 per cent FC twice a day.

Note - All values are expressed per pot.
Values in paranthesis indicate per cent change from control.

S - Species
M - Moisture regime.

The LAD in amaranthus was significantly higher than in sunflower under both moisture regimes. The LAD values for amaranthus were 1057.86 $dm^2$ days in plants grown at FC and 904.02 $dm^2$ days for plants grown at 60 per cent FC. Corresponding values for sunflower were 811.11 $dm^2$ days and 577.34 $dm^2$ days respectively. The reduction in LAD under stress was 28.82 per cent in sunflower but only 14.55 per cent in amaranthus.

## Dry matter accumulation (DMA)

The initial dry matter determined on 28th DAS was 4.08 g in sunflower and 4.68 g in amaranthus. Final DM was recorded on 56th DAS. There was no significant difference in DM produced under control but under stress, amaranthus produced significantly higher DM than sunflower. The values were 69.82 g and 53.41 g in amaranthus for control and stress conditions. Corresponding values for sunflower were 65.35 g and 43.7 g. Again as in the case of final leaf area and LAD, the per cent reduction in final DM was less in amaranthus.

The $\Delta$ DM (dry matter accumulated during the duration of the experiment), computed from the initial and final dry matter, showed a trend similar to that of final dry matter. The per cent reduction under stress was 35.34 in sunflower and 25.19 in amaranthus.

### Cumulative water transpired (CWT)

Cumulative water transpired is the amount of total water transpired by the plants during the entire duration of the experiment (28 DAS to 56 DAS). This was determined employing a gravimetric technique, giving appropriate corrections for possible evaporative losses.

Under both stressed and nonstressed conditions sunflower recorded higher CWT than amaranthus. But per cent reduction in CWT under stress was less in amaranthus (32.23%) compared to sunflower (37.76%).

### TQ, WUE, NAR, DM/LAD ratio and rate of water loss (CWT/LAD)

### TQ and WUE

TQ is the amount of water expended in the production of unit amount of dry matter. Lower the TQ, higher the intrinsic ability of a species to produce unit amount of dry matter with less water use. In other words, lower the TQ, higher is the WUE. The TQ is computed from the data on dry matter produced and CWT during the experimental period.

The TQ values showed significant differences between the two species but within each species the difference in TQ between the two moisture regimes was found to be

Table 2. Transpiration quotient (TQ), water use efficiency (WUE), net assimilation rate (NAR), DM/LAD ratio and rate of water loss (CWT/LAD) in sunflower and amaranthus.

| Species | Moisture regime | TQ (ml g$^{-1}$) | WUE (g DM kg $H_2O^{-1}$) | NAR (mg dm$^{-2}$ d$^{-1}$) | DM/LAD (mg dm$^{-2}$d) | CWT/LAD (ml dm$^{-2}$d) |
|---|---|---|---|---|---|---|
| Sunflower | Control | 439.97 | 2.2729 | 96 | 75.47 | 33.67 |
|  | Stress | 424.27 | 2.3570 | 80 | 68.88 | 29.15 |
|  |  | ( 3.57) | (3.70) | (16.67) | (8.73) | (13.42) |
| Amaranthus | Control | 283.12 | 3.5321 | 91 | 61.58 | 17.40 |
|  | Stress | 257.29 | 3.8867 | 76 | 54.24 | 13.99 |
|  |  | ( 9.12) | (10.03) | (16.48) | (11.92) | (19.60) |
| CD (P=0.05) | S | 21.59 | 0.20 | 5.98 | 4.97 | 2.06 |
|  | M | 21.59 | 0.20 | 5.99 | 4.97 | 2.06 |
|  | S x M | 30.54 | 0.28 | 8.47 | 7.03 | 2.92 |

nonsignificant (Table-2). Amaranthus recorded lower TQ values than sunflower under both stressed and nonstressed conditions. TQ values were 439.97 and 424.27 at FC and 60 per cent FC for sunflower whereas the corresponding values were 283.12 and 257.29 for amaranthus. So, per cent reduction in TQ under stress was higher in amaranthus (9.12%) than in sunflower (3.57%).

WUE, is an expression, just the reciprocal of TQ. So, the relative differences in WUE in the two species were similar to that of TQ.

Net assimilation rate (NAR)

Net assimilation rate is the amount of dry matter produced per unit functional leaf area per unit time. This is a reflection of the photosynthetic efficiency of the species if the differences in dark respiration are not marked. NAR was computed based on $\Delta$DM, initial LA and final leaf areas recorded during the experiment. The NAR values for sunflower were 96 mg dm$^{-2}$ day$^{-1}$ and 80 mg dm$^{-2}$ day$^{-1}$ in control and stress, respectively. The values for amaranthus were 91 and 76 for the same moisture regimes. Both sunflower (16.67%) and amaranthus (16.48%) showed similar reductions in NAR when plants were grown at 60 per cent field capacity.

## DM/LAD ratio

It is the amount of DM produced per unit functional leaf area. This again, like NAR, is a direct reflection of the assimilation capacity of the leaf. This was computed based on the data recorded on $\Delta$DM and LAD. In plants grown at both FC and 60 per cent FC, DM/LAD ratio was greater in sunflower. Also, sunflower showed a lesser reduction (8.73%) under stress as compared to amaranthus (11.92%).

## Rate of water loss (CWT/LAD)

This reflects the mean transpiration rate per unit functional leaf area for the entire growth period. Rate of water loss was computed from the data on CWT and LAD. Rate of water loss in sunflower and amaranthus differed significantly under both control and stress conditions. Very high rates of water loss were observed in sunflower. The percentage reduction in CWT/LAD under stress was higher in amaranthus (19.6%) than sunflower (13.42%).

The results obtained on different growth characteristics indicate that:

Gravimetric technique (based on CWT and DM) is an appropriate technique to determine the TQ differences in different species.

There was a distinct species variation in TQ. Though the CWT was less in amaranthus, DM produced was more resulting in high WUE in amaranthus.

In both the species, under stress, the TQ decreased. However, the per cent decrease in TQ was more in amaranthus compared to sunflower. Lesser decrease in TQ or a greater increase in WUE under stress in amaranthus was associated with a greater per cent reduction in rate of water loss in amaranthus, as compared to sunflower. As the NAR recorded showed similar reductions for both the species under stress, a greater reduction in CWT/LAD alone contributed for a substantial increase in WUE under stress in amaranthus.

Reduction in DM was less in amaranthus under stress compared to sunflower. Percentage NAR decrease under stress was similar in both the species. But LAD decreased to a lesser extent in amaranthus possibly due to maintenance of higher soil moisture content throughout the day and this alone was responsible for lesser reduction in DM under stress in amaranthus.

Sunflower showed higher NAR and DM/LAD both under control and stress conditions. But NAR and DM/LAD decreased under stress in both the species indicating that photosynthetic efficiency decreased along with decrease in leaf area duration under stress.

Similarly, CWT decreased under stress in both the species. Lesser percentage decrease observed in amaranthus was associated with a greater per cent of control LAD maintained in amaranthus under stress (85.45%) than in sunflower (71.18%).

## Gas exchange characteristics

### Differences in photosynthetic rate(A), conductance ($g_s$) and assimilation to conductance ratio ($A/g_s$) in sunflower and amaranthus grown at FC and 60 per cent FC

An attempt was made in this experiment to determine the differences in gas exchange characteristics, assimilation rate and stomatal conductance in sunflower and amaranthus and also to study the behaviour of these parameters under stress. Intrinsic water use efficiency ($A/g_s$) was computed from A and $g_s$ with an objective to compare it with the observed TQ values in the two species.

Every day in the morning the pots were weighed and required amount of water was added to each pot to bring back to FC or 60 per cent FC. The amount of water to be added however, varied between these crops both in the control and stressed plants mainly because of differences in the amount of water transpired per day. The amount of water required to bring the pots to any one moisture regime was always less in amaranthus because they maintained high soil moisture content. This suggests that in amaranthus grown pots the soil moisture was maintained high throughout the day compared to sunflower. This in turn might have facilitated higher plant tissue water status in amaranthus. Moreover in this experiment the watering schedule is such that the plants are subjected to a cycle of stress in between two successive waterings. Hence, to see if this had any bearing on the gas exchange characteristics in amaranthus and to what extent it did influence the observed lesser decrease in DM in amaranthus under stress, observations were made prior to watering and also one hour after watering.

'A' and $g_s$ were measured using a portable photosynthesis system, LI-6000. The data on 'A' and $g_s$ and also the environmental conditions under which the measurements were made are given in Table-3.

Observations recorded before watering indicate 'A' values of 28.04 and 18.18 for sunflower and amaranthus in control and 22.04 and 18.34 in stress. The corresponding conductance values were 0.4321 and 0.1627 in control and 0.3898 and 0.2051 in stress. $A/g_s$ values computed from 'A' and $g_s$ were 66.02 and 56.53 for sunflower in control and stress respectively whereas amaranthus recorded correspondingly, values of 112.23 and 89.14.

**Table 3.** Gas exchange parameters recorded before and after watering in sunflower and amaranthus grown at two different moisture regimes.

(Values are means of 3 to 6 replications)

| | Before watering (47 DAS) | | | | After watering (49 DAS) | | | |
|---|---|---|---|---|---|---|---|---|
| | Sunflower | | Amaranthus | | Sunflower | | Amaranthus | |
| | Control | Stress | Control | Stress | Control | Stress | Control | Stress |
| **A** | 28.04 | 22.04<br>(21.39) | 18.18 | 18.34<br>(0.88) | 25.75 | 23.17<br>(10.01) | 22.21 | 23.05<br>( 3.78) |
| $g_s$ | 0.4321 | 0.3898<br>(9.79) | 0.1627 | 0.2051<br>(26.06) | 0.5477 | 0.4865<br>(11.17) | 0.1599 | 0.1359<br>(15.01) |
| LT | 31.2 | 31.7 | 31.7 | 30.6 | 31.2 | 31.7 | 31.7 | 30.6 |
| RH | 68.7 | 62.4 | 56.8 | 53.8 | 71.9 | 70.2 | 66.3 | 67.3 |
| $CO_2$ | 320.8 | 316.3 | 317.8 | 310.5 | 326.3 | 324.3 | 332.0 | 323.5 |
| PAR | 633.1 | 706.4 | 647.8 | 761.1 | 786.9 | 944.0 | 819.8 | 950.6 |
| $A/g_s$ | 66.02 | 56.53<br>(14.37) | 112.23 | 89.14<br>(20.57) | 47.0 | 47.69<br>(1.46) | 142.22 | 172.22<br>(21.8) |

A - Photosynthetic rate - μ moles $m^{-2}s^{-1}$
$g_s$ - Stomatal conductance - moles $m^{-2}s^{-1}$
LT - Leaf temperature - $^{o}C$
RH - Relative humidity - %
PAR - Photosynthetically active - μ Ein $m^{-2}s^{-1}$
$CO_2$ - Chamber $CO_2$ concentration - ppm
$A/g_s$ - Assimilation to conductance ratio - moles $CO_2$ $mole^{-1}$ $H_2O$.

Observations made after watering indicated 'A' values of 25.75 and 23.17 in control and stress for sunflower whereas they were 22.21 and 23.05 in amaranthus. Sunflower recorded intrinsically high conductances than amaranthus under both the moisture regimes. Amaranthus recorded $A/g_s$ values of 142.22 and 173.22 under control and stress as compared to 47.00 and 47.69 recorded by sunflower. Thus, amaranthus recorded a greater per cent increase (21.80) in $A/g_s$ under stress than sunflower (1.46).

The results obtained on gas exchange characteristics indicate that;

In general, sunflower had higher A and $g_s$ both under control and stress. In this experiment in control amaranthus plants, upper leaves showed some yellowing during the later stages of growth. Hence, amaranthus recorded marginally higher A values under stress.

$A/g_s$ recorded both before and after watering showed a greater value for amaranthus than sunflower. $A/g_s$ values recorded before watering were higher than those recorded after watering in both control and stress for sunflower but amaranthus showed a reverse trend.

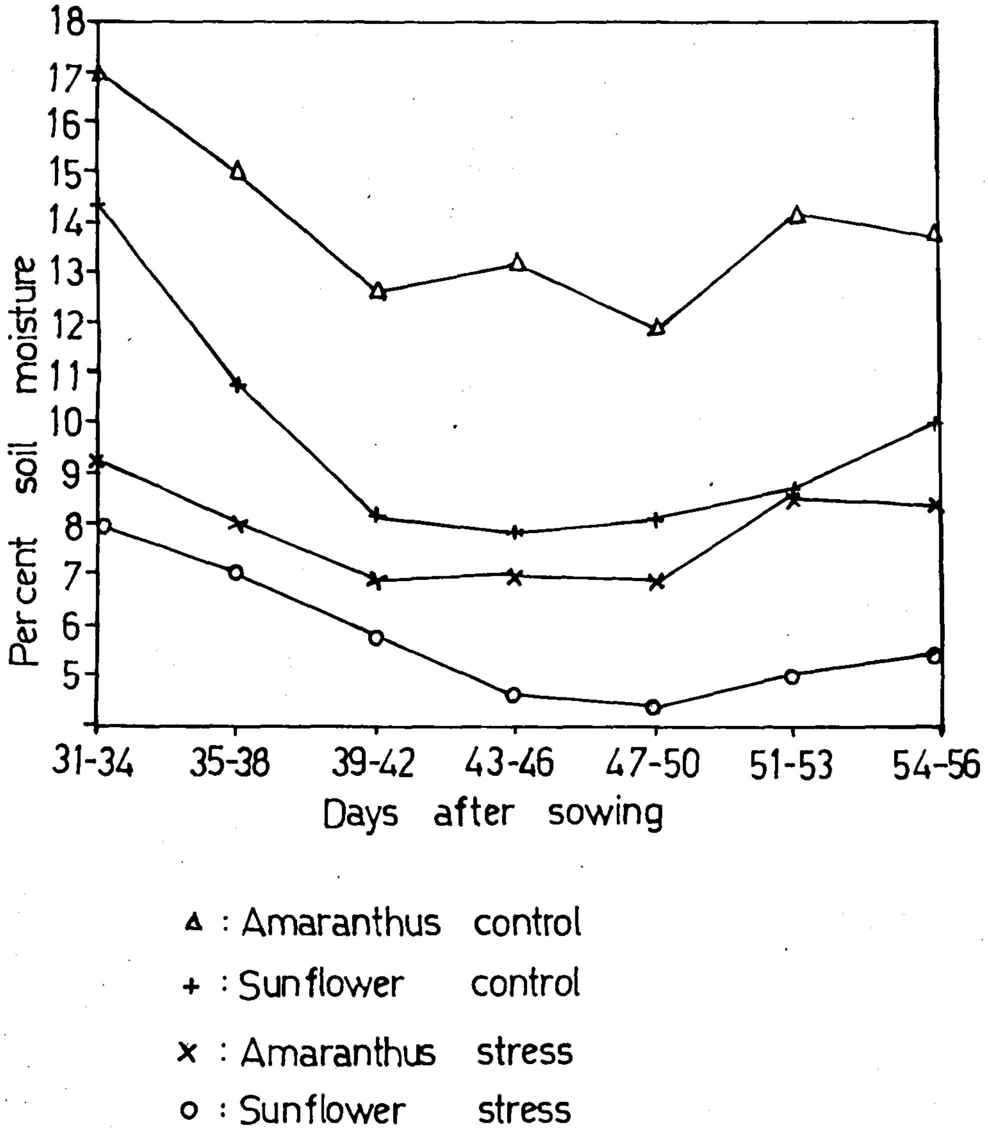

Fig. 1. Soil moisture content in sunflower and amaranthus during the experimental period prior to bringing the pots to respective soil moisture regimes.

Amaranthus showed a greater per cent increase in $A/g_s$ under stress than sunflower but observations recorded before watering showed a greater per cent decrease (20.56) in $A/g_s$ for amaranthus under stress than sunflower (14.37).

Higher $A/g_s$ recorded in amaranthus can be related with higher water use efficiency recorded in amaranthus.

## Soil moisture determination

Soil moisture content in different treatments prior to early morning watering was computed either as per cent of FC or per cent of soil dry weight and this prewatering soil moisture content in sunflower and amaranthus over the entire duration of the experiment has been presented in Fig.1, and also Appendix-1. The determinations were made to find out if the amount of water retained in the pots between two successive irrigations did have a significant effect on leaf expansion rates observed in the two species.

In general amaranthus grown pots maintained higher soil water content than sunflower grown pots. The mean soil moisture content over the entire duration of the experiment was 13.98 and 9.71 (per cent of soil dry weight) for amaranthus and sunflower under control moisture regime whereas the corresponding values were 7.81 and 5.78 under stress moisture regime. The same was reflected over different stages of the experiment.

## Leaf area development and relative leaf expansion rates (RLER)

Leaf expansion is very sensitive to decrease in water potential. Maintenance of leaf tissue water status is therefore essential for its expansion rates. To assess the differences in leaf expansion rates in sunflower and amaranthus at different moisture regimes, the leaf area development of the plant in different treatments was monitored once in two days over the entire duration of the experiment by measuring leaf area in situ. The leaf area of individual leaves was measured and from these primary values the leaf expansion rates of different leaves of the plant were computed. The RLER of just emerging first, fourth and seventh leaves were computed later on.

The absolute leaf area development in the two species showed wide variation. There was not much difference in leaf area development in the two species at FC. However, there was a greater reduction in leaf area development in 60 per cent FC from control in sunflower than amaranthus during any stage of the experiment. For example, on the 14th day after treatment imposition sunflower under stress exhibited a 45.80 per cent reduction in LA whereas amaranthus exhibited only 13.38 per cent reduction. The above pattern of leaf area

Table 4 . Leaf area development and **relative leaf expansion rates (RLER) in sunflower and amaranthus grown at two different moisture regimes.**

| Species | Moisture regime | Days after treatment imposition | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2 | 4 | 6 | 8 | 10 | 12 | 14 | 16 | 18 | 20 | 22 | 24 | 26 |
| Leaf area (cm$^2$ plant$^{-1}$): | | | | | | | | | | | | | | |
| Sunflower | FC | 82.03 | 256.24 | 460.61 | 708.5 | 994.99 | 1256.14 | 1478.42 | 1652.16 | 1819.64 | 1998.64 | 2146.38 | 2212.63 | 2278.72 |
| | 60%FC | 73.99 | 161.04 | 296.25 | 422.6 | 584.04 | 691.23 | 801.29 | 897.03 | 973.86 | 1048.69 | 1093.37 | 1156.52 | 1210.84 |
| | | (9.8) | (37.19) | (35.68) | (40.35) | (41.30) | (44.93) | (45.80) | (45.71) | (46.48) | (47.53) | (49.06) | (47.75) | (46.86) |
| **Amaranthus** | FC | 50.97 | 170.67 | 356.33 | 630.3 | 912.99 | 1194.43 | 1423.55 | 1638.79 | 1865.49 | 2096.58 | 2218.38 | 2288.29 | 2351.32 |
| | 60% FC | 49.46 | 160.22 | 344.78 | 601.7 | 802.75 | 1043.01 | 1233.05 | 1393.41 | 1610.67 | 1804.19 | 1899.68 | 2007.76 | 2103.68 |
| | | (2.96) | (6.12) | (3.24) | (4.54) | (12.07) | (12.68) | (13.38) | (17.60) | (13.66) | (13.95) | (14.37) | (12.26) | (10.53) |

| | | Days after treatment imposition | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 2-4 | 4-6 | 6-8 | 8-10 | 10-12 | 12-14 | 14-16 | 16-18 | 18-20 |
| RLER (cm$^2$.cm$^{-2}$.day$^{-1}$): | | | | | | | | | | |
| Sunflower | FC | 0.6950 | 0.2932 | 0.2153 | 0.1698 | 0.1166 | 0.0815 | 0.0555 | 0.0483 | 0.0469 |
| | 60% FC | 0.3888 | 0.3048 | 0.1785 | 0.1618 | 0.0849 | 0.0735 | 0.0565 | 0.0411 | 0.0370 |
| Amaranthus | FC | 0.6043 | 0.3681 | 0.2852 | 0.1853 | 0.1344 | 0.0877 | 0.0704 | 0.0648 | 0.0584 |
| | 60% FC | 0.5877 | 0.3832 | 0.2785 | 0.1441 | 0.1309 | 0.0837 | 0.0612 | 0.0725 | 0.0568 |

Note: Values in paranthesis represent per cent reduction from control(FC).

**Table 5(a).** Leaf area development and RLER in just emerging first leaf in sunflower and amaranthus.

| Species | Moisture regime | Days after treatment imposition | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 2 | 4 | 6 | 8 | 10 | 12 | 14 |
| Leaf area ($cm^2$): | | | | | | | | |
| Sunflower | FC | 32.97 | 62.87 | 89.96 | 106.47 | 122.72 | 129.64 | 131.86 |
| | 60% FC | 28.69 | 46.89 | 58.07 | 67.56 | 75.71 | 81.27 | 82.83 |
| | | (12.98) | (25.42) | (35.46) | (36.55) | (38.31) | (37.31) | (37.18) |
| Amaranthus | FC | 26.96 | 58.28 | 86.10 | 112.79 | 130.45 | 140.99 | 143.83 |
| | 60% FC | 24.34 | 54.70 | 77.46 | 95.97 | 107.14 | 112.23 | 114.93 |
| | | (9.71) | (6.14) | (10.03) | (14.91) | (17.87) | (20.39) | (20.09) |

| | | Days after treatment imposition | | | | | |
|---|---|---|---|---|---|---|---|
| | | 2-4 | 4-6 | 6-8 | 8-10 | 10-12 | 12-14 |
| RLER ($cm^2\ cm^{-2}\ day^{-1}$): | | | | | | | |
| Sunflower | FC | 0.3228 | 0.1792 | 0.0843 | 0.0710 | 0.0275 | 0.0085 |
| | 60% FC | 0.2457 | 0.1058 | 0.0758 | 0.0569 | 0.0354 | 0.0095 |
| Amaranthus | FC | 0.3855 | 0.1952 | 0.1350 | 0.0728 | 0.0389 | 0.0099 |
| | 60% FC | 0.4048 | 0.1740 | 0.1072 | 0.0551 | 0.0232 | 0.0119 |

Table 5(b). Leaf area development and RLER in just emerging fourth leaf in sunflower and amaranthus.

| Species | Moisture regime | Days after treatment imposition | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 6 | 8 | 10 | 12 | 14 | 16 | 18 | 20 |
| **Leaf area ($cm^2$):** | | | | | | | | | |
| Sunflower | FC | 35.25 | 61.72 | 89.52 | 106.93 | 121.13 | 130.84 | 138.37 | 146.94 |
| | 60% FC | 24.16 | 37.39 | 55.04 | 64.09 | 69.67 | 74.21 | 79.76 | 83.98 |
| | | (31.46) | (39.41) | (38.52) | (40.06) | (42.78) | (43.28) | (42.36) | (42.85) |
| Amaranthus | FC | 31.88 | 69.99 | 99;71 | 120.64 | 141.16 | 151.34 | 163.55 | 170.12 |
| | 60% FC | 30.79 | 64.10 | 88.52 | 104.31 | 116.45 | 128.54 | 141.94 | 146.69 |
| | | (3.42) | (8.42) | (11.22) | (13.54) | (17.50) | (15.05) | (13.21) | (13.77) |

| | | Days after treatment imposition | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **RLER ($cm^2$ $cm^{-2}$ $day^{-1}$):** | | | | | | | | |
| Sunflower | FC | 0.2801 | 0.1860 | 0.0889 | 0.0624 | 0.0386 | 0.0280 | 0.0301 |
| | 60% FC | 0.2184 | 0.1934 | 0.0761 | 0.0419 | 0.0316 | 0.0361 | 0.0258 |
| Amaranthus | FC | 0.3932 | 0.1770 | 0.0953 | 0.0786 | 0.0348 | 0.0388 | 0.0197 |
| | 60% FC | 0.3666 | 0.1614 | 0.0821 | 0.0551 | 0.0494 | 0.0496 | 0.0165 |

Table 5(c). Leaf area development and RLER in just emerging seventh leaf in sunflower and amaranthus.

| Species | Moisture regime | Days after treatment imposition | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 10 | 12 | 14 | 16 | 18 | 20 | 22 | 24 |
| Leaf area (cm$^2$): | | | | | | | | | |
| Sunflower | FC | 35.18 | 55.28 | 72.96 | 86.78 | 97.49 | 108.11 | 114.05 | 120.47 |
| | 60% FC | 18.29 | 25.07 | 32.31 | 39.28 | 45.87 | 49.90 | 52.75 | 56.32 |
| | | (47.73) | (54.65) | (55.72) | (54.74) | (52.95) | (53.84) | (53.75) | (53.25) |
| Amaranthus | FC | 34.41 | 66.35 | 84.15 | 96.13 | 118.37 | 134.68 | 148.91 | 160.50 |
| | 60% FC | 32.82 | 58.48 | 82.21 | 92.49 | 110.58 | 124.63 | 136.43 | 144.43 |
| | | (4.62) | (11.86) | (2.31) | (3.79) | (6.58) | (7.46) | (8.38) | (10.00) |

| | | Days after treatment imposition | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 10-12 | 12-14 | 14-16 | 16-18 | 18-20 | 20-22 | 22-24 |
| RLER (cm$^2$ cm$^{-2}$ day$^{-1}$) | | | | | | | | |
| Sunflower | FC | 0.2260 | 0.1388 | 0.0868 | 0.0582 | 0.0517 | 0.0268 | 0.0274 |
| | 60% FC | 0.1550 | 0.1269 | 0.0977 | 0.0776 | 0.0421 | 0.0278 | 0.0327 |
| Amaranthus | FC | 0.3283 | 0.1189 | 0.0666 | 0.1041 | 0.0646 | 0.0502 | 0.0376 |
| | 60% FC | 0.2888 | 0.1703 | 0.0589 | 0.0893 | 0.0598 | 0.0453 | 0.0285 |

development was associated with a higher RLER maintained in amaranthus over the major part of the experimental period compared to sunflower. In both the species there was a sudden decrease in RLER after an initial higher value.

The differences in absolute leaf area development and RLER in two species were reflected largely unchanged in the leaf area development of just emerging 1st, 4th and 7th leaves of the plant in the two species grown at two moisture regimes.

Owing to differences in amount of water transpired per day the amount of water required to bring the pots to the respective moisture regimes was always less in amaranthus because they maintained higher soil moisture. This might have facilitated higher plant or tissue water status resulting in a lesser reduction in leaf area developed in amaranthus under stress.

## CORRELATIONS

To study the relationship between different growth characteristics and to identify the growth parameters influencing TQ, correlations were done and the data for the same are presented in Table-6.

TQ showed a positive significant relation with CWT and rate of water loss indicating that water use efficiency

Table 6. Relationship among growth and physiological characteristics in sunflower and amaranthus grown at two different moisture regimes.

| | CWT | DM | TQ | LAD | CWT/LAD | DM/LAD |
|---|---|---|---|---|---|---|
| CWT | - | 0.597* | 0.652* | 0.072 | 0.743** | 0.729** |
| △ DM | - | - | -0.198 | 0.781** | -0.035 | 0.185 |
| TQ | - | - | - | -0.657* | 0.954** | 0.754** |
| LAD | - | - | - | - | -0.602* | -0.464* |
| CWT/LAD | - | - | - | - | - | 0.905* |

*Significant at 5% level
**Significant at 1% level

is inversely related to the rate of water loss. A positive relation was found between $\Delta$DM and CWT and also between rate of water loss and DM/LAD suggesting that higher the CWT, higher the DM production or higher the rate of water loss higher is the assimilation capacity. A positive relation was found between LAD and $\Delta$DM and a negative relation was observed between TQ and LAD and also between LAD and rate of water loss.

The relationship between gas exchange parameters and the major components of TQ, i.e., assimilation (DM/LAD) and rate of water loss (CWT/LAD) was studied and presented in Table-7.

Photosynthetic rate (A) was significantly correlated ($r = 0.644$) with leaf assimilation efficiency (DM/LAD). Stomatal conductance ($g_s$) was positively correlated with rate of water loss (CWT/LAD) ($r = 0.964$) and negatively with TQ, whereas $A/g_s$ (intrinsic WUE) had a significantly negative correlation with TQ ($r = -0.965$). The relationship between A and DM/LAD, $g_s$ and CWT/LAD, $g_s$ and TQ, and between $A/g_s$ and TQ indicate that gas exchange measurements are related to similar physiological traits determined by gravimetric techniques.

Table 7. Relationship between gas exchange parameters and TQ associated growth characteristics in sunflower and amaranthus at two different moisture regimes.

| | TQ | DM | DM/LAD | CWT/LAD | CWT |
|---|---|---|---|---|---|
| A | 0.685** | 0.149 | 0.644** | 0.750** | 0.741** |
| $g_s$ | 0.967** | -0.178 | 0.810** | 0.964** | 0.646** |
| $A/g_s$ | -0.965** | 0.174 | -0.815** | -0.953** | -0.623** |

A V/s $g_s$ = 0.755**; A V/s x $A/g_s$ = -0.636**; $g_s$ V/s $A/g_s$ = -0.983**

Note: Gas exchange parameters used here were recorded after watering on 49th DAS.

Experiment-II: Differences in water use efficiency in selected $C_3$ and $C_4$ species at a given level of moisture input

Under field conditions (rainfed situations) the input of water to all the species, by precipitation will remain same. So with an objective to assess differences in WUE amongst selected $C_3$ and $C_4$ species at a given input of water rather than maintaining plants at a particular moisture regime, this pot culture experiment was conducted. All the crops were supplied with a similar input of water and differences in WUE were estimated under two different inputs of water, one in which the pots were watered to near field capacity and in the other, pots were supplied with 60 per cent of water given under the former regime. The pots which were watered to near field capacity can be considered as nonstressed and pots which were supplied with 60 per cent of water given to nonstressed, can be considered as stressed. In the initial stages the water required for sorghum, soybean and cowpea was less and hence to give the same total input of water the duration of the crop growth period was slightly extended for the above said crops. Excepting ragi, for all the remaining crops experiment was initiated on 31st DAS and was terminated on 57th DAS for sunflower and amaranthus; on 58th DAS for cowpea; on 60th DAS for soybean and on 61st DAS for sorghum, Duration of the experiment for ragi was from 38th DAS to 64th DAS.

As in the first experiment observations on different growth characteristics were made in addition to gas exchange parameters. Unlike in the first experiment emphasis here was given to determinations of WUE and other DM related parameters like NAR and DM/LAD ratio based on total biomass (shoot + root DM). For this purpose root DM was also determined.

### Leaf area (LA), functional leaf area (LAD) and cumulative water transpired (CWT)

Initial leaf areas recorded in six species at the start of the experiment showed a range of 313 to 607 $cm^2$ per pot. Sorghum had the lowest of 313 $cm^2$ $pot^{-1}$ whereas soybean had the highest initial LA of 607 $cm^2$ per pot (Table-8).

The final leaf areas recorded at the end of the experiment showed significant differences both among the species and also between two moisture regimes within a species. The range in LA for three $C_3$ species in the first moisture regime (near field capacity) was 1976.88 $cm^2$ to 4138.00 $cm^2$ and 1281.00 to 2710.63 $cm^2$ under the second moisture regime. The corresponding ranges for the three $C_4$ species were 4301.88 to 5354.75 $cm^2$ and 3180.63 to 441.50 $cm^2$ respectively. Soybean showed the highet per cent reduction in LA (38.40) in the second moisture regime when

Table 8. Leaf area, functional leaf area and cumulative water added in six species grown at two different inputs of water.

| Species | Moisture regime | Initial LA ($cm^2$) | Final LA ($cm^2$) | LAD ($dm^2$ days) | CWA (ml) |
|---|---|---|---|---|---|
| Sunflower | 1 | 427 | 1976.88 | 312.50 | 14800 |
| | 2 | 427 | 1281.00 | 222.04 | 8880 |
| | | | (35.20) | (28.95) | |
| Cowpea | 1 | 354 | 4138.00 | 606.42 | 14800 |
| | 2 | 354 | 2710.63 | 413.73 | 8880 |
| | | | (34.51) | (31.78) | |
| Soybean | 1 | 607 | 3062.13 | 532.02 | 14800 |
| | 2 | 607 | 1886.25 | 361.52 | 8880 |
| | | | (38.40) | (32.05) | |
| Amaranthus | 1 | 507 | 4301.88 | 625.15 | 14800 |
| | 2 | 507 | 3180.63 | 479.39 | 8880 |
| | | | (26.06) | (23.32) | |
| Sorghum | 1 | 313 | 5222.13 | 830.29 | 14800 |
| | 2 | 313 | 4441.50 | 713.15 | 8880 |
| | | | (14.95) | (14.11) | |
| Ragi | 1 | 514 | 5354.75 | 762.94 | 14800 |
| | 2 | 514 | 3396.38 | 508.22 | 8880 |
| | | | (36.57) | (33.39) | |
| CD ($P=0.05$) | S | - | 144.00 | 20.10 | - |
| | M | - | 83.14 | 11.60 | - |
| | S x M | -- | 203.65 | 28.43 | |

Moisture regime 1 - Pots watered to near FC twice a day
Moisture regime 2 - Pots watered to 60% of that supplied in moisture regime 1
S - Species
M - Moisture regime

<u>Note</u>: All values are per pot. Values in paranthesis indicate per cent change under moisture regime 2 from moisture regime 1.

the input of water was less. Sorghum showed the least reduction (14.95%) followed by amaranthus (26.06%).

## Lear area duration

In general $C_4$ crops recorded higher LAD than $C_3$ crops in both the moisture regimes. Among the $C_4$ crops only cowpea recorded higher LAD of 606.42 and 413.73 $dm^2$ days for the first and second moisture regimes respectively. Soybean recorded 532.02 and 361.52 $dm^2$ days and sunflower recorded 312.50 and 222.04 $dm^2$ days for the two moisture regimes. Among the $C_4$ species sorghum had the highest LAD of 830.29 $dm^2$ days under moisture regime-1 and it also showed the lowest reduction (14.11%) under the second moisture regime. Amaranthus also showed less reduction under stress regime (23.32%) but ragi showed a 33.39 per cent reduction.

## Cumulative water applied (CWA)

As the major objective of this experiment was to study differences in water use efficiency and associated parameters at a given input of water, the amount of water supplied for all the six species was uniform. CWA in two moisture regimes was 14800 ml and 8880 ml respectively. Care was taken to minimize evaporation. However, some evaporation losses occur in this type of container experiments.

But, for this experiment no correction for possible E was given. The CWT was considered same as CWA for computing different parameters.

## Dry matter accumulation in shoot and root in six species grown at two moisture regimes

The two major components of TQ or WUE determinations are $\Delta$DM and CWA. As the CWA was similar for all the species in this experiment, any difference in DM accumulation among the species would result in a change in WUE.

Initial shoot DM recorded in six species at the start of the experiment showed a range of 1.3 g to 3.6 g, soybean recorded the highest initial DM of 3.6 g and sorghum recorded the lowest initial DM of 1.3 g.

### Final shoot DM

Amaranthus, sorghum and ragi produced higher final shoot DM than sunflower, cowpea and soybean in both the moisture regimes. There was not much difference between amaranthus and sorghum at any given moisture regime but ragi produced significantly lower final shoot DM than the other two $C_4$ crops (Table-9). The per cent reduction in final shoot DM in the second moisture regime was 33.3, 34.6 and 36.3 for amaranthus, sorghum and ragi. Among the $C_3$ crops sunflower produced higher final shoot DM than cowpea and

Table 9. Shoot dry matter (SDM)' root dry matter and total dry matter (TDM) in six species grown at two different inputs of water.

| Species | Moisture regime | Initial DM (g) | Final SDM (g) | Root DM (g) | Final TDM (g) | △ SDM (g) | △TDM (g) |
|---|---|---|---|---|---|---|---|
| Sunflower | 1 | 3.0 | 37.90 | 6.06 | 43.96 | 34.90 | 40.96 |
| | 2 | 3.0 | 24.00 | 3.65 | 27.70 | 21.13 | 24.70 |
| | | | (36.70) | (38.80) | (37.00) | (39.50) | (39.70) |
| Cowpea | 1 | 2.1 | 31.35 | 6.18 | 37.53 | 29.25 | 35.43 |
| | 2 | 2.1 | 22.25 | 5.14 | 27.39 | 20.15 | 25.29 |
| | | | (29.00) | (16.80) | (27.00) | (31.10) | (28.60) |
| Soybean | 1 | 3.6 | 31.05 | 7.18 | 38.23 | 27.45 | 34.63 |
| | | | 19.58 | 5.33 | 24.90 | 15.98 | 21.30 |
| | | | (37.00) | (25.80) | (34.90) | (41.80) | (38.50) |
| Amaranthus | 1 | 2.5 | 58.86 | 13.31 | 72.18 | 56.36 | 69.68 |
| | 2 | 2.5 | 39.25 | 11.16 | 50.54 | 36.38 | 47.54 |
| | | | (33.30) | (16.20) | (30.00) | (35.50) | (31.80) |
| Sorghum | 1 | 1.3 | 60.43 | 11.48 | 71.90 | 59.13 | 70.60 |
| | 2 | 1.3 | 39.55 | 9.78 | 49.24 | 38.16 | 47.94 |
| | | | (34.60) | (14.80) | (31.50) | (35.50) | (32.10) |
| Ragi | 1 | 2.4 | 55.78 | 12.93 | 68.70 | 53.38 | 66.30 |
| | 2 | 2.4 | 35.55 | 10.19 | 45.85 | 33.15 | 43.45 |
| | | | (36.30) | (21.20) | (33.30) | (37.90) | (34.50) |
| CD (P=0.05) | S | - | 1.35 | 0.32 | 1.48 | 1.28 | 1.38 |
| | M | - | 0.78 | 0.18 | 0.85 | 0.74 | 0.80 |
| | S x M | - | 1.91 | 0.45 | 2.09 | 1.82 | 1.95 |

soybean under both the moisture regimes. Cowpea recorded the lowest reduction in DM under the second moisture regime (29.00%).

### Root DM

Root DM observed at the end of the experiment showed almost a two fold higher value in $C_4$ species over $C_3$ species. The range in root DM recorded among the different species was 6.06 to 13.31 g in sunflower and amaranthus, in the first moisture regime and 3.65 g in sunflower to 11.16 g in amaranthus in the second moisture regime. Lesser reduction under stress was found in sorghum (14.8%) followed by amaranthus (16.2%), cowpea (16.8%) and ragi (21.2%).

### Final total DM

The final total dry matter was arrived at by summing up the final above ground shoot DM with final root DM. The trend here, in general, was similar to final shoot DM but for sunflower where owing to a greater reduction in root DM (39.8%) in plants grown in second moisture regime, final TDM also showed a greater decrease (37.0%) in this moisture regime.

### $\Delta$ SDM

Dry matter accumulation in shoot during the experimental duration was computed based on initial and final shoot DM. The highest $\Delta$SDM was noticed in sorghum (59.13 g and 38.16 g) in both the moisture regimes and the lowest was noticed in soybean (27.45 g and 15.98 g). Soybean also recorded the greatest per cent decrease in $\Delta$ SDM (41.8) under stress whereas cowpea recorded the lowest decrease (31.1%).

### $\Delta$ TDM

Total dry matter accumulated during the experimental duration was computed based on the initial SDM and final TDM. The relative differences between the species at any given moisture regime was similar to that observed in final TDM. Accordingly, sunflower showed a greater decrease in TDM (39.7%) under the second moisture regime. The decrease in the remaining species was 38.5 per cent (soybean), 45.5 per cent (ragi), 32.1 per cent (sorghum), 31.8 per cent (amaranthus) and 28.6 per cent (cowpea).

## Root to shoot ratio and root to leaf area ratio in six species grown at two different moisture regimes

### Root to shoot ratio (mg $g^{-1}$)

Root to shoot ratio is just a reflection of the amount of root DM produced in a given species at a particular moisture regime per unit amount of shoot DM produced. Proportionately a lesser decrease in root DM than shoot DM

Table 10. Root to shoot ratio and root to leaf area ratio in six species grown at two different inputs of water.

| Species | Moisture regime | Root/shoot (mg g$^{-1}$) | Per cent root DM (%of TDM) | Root/leaf area (mg dm$^{-2}$) |
|---|---|---|---|---|
| Sunflower | 1 | 159.88 | 13.79 | 307 |
| | 2 | 152.25 | 13.18 | 285 |
| | | (4.77) | | (7.17) |
| Cowpea | 1 | 197.00 | 16.47 | 149 |
| | 2 | 232.25 | 18.77 | 190 |
| | | (17.89) | | (27.52) |
| Soybean | 1 | 231.38 | 18.78 | 234 |
| | 2 | 272.25 | 21.41 | 283 |
| | | | | (20.94) |
| Amaranthus | 1 | 226.50 | 18.44 | 309 |
| | 2 | 285.63 | 22.08 | 351 |
| | | (26.10) | | (13.59) |
| Sorghum | 1 | 190.38 | 15.97 | 220 |
| | 2 | 248.25 | 19.86 | 220 |
| | | (30.40) | | (-) |
| Ragi | 1 | 232.00 | 18.82 | 241 |
| | 2 | 287.00 | 22.22 | 300 |
| | | (23.71) | | (24.48) |
| CD (P=0.05) | S | 10.17 | - | - |
| | M | 5.87 | - | - |
| | S x M | 14.38 | - | - |

under stress will cause an increase in root to shoot ratio under stress.

But for sunflower, root/shoot ratio increased in the second moisture regime in all the species. Sunflower recorded a 4.77 per cent decrease in root/shoot ratio under stress. The increase observed varied from 17.66 per cent in soybean to 30.40 per cent in sorghum. Amaranthus recorded 26.10 per cent increase, ragi recorded 23.71 per cent increase and cowpea a 17.89 per cent increase. Root DM when expressed as a per cent of TDM also showed a decrease under stress in sunflower and an increase in the remaining five species. An increase in root to shoot ratio under stress is of significance. It facilitates better water harvesting under limited water input conditions.

## Root to leaf area ratio (mg dm$^{-2}$)

Root to leaf area ratio is a reflection of the amount of root DM putforth by a species to meet the transpirational need of unit amount of leaf area.

There was no change in the ratio in sorghum in the second moisture regime. There was a decrease in the ratio under stress in sunflower (7.17%). In the remaining four species there was an increase in the ratio under stress, the per cent increase being 27.52 in cowpea, 24.48 in ragi, 20.94 in soybean and 13.59 in amaranthus.

## Transpiration quotient (TQ) and water use efficiency (WUE) in six species grown at two different inputs of water

TQ and WUE were computed using dry matter accumulation and cumulative water applied. Here, TQ and WUE have been expressed based on both above ground biomass and also total biomass. To that extent the drawback in the expression of WUE based on SDM alone due to noninclusion of root DM was eliminated. TQ has been expressed as ml $H_2O$ $g^{-1}$ DM and WUE as g DM $kg^{-1}$ $H_2O$.

## Transpiration quotient (TQ)

TQ based on $\Delta$ SDM showed a range of 251.13 to 539.96 for the six species in moisture regime-1 and 232.97 to 557.11 in the second moisture regime. Corresponding ranges for $C_3$ crops were 424.24 to 539.96 and 424.12 to 557.11; for $C_4$ crops ranges were 251.13 to 277.6 and 232.97 to 268.26. Cowpea recorded highest decrease in TQ under stress (13.10%), soybean showed a net increase in TQ (3.18%) under stress, sunflower exhibited similar TQ values for both the moisture regimes. All other species showed a general decrease in TQ under stress.

There was a decrease in TQ under stress in all the species when it was computed based on $\Delta$ TDM (Table-11). However, sunflower showed only a marginal decrease (0.49%)

Table 11. Transpiration quotient (TQ) and water use efficiency (WUE) in six species grown at two different inputs of water.

| Species | Moisture regime | TQ based on Δ SDM (ml g$^{-1}$) | TQ based on Δ TDM (ml g$^{-1}$) | WUE based on Δ SDM (g DM kg$^{-1}$ $H_2O$) | WUE based on Δ TDM (g DM kg$^{-1}$ $H_2O$ |
|---|---|---|---|---|---|
| Sunflower | 1 | 424.24 | 361.41 | 2.358 | 2.768 |
|  | 2 | 424.12 | 359.65 | 2.379 | 2.782 |
|  |  | (0.03) | (0.49) | (0.89) | (0.51) |
| Cowpea | 1 | 508.38 | 429.88 | 1.976 | 2.394 |
|  | 2 | 441.77 | 351.48 | 2.269 | 2.848 |
|  |  | (13.10) | (18.24) | (14.83) | (18,96) |
| Soybean | 1 | 539.96 | 427.86 | 1.855 | 2.340 |
|  | 2 | 557.11 | 417.53 | 1.799 | 2.309 |
|  |  | (3.18) | (2.41) | (3.02) | (2.52) |
| Amaranthus | 1 | 263.16 | 212.75 | 3.808 | 4.708 |
|  | 2 | 244.86 | 187.30 | 4.096 | 5.353 |
|  |  | (7.00) | (12.06) | (7.56) | (13.70) |
| Sorghum | 1 | 251.13 | 209.63 | 3.995 | 4.770 |
|  | 2 | 232.97 | 184.87 | 4.298 | 5.398 |
|  |  | (7.23) | (11.81) | (7.58) | (13.17) |
| Ragi | 1 | 277.60 | 223.35 | 3.606 | 4.480 |
|  | 2 | 268.26 | 204.55 | 3.733 | 4,893 |
|  |  | (3.36) | (8.42) | (3.52) | (9.22) |
| CD ($P=0.05$) | S | 12.53 | 9.55 | 0.104 | 0.110 |
|  | M | 7.23 | 5.51 | 0.060 | 0.070 |
|  | S x M | 17.72 | 13.51 | 0.150 | 0.160 |

whereas cowpea showed the maximum decrease (18.24%). The range in TQ observed under the two moisture regimes was 184.87 in sorghum in the second moisture regime to 429.88 in cowpea in the first moisture regime. The greater decrease observed under stress as compared to TQ based on $\Delta$ SDM alone can be attributed to a lesser decrease in root DM under stress.

### Water use efficiency

This is an expression just the reciprocal of transpiration quotient. Thus the relative differences observed among the six species in TQ at any given moisture regime were also observed in WUE.

### Net assimilation rate, DM/LAD ratio and rate of water loss (CWA/LAD) in six species

### Net assimilation rate (mg dm$^{-2}$ d$^{-1}$)

NAR was computed based on $\Delta$ SDM and initial and final leaf areas. NAR was calculated separately for $\Delta$SDM and $\Delta$TDM.

Sunflower showed the highest NAR among the six species at any given moisture regime when NAR was based on $\Delta$ SDM. Sunflower recorded 132.75 and 104.75 for the two moisture regimes. It was closely followed by amaranthus with NAR values of 122.85 and 96.13, soybean recorded the

Table 12. Net assimilation rate, DM/LAD ratios and rate of water loss (CWA/LAD) in six species grown at two different inputs of water.

| Species | Moisture regime | NAR (mg $dm^{-2} day^{-1}$) | | DM/LAD (mg $dm^{-2}$ day) | | Rate of water loss CWA/LAD (ml $dm^{-2}d$) |
|---|---|---|---|---|---|---|
| | | Based on SDM | Based on TDM | Based on SDM | Based on TDM | |
| Sunflower | 1 | 132.75 | 156.00 | 111.80 | 131.23 | 47.41 |
| | 2 | 104.75 | 121.88 | 95.17 | 111.04 | 40.05 |
| | | (21.09) | (21.88) | (14.87) | (15.38) | (15.52) |
| Cowpea | 1 | 70.50 | 85.00 | 48.20 | 58.38 | 24.44 |
| | 2 | 64.50 | 80.75 | 48.72 | 61.17 | 21.49 |
| | | ( 8.51) | ( 5.00) | ( 1.08) | ( 4.79) | (12.04) |
| Soybean | 1 | 62.38 | 78.75 | 51.62 | 65.12 | 27.87 |
| | 2 | 48.75 | 65.00 | 44.80 | 58.92 | 24.59 |
| | | (21.84) | (17.46) | (13.21) | ( 9.52) | (11.75) |
| Amaranthus | 1 | 122.25 | 151.25 | 90.30 | 111.69 | 23.77 |
| | 2 | 96.13 | 125.63 | 75.94 | 99.17 | 18.55 |
| | | (21.37) | (16.94) | (15.89) | (11.20) | (21.94) |
| Sorghum | 1 | 113.13 | 135.13 | 71.29 | 85.16 | 17.87 |
| | 2 | 81.88 | 103.00 | 53.74 | 67.52 | 12.50 |
| | | (27.62) | (23.77) | (24.62) | (20.71) | (30.06) |
| Ragi | 1 | 99.13 | 123.63 | 69.98 | 86.09 | 19.42 |
| | 2 | 83.50 | 109.63 | 65.26 | 85.63 | 17.50 |
| | | (15.76) | (11.32) | (6.74) | (0.53) | (9,92) |
| CD (P=0.05) | S | 2.53 | 3.12 | 2.11 | 2.74 | 0.79 |
| | M | 1.46 | 1.80 | 1.22 | 1.58 | 0.46 |
| | S x M | 3.58 | 4.41 | 2.98 | 3.88 | 1.12 |

lowest amongst the six species and the NAR values were 62.38 and 48.73 for the two first and second moisture regimes. In general, all the species showed a reduction in NAR under stress ranging from 8.51 per cent (cowpea) to 27.62 per cent (sorghum).

When NAR was computed based on $\Delta$ TDM, again sunflower showed the highest NAR of 156.00 followed by amaranthus with 151.25 in the first moisture regime. In the second moisture regime amaranthus showed a greater NAR (125.63) than sunflower (121.88). Again, soybean recorded lowest NAR values at any given moisture regime and there was a general decrease in NAR under stress ranging from 5 per cent in cowpea to 23.77 per cent in sorghum. Sunflower showed a 21.88 per cent decrease as compared to 21.09 per cent decrease when NAR was based on $\Delta$ SDM. This was mainly due to a greater reduction in root DM under stress in sunflower.

## DM/LAD ratio

DM/LAD ratio is the amount of DM produced per unit functional leaf area. Thus, it reflects the mean assimilation efficiency of a species. Higher the ratio, higher the assimilation efficiency.

Sunflower recorded higher DM/LAD ratios under both the water regimes when it was based on either $\Delta$ SDM on $\Delta$ TDM. Cowpea showed a net increase in DM/LAD ratio under stress. The increase was 1.08 per cent (based on $\Delta$ SDM) and 4.79 per cent (based on $\Delta$ TDM). DM/LAD was affected to a lesser extent in ragi under stress and the decrease noticed was 6.74 per cent (based on $\Delta$ SDM) and only 0.53 per cent (based on $\Delta$ TDM). There was a decrease in DM/LAD in the remaining four species under stress.

## Rate of water loss (CWA/LAD)

Rate of water loss was computed from the data on CWA and LAD. This parameter reflects the mean transpiration rate per unit leaf area during the experimental growth period. As CWA in this experiment was uniform for all the species rate of water loss at a given moisture regime was inversely proportional to LAD. Hence, higher the LAD lower the rate of water loss.

CWA/LAD observed in sunflower was almost two times more than any other species at any given water regime. It recorded CWA/LAD values of 47.41 and 40.05 under the first and second moisture regimes respectively. The range in CWA/LAD for the remaining five crops was 17.87 to 27.87 under the first water regime and 12.50 to 24.59 in the second moisture regime. CWA/LAD was affected by stress in all the species and stress reduced the rate of water loss. The reduction was maximum in sorghum (30.06%) and minimum in ragi (9.92%).

Observations on TQ and associated growth parameters indicate tha;

$C_4$ crops in general recorded higher LA and LAD than $C_3$ crops. Only exception among $C_3$ crops was cowpea which also recorded higher LA and LAD. Sorghum recorded the least reduction in LA and LAD under stress.

Even DM accumulation was higher in $C_4$ crops and as the CWA was uniform in all species this higher DM accumulation resulted in higher WUE among the $C_4$ crops. There was a decrease in DM under stress in all the species and this was partly due to reduction in NAR and mainly due to reduction in LAD and only in sorghum there was a greater decrease in NAR and a lesser decrease in LAD.

There was a decrease in root DM under stress in all the species but there was an increase in root to shoot ratios in all but one species. Only in sunflower there was a substantial decrease in root DM resulting in reduced root to shoot ratio under stress.

WUE was higher in $C_4$ than $C_3$ under both the regimes. Ranking done in $C_3$ under the first moisture regime did not remain the same in the second moisture regime. Cowpea recorded the highest per cent increase in WUE under stress.

Sunflower and amaranthus recorded higher NAR and DM/LAD ratios than other crops. Cowpea recorded very low decrease in NAR and in fact an increase in DM/LAD under stress.

Rate of water loss was very high in sunflower and very low in sorghum. But for these two crops the range in CWA/LAD was not much in the remaining four crops. CWA/LAD decreased under stress in all the crops.

Root to shoot ratio and root to leaf area ratio in general tended to increase under stress. Exception in both the cases was sunflower.

## Differences in gas exchange parameters in six species grown under two different moisture regimes

One of the major objective was to ascertain the relative differences in photosynthetic rates and stomatal conductances in the six different species both under a relatively nonstressed regime and a comparatively stressed regime. In turn, these parameters were to be related to TQ

Table 13. Gas exchange parameters recorded between 47th DAS to 49th DAS in six species grown at two different inputs of water.

| Species | M | A | $g_s$ | $A/g_s$ | PAR | RH | LT | $CO_2$ |
|---|---|---|---|---|---|---|---|---|
| Sunflower | 1 | 30.46 | 0.4878 | 62.44 | 1406 | 66.4 | 29.92 | 328.9 |
| | 2 | 27.27 | 0.3915 | 69.65 | 1495 | 64.5 | 31.36 | 334.0 |
| | | (10.47) | (19.74) | (11.54) | | | | |
| Cowpea | 1 | 20.60 | 0.2230 | 92.40 | 1436 | 57.0 | 31.46 | 325.2 |
| | 2 | 15.56 | 0.1277 | 114.01 | 1605 | 44.7 | 35.56 | 328.2 |
| | | (29.32) | (42.74) | (23.38) | | | | |
| Soybean | 1 | 19.51 | 0.3578 | 54.53 | 1533 | 58.7 | 34.89 | 326.3 |
| | 2 | 12.84 | 0.2557 | 50.20 | 1664 | 51.4 | 36.94 | 319.9 |
| | | (34.18) | (28.54) | (7.9) | | | | |
| Amaranthus | 1 | 35.05 | 0.2433 | 144.06 | 1619 | 51.8 | 32.98 | 334.7 |
| | 2 | 32.05 | 0.1924 | 166.60 | 1795 | 42.1 | 34.78 | 337.0 |
| | | (8.56) | (20.92) | (15.65) | | | | |
| Sorghum | 1 | 34.98 | 0.2203 | 158.79 | 1521 | 52.5 | 33.15 | 325.2 |
| | 2 | 28.31 | 0.1488 | 190.24 | 1585 | 50.2 | 35.52 | 313.8 |
| | | (19.07) | (32.45) | (19.81) | | | | |
| Ragi | 1 | 29.14 | 0.1676 | 173.90 | 1496 | 57.9 | 25.32 | 328.3 |
| | 2 | 18.71 | 0.0874 | 214.07 | 1666 | 51.1 | 27.30 | 338.2 |
| | | (35.79) | (47.84) | (23.10) | | | | |

M - Moisture regime
A - Photosynthetic rate (μ moles $m^{-2}s^{-1}$)
$g_s$ - Stomatal conductance(μ moles $m^{-2}s^{-1}$)
$A/g_s$ - Rate of assimilation rate to stomatal conductance (u moles $CO_2$ per mole $H_2O$)
PAR - Photosynthetically active ratiation (μ Ein $m^{-2}s^{-1}$)
RH - Relative humidity(%)
LT - Leaf temperature(°C)
$CO_2$- Chamber $CO_2$ concentration (ppm)

Note - Values are means of 6 to 12 replications.

and rate of water loss. The gas exchange parameters were recorded between 47 DAS to 49 DAS using a LI-6000 Portable Photosynthesis System. There was not much difference in the environmental conditions during these days (Table-13).

All the $C_4$ species/crops and sunflower among the $C_3$ showed greater 'A' values than cowpea and soybean. There was a decrease in 'A' under stress in all the species, the decrease being just 8.56 per cent in amaranthus to 35.79 per cent in ragi.

Stomatal conductances, irrespective of the species showed a general decrease under stress. The decrease was greater in ragi (47.84%) and cowpea (42.74%) and least in sunflower (19.74%).

The range in $A/g_s$ varied from 54.53 in soybean to 173.90 in ragi in the first moisture regime and from 50.20 in soybean to 214.07 in ragi under stress (in second moisture regime). In soybean, under stress there was a decrease in $A/g_s$ by 7.9 per cent. In all other crops $A/g_s$ showed an increase under stress. Highest increase was recorded by cowpea (23.38%).

Thus, $C_4$ crops recorded higher 'A' rates than $C_3$ crops. Exception among the $C_3$ was sunflower. Sunflower recorded the highest stomatal conductance among the six species. $A/g_s$, a reflection of intrinsic WUE was greater in $C_4$ than in $C_3$.

There was a general decrease in A and $g_s$ in all the species under stress. Only in soybean there was a greater decrease in A than $g_s$. Thus, $A/g_s$ in soybean under stress decreased. In all other crops there was a proportionately lesser reduction in $g_s$ than A and thus they recorded an increase in $A/g_s$ under stress.

## Correlations

To arrive at the importance of different physiological traits associated with WUE the correlations between TQ and different growth and gas exchange parameters were determined.

## Relationship of WUE, Δ TDM, NAR, CWA/LAD in nonstressed conditions with those under stressed conditions

An attempt was made to study the relationship between TQ associated growth parameters in stressed conditions with those under nonstressed conditions. When all the six species were analysed together, there was a very high relationship between parameters under control and stress. The 'r' value of 0.961 for TQ suggested that intrinsically low TQ types are advantageous.

Table 14. Relationship of TQ associated parameters in nonstressed conditions with those under stressed conditions.

| Parameters | All six species | $C_3$ species | $C_4$ species |
|---|---|---|---|
| TQ | 0.961** | 0.413* | 0.340 |
| $\Delta$ TDM | 0.970** | 0.398 | 0.141 |
| NAR | 0.942** | 0.974** | 0.615** |
| CWA/LAD | 0.975** | 0.984** | 0.709** |

*Significant at 5 per cent
**Significant at 1 per cent

In $C_3$ species TQ under control was related to TQ under stress with a 'r' value of 0.413. NAR and CWA/LAD had 'r' values of 0.974 and 0.984 respectively.

In $C_4$ species the relationship for TQ and $\Delta$ TDM was nonsignificant but NAR and CWA/LAD had significant 'r' values.

The results indicate that a species with higher TQ in control can have a lower TQ under stress and vice versa. NAR and CWA/LAD changed only to a lesser extent in stress in $C_3$ species and to a greater extent in $C_4$ species.

## Physiological and gas exchange traits associated with WUE

This experiment involved such species which had intrinsically greater variation in TQ and certain growth parameters like NAR and rate of water loss. Hence, it was thought fit to study the relationship between growth and physiological characters with TQ not only in the pooled data from all the six species but also separately in $C_3$ and $C_4$ species. Thus, correlations were worked for these distinct groups. Within each group data from stressed and nonstressed treatments were studied separately. Correlation matrices are presented in Appendices-2(a) to 2(i). The same was followed to relate gas exchange characters and growth characters associated with TQ.

Table 15. Relationship between TQ and growth characteristics in six species grown at two different inputs of water.

| Parameters | All six species | $C_3$ species | $C_4$ species |
|---|---|---|---|
| TQ Vs TDM | -0.741** | 0.040 | 0.569** |
| TQ Vs NAR | -551** | -0.576** | 0.305* |
| TQ Vs LAD | -554** | 0.610** | 0.409** |
| TQ Vs CWA/LAD | 0.575** | -0.418** | 0.518** |

WUE Vs growth paramters

When all the species were studied together, TQ had significant relationships with $\Delta$TDM (r = -0.741), NAR (r = -0.551), rate of water loss (r = 0.575) and LAD (r = -0.554).

In the $C_3$ species TQ was negatively related to NAR and also with rate of water loss (CWA/LAD).

In the $C_4$ species, TQ was positively related to NAR (r = 0.305) and also rate of water loss (r = 0.518).

The results from the six species indicate that both NAR and CWA/LAD contribute for differences in TQ. Among the $C_4$ species rate of water loss seems to be an important physiological parameter contributing for differences in TQ whereas in $C_3$ the major contributing factor for variations in TQ seems to be differences in NAR.

In this experiment we have noticed that at low water input TQ showed only a slight reduction but TDM production reduced drastically. This $\Delta$ TDM was dependent solely on CWA whereas TQ is a parameter computed based on both CWA and $\Delta$ TDM. So, any study relating TQ and $\Delta$ TDM has little relevance.

### Gas exchange characters versus growth parameters associated with WUE

The relationship of the gas exchange parameters contributing for intrinsic WUE, 'A' and $g_s$ with the growth parameters NAR and CWA/LAD reflecting 'A' and $g_s$ for the entire experimental period was studied. Also the relationship between TQ and intrinsic WUE in the form of $A/g_s$ ratio was studied.

When the relations were studied in all the six species together, 'A' showed a significant positive relation with NAR; $g_s$ showed a positive relation with rate of water loss (CWA/LAD), and $A/g_s$ in instantaneous measurement of WUE showed a negative relationship ($r = -0.916$) with TQ.

Even in $C_3$ crops 'A' and NAR ($r = 0.915$), $g_s$ and CWA/LAD ($r = 0.909$) were positively related. However, the relationship between $A/g_s$ and TQ, though significant ($r = -0.365$), was not strong.

In $C_4$ crops similar results were obtained in $C_3$ species with regards to 'A' and NAR, and $g_s$ and CWA/LAD. But the relationship between $A/g_s$ and TQ was nonsignificant.

Within each group of either $C_3$ or $C_4$ species A was positively related with $g_s$. This meant that as $g_s$ increased Ci increased promoting higher assimilation rates.

Table 16. Relationship between gas exchange characteristics and TQ associated growth characteristics in six species grown at two different inputs of water.

| Parameters | All six species | $C_3$ species | $C_4$ species |
|---|---|---|---|
| 'A' Vs NAR | 0.871** | 0.915** | 0.702** |
| $g_s$ Vs CWA/LAD | 0.914** | 0.909** | 0.561** |
| $A/g_s$ Vs TQ | -0.916** | -365* | -0.208 |
| A Vs $g_s$ | 0.167 | 0.826** | 0.968** |

The significant relationship obtained between gas exchange and growth parameters associated with TQ further validates the use of instantaneous gas exchange measurements to study the intrinsic differences in WUE.

Experiment-III: Effect of moisture stress on gas exchange characteristics in sunflower and amaranthus

Assimilation to conductance ratio is a reflection of WUE. In plants subjected to stress a lesser decrease in 'A' compared to $g_s$ results in an increased $A/g_s$ ratio and hence enhanced WUE. Lesser decrease in 'A' under stress depends on the stability of mesophyll factors. An attempt was made in this experiment to assess intrinsic differences if any, in 'A' and $g_s$ in amaranthus and sunflower subjected to different degrees of moisture stress. The effect of moisture stress on gas exchange characteristics like assimilation rate (A), stomatal conductance ($g_s$) and intercellular $CO_2$ concentration (Ci) in sunflower and amaranthus was determined.

Plants were raised in pots and 40 day old plants were subjected to four moisture regimes. Plants in the first regime were irrigated to FC (determined gravimetrically) twice a day and in the subsequent three regimes the plants were supplied with 75, 50 and 25 per cent of water given to

Table 17. Gas exchange parameters as influenced by leaf water status in sunflower and amaranthus.

| Sunflower | | | | Amaranthus | | | |
|---|---|---|---|---|---|---|---|
| $\Psi$ | A | $g_s$ | Ci | $\Psi$ | A | $g_s$ | Ci |
| -0.840 | 27.73 | 0.6395 | 250 | -0.744 | 22.31 | - | - |
| -1.080 | 25.25 | 0.4921 | 238 | -0.852 | 16.97 | - | - |
| -1.164 | 15.01 | 0.2482 | 223 | -1.032 | 11.69 | 0.0752 | 71.28 |
| -1.248 | 11.44 | 0.1764 | 216 | -1.140 | 7.91 | 0.0603 | 110.12 |
| -1.332 | 8.09 | 0.1316 | 222 | -1.164 | 7.90 | - | - |
| -1.428 | 6.60 | 0.0833 | 193 | -1.212 | 5.92 | 0.0564 | 152.06 |
| -1.500 | 5.11 | 0.0597 | 183 | -1.224 | 6.03 | - | - |
| -1.668 | 6.28 | 0.0617 | 157 | -1.524 | 3.10 | 0.0336 | 172.38 |
| -1.716 | 5.41 | 0.0482 | 140 | -1.536 | 3.42 | 0.0456 | 200.00 |
| -1.766 | 2.69 | 0.0346 | 196 | | | | |
| -1.812 | 2.65 | 0.0477 | 231 | | | | |

Note: All observations were recorded under the following conditions;
i) Ambient $CO_2$ concentration of 320 ppm and
ii) Light intensity of 1600 to 1650 μEin $m^{-2}s^{-1}$.

= Leaf water potential (Megapascals)
A = Assimilation rate (μ mol $m^{-2}s^{-1}$)
$g_s$ = Stomatal conductance (mol $m^{-2}s^{-1}$)
Ci = Intercellular $CO_2$ concentration (ppm)

control (first regime) plants. The various moisture regimes were imposed for two days and on the third day photosynthetic rate and stomatal conductance were measured in the topmost fully expanded leaves using a LI-6000 Portable Photosynthetic System. In the same leaves water potential was also recorded using a psychrometer (HP-115 water potential data system) in the dew point mode. Gas exchange measurements were made at different times on the day with an objective to vary the tissue water potential prior to taking gas exchange measurements. From the data on 'A', $g_s$ and ambient $CO_2$ concentration (Ca), Ci was calculated. The data on A, $g_s$ and Ci at different leaf water potentials has been presented in Table-17.

Stress effect on gas exchange characteristics in sunflower

Gas exchange characteristics in sunflower were studied in a range of -0.84 to -1.812 MPa leaf water potential (LWP). At a LWP of -0.84 MPa sunflower recorded A and $g_s$ values of 27.73 $\mu$ moles $m^{-2}$ $s^{-1}$ and 0.6395 moles $m^{-2}$ $s^{-1}$, respectively. When LWP decreased from - 1.08 to -1.164 MPa, 'A' drastically decreased from 25.25 to 15.10 $\mu$ moles $m^{-2}$ $s^{-1}$ and $g_s$ decreased from 0.4921 to 0.2482 suggesting that threshold LWP for a decrease in A and $g_s$ could be somewhere between -1.08 to -1.164 MPa. Thereafter there was a gradual decrease in A and $g_s$ with decreasing LWP.

However, the Ci decreased progressively indicating that $g_s$ was affected to a greater extent than mesophyll facters but at LWP below -1.716 MPa, Ci increased indicating that at this LWP even mesophyll factors were affected.

### Stress effect on gas exchange characteristics in amaranthus

Unlike in sunflower, in amaranthus there was no indication of any threshold, but a gradual decrease in 'A' and $g_s$ was noticed when LWP decreased from -0.744 to -1.536 MPa. Stomatal conductance could not be measured for certain LWP values and thus Ci for the corresponding LWP could not be calculated from the available data.

Thus, sunflower showed higher 'A' and $g_s$ values than amaranthus at any given LWP. The effect on 'A' when LWP decreased was more or less, similar in both the species. For example when LWP decreased from -0.84 to -1.248 MPa in sunflower, 'A' decreased by 59 per cent; in amaranthus 'A' decreased by 64 per cent when LWP decreased from -0.852to -1.224 MPa. Hence, the superiority in terms of greater mesophyll stability in any one species over the other could not be ascertained.

### Experiment-IV: Shade effect on the extent of stomatal closure in selected $C_3$ and $C_4$ species

Changes in stomatal behaviour as a response to environmental factors is an adaptation to optimize carbon assimilation and water loss. The light intensity, tissue water potential (moisture stress) and ambient $CO_2$ concentration (Ca,,which in turn alters intercellular $CO_2$ concentration, Ci) affect the stomatal conductance. The threshold level for an environmental factor for decreasing stomatal conductance or closing the stomata may vary in different species. Often the species which show a higher threshold for a particular environmental factor for decrease in stomatal conductance ($g_s$) are termed as sensitive crops for stomatal behaviour. The stomatal sensitivity can be assessed by altering either light intensity, ambient $CO_2$ concentration or tissue water status. However, it is not very clear whether the response of stomata is the same in the alterations in different environmental factors which influence stomatal behaviour. In this experiment an attempt was made to assess the relative sensitivity of these species to light with regards to their stomatal behaviour.

The experiment was conducted on a bright sunny day. plants were exposed to full sunlight for a period of three

Table 18. Stomatal resistances in sunflower and amaranthus at saturated light intensity and on transfer to low light.

| Crop | | Light treatment | Shade treatment | | |
|---|---|---|---|---|---|
| | | | Duration of shade treatment | | |
| | | | 10 minutes | 40 minutes | 70 minutes |
| Sunflower | $r_s$ | 1.638 | 1.593<br>(2.75) | 1.933<br>(18.01) | 2.133<br>(30.22) |
| | PAR | 1600 | 40.0 | 35.0 | 40.0 |
| | RH | 52.0 | 41.3 | 43.4 | 42.0 |
| | LT | 31.5 | 24.4 | 25.8 | 26.9 |
| Amaranthus | $r_s$ | 3.705 | 4.565<br>(23.21) | 5.648<br>(52.44) | 8.583<br>(131.65) |
| | PAR | 1550 | 35.0 | 30.0 | 45.0 |
| | RH | 52.8 | 40.8 | 43.0 | 42.8 |
| | LT | 34.0 | 28.1 | 28.2 | 30.1 |

$r_s$ - Stomatal resistance (s $cm^{-1}$)
PAR - Photosynthetically active radiation ($\mu$ Ein $m^{-2}s^{-1}$)
RH - Relative humidity (%)
LT - Leaf temperature (°C)

Note - Observations were made on the abaxial surface of top most fully expanded leaves. Values in paranthesis represent per cent change in $r_s$ under shade treatment from light treatment.

hours and were transferred to shade with a light intensity of 50 ± 30 $\mu$ Ein $m^{-2}$ $s^{-1}$. After transfering the plants to shade the stomatal conductance was monitored as a measure of sensitivity. Stomatal resistances ($r_s$) were measured in the topmost expanded leaf in all the treatments before transfering to shade and at regular intervals after transfering to low light, using a portable steady state porometer (LICOR-1600).

## Stomatal sensitivity to light in sunflower and amaranthus

Before transferring to shade amaranthus had a higher $r_s$ (3.705 s $cm^{-1}$) than sunflower (1.638 s $cm^{-1}$). Within 10 minutes after transferring to shade $r_s$ in amaranthus increased by 23.21 per cent and by 52.44 per cent after 40 minutes. But increase in $r_s$ in sunflower even after 70 minutes was just 30.22 per cent whereas the corresponding value for amaranthus was 131.65 per cent. The results indicate that although stomata of sunflower responded to changes in light intensity, the sensitivity of the stomata was much lesser as compared to amaranthus.

## Stomatal sensitivity to light in selected $C_3$ and $C_4$ species

The diffusive resistances recorded in species exposed to full light intensity showed a wide range of 2.25 s $cm^{-1}$ (sorghum) to 4.17 s $cm^{-1}$ (cowpea). In shade, within

Table 19. Stomatal resistances in $C_3$ and $C_4$ species at saturated light intensity and on transfer to low light.

| | | Sunflower | Cowpea | Soybean | Amaranthus | Sorghum | Ragi |
|---|---|---|---|---|---|---|---|
| <u>Light treatment</u>: | | | | | | | |
| | $r_s$ | 2.39 | 4.17 | 3.98 | 2.89 | 2.25 | 4.04 |
| | PAR | 1510 | 1660 | 1410 | 1605 | 1440 | 1540 |
| <u>Shade treatment</u>: | | | | | | | |
| 1. | $r_s$ | 2.73<br>(14.23) | 5.68<br>(36.21) | 7.30<br>(83.40) | 3.83<br>(32.53) | 5.24<br>(132.88) | 9.89<br>(144.80) |
| | PAR | 29 | 22 | 19 | 41 | 48 | 24 |
| 2. | $r_s$ | 2.91<br>(21.75) | 6.09<br>(36.04) | 7.86<br>(97.48) | 4.17<br>(44.29) | 5.38<br>(139.11) | 13.25<br>(227.97) |
| | PAR | 28 | 66 | 21 | 61 | 63 | 49 |

Shade treatment 1. Observations recorded 10 minutes after transfer to shade.
2. Observations recorded 40 minutes after transfer to shade.

10 minutes ragi, sorghum and soybean showed an increase in $r_s$ by 144.8, 132.88 and 83.4 per cent respectively. Stomatal resistance in amaranthus, cowpea and sunflower increased only to a lesser extent. The trend in the increase in $r_s$ continued even after 40 minutes in shade.

In all, sunflower was the least sensitive and ragi the most sensitive. Sorghum and soybean also showed higher sensitivity than cowpea and amaranthus.

# DISCUSSION

# V. DISCUSSION

Among the several abiotic stresses, drought stress is the major constraint affecting the yield of all the rainfed crops. Although drought causes more yield reduction than the combined effect of all other abiotic stresses, the progress made in enhancing the productivity under water limited environment has not been significant. The main reason being the complexity of the problem, since the magnitude, duration and time of occurence of stress under rainfed conditions is unpredictable and is often compounded by the variations in temperature and relative humidity. It is only during this decade, the diverse implications associated with drought stress have been clearly understood and the different adaptive mechanisms and their importance extensively studied and reviewed in recent papers (Turner and Begg, 1981; Udaya Kumar _et al_., 1986; Paleg and Aspinall, 1981).

Plants adapt different mechanisms depending on magnitude and duration of stress to optimize growth rates or to ensure survival. These mechanisms, escape, avoidance and tolerance either favour survival under stress situations or help to optimize productivity under water limited conditions. It is important for a drought resistant crop to have characteristics associated with maximizing productivity under stress situations rather than to ensure mere survival.

Avoidance mechanisms often maintain higher tissue water potentials and avoid any substantial reduction in physiological processes. Water uptake mechanisms associated with root characters are very important in maintaining high tissue water potential. Therefore water conservation practices to minimize the water loss and plant characters which enhance its extraction have been useful approaches to enhance productivity under rainfed conditions.

It is well established that biomass production of any crop species is directly related to amount of water expended in evapotranspiration which in turn depends on the availability of water and its uptake. Enhancement of ET has been shown to predominantly depend on water conservation practices and root characteristics associated with water extraction from the soil. However, at a given E and ET productivity of different crops again differs based on differences in WUE (Johnson et al., 1983; Garrity et al., 1982; Nagamani, 1985; Uma, 1987; Sashidhar, 1987).

WUE reflects the amount of DM produced per unit of water transpired (or lost in ET). In genotypes with high WUE, the carbon gain per unit amount of water transpired will be high. Intrinsic differences in WUE are found to exist amongst different species (Shantz and Piemeisel, 1927;

Sashidhar, 1987). Also, $C_4$ species are found to have a higher WUE than $C_3$ species (Teare et al., 1973; Hattendorf et al., 1988; Ku and Edward, 1978; Hitoshi Oguro, 1985; Uma, 1987). Significant varietal differences within a particular species has also been reported in cowpea (Nagamani, 1985) soybean (Arun, 1985), wheat (Farquhar and Richards, 1984), finger millet (Sashidhar, 1987).

WUE will be affected by several environmental factors like VPD (Rawson, 1977) temperature (Khairi and Hall, 1976; Jones et al., 1985) and light (Fischer and Turner, 1978). Moisture stress is one such environmental factor influencing WUE. Differences in WUE under a given abiotic stress depend on the effect of this abiotic factor on some physiological processes of the plant associated with WUE. So WUE in species or genotypes may differ under a particular stress situation.

Under rainfed conditions in a given zone input of water for all crops will be uniform. If the factors associated with evaporation and water extraction are not different from species to species then the biomass differences at a given input of water are predominantly due to differences in WUE. So at a given input of water a species or a genotype with a higher WUE may be productive. Keeping this in view experiments were carried out in this study with the following objectives.

i) To assess the differences in WUE in sunflower and amaranthus at two different moisture regimes (at 100 per cent field capacity and at 60 per cent field capacity).

ii) To assess the differences in WUE in the 6 species under adequate and inadequates input of water, but similar to all the species.

iii) To identify the physiological and gas exchange characteristics associated with WUE.

To meet the above objectives, in all, four pot culture experiments were conducted.

In the first experiment differences in WUE were determined in sunflower and amaranthus grown at two moisture regimes. One set of the plants were grown at near field capacity and the other set at 60 per cent field capacity.

In the second experiment several $C_3$ and $C_4$ species were included. The differences in WUE were studied at two different inputs of water.

Gas exchange characteristics at different leaf water potentials in sunflower and amaranthus were studied in the third experiment. An attempt was also made to determine the light effect on relative stomatal sensitivity in all six species in the fourth experiment.

## Determination of WUE in container studies

In this study, in the first experiment WUE was determined employing a gravimetric technique. The CWT and $\Delta$ DM during the experimental period were determined to compute WUE. To determine CWT, the pots were weighed daily using a bench platform type balance. Care was taken to minimize evaporation and appropriate correction was given to delineate E from ET.

In the second experiment where similar input of water was given to all the species WUE was determined from cumulative water added (CWA) and DM produced during the growth period. Since, care was taken to minimize E, CWA itself was considered as CWT by the different species.

At the whole plant level, in field studies, WUE is determined by assessing the ratio between ET and DM produced. The major drawback of such studies is delineation of Eand T from ET and other problems associated with extraction of root system which otherwise would have contributed for DM produced (Fischer and Turner, 1978; Passioura, 1983). Hence, a better picture on WUE at whole plant level can be obtained through container studies though a limitation in the form of inadequate soil volume may come in the way for full expression of the plant. Distinct differences in WUE have been established using this technique by Lawes (1850), Farquhar and Richards (1984), Arun (1985) and Sashidhar (1987).

Even in this study, both by maintaining plants at a particular soil moisture level by adopting gravimetric technique or when a known input of water was given for a specific growth period distinct differences in WUE were observed (Tables-2 and 10). Thus the results obtained in this study indicate that differences in WUE can be accurately assessed in container studies by determining CWA and $\Delta$DM for a specific growth period. Often the cumulative water to be added to maintain a particular water regime is determined by adopting a gravimetric method. However, even when a known input of water was periodically supplied and arrived at CWA, the differences in WUE can be accurately determined by using the container studies. Such an approach has two advantages; the daily weighing can be avoided and since the input of water is same for all crops, we can simulate the rainfed field situations where the input of water for different crops always remains the same.

## Variation in WUE in sunflower and amaranthus when soil moisture was maintained at 100 per cent FC or 60 per cent FC

In this experiment differences in WUE in sunflower and amaranthus were determined at two different moisture regimes. One set of pots were brought back to field capacity twice a day and were considered as control plants (or plants grown at field capacity). In the other set plants were brought back to 60 per cent field capacity twice a day and were considered as stressed plants (or plants grown at 60 per cent field capacity).

Distinct species variation was observed in TQ. CWT was generally less in amaranthus but DM was higher than in sunflower. This results in a lower TQ in amaranthus, compared to sunflower (Table-2).

Since, TQ is the ratio between transpiration and assimilation, the two associated physiological parameters, NAR and mean rate of water loss (CWT/LAD) were determined. The assimilation rates as measured by NAR (and DM/LAD) were not distinctly different between these two species. However, it was not the same with CWT/LAD. Amaranthus recorded significantly lower CWT/LAD than sunflower which resulted in a greater WUE in amaranthus. As the NAR recorded showed similar reductions for both the species under stress, a greater reduction in CWT/LAD alone contributed for the substantial increase in WUE under stress in amaranthus. Thus, a reduction in transpiration rate due to stomatal regulation of water loss is associated with an increase in WUE.

The NAR however, decreased to the same extent in both the species. The results obtained on carbon exchange rate at different tissue water potentials in these two species also suggests that in both the species with decrease in tissue water potential CER also decreased (Table-17).

Amaranthus did not show higher stability of chloroplast functions at low tissue water potentials compared to sunflower.

Though the per cent decrease in NAR was similar for both the species the major contributing factor was the differences in CWT/LAD which decreased by 19.6 per cent in amaranthus whereas in sunflower it decreased only by 13.42 per cent. The intrinsic low CWT/LAD and the greater decrease in this parameter at 60 per cent field capacity was responsible in better soil moisture conservation which possibly facilitated a greater LAD and its metabolic activity. LAD decreased by 14.55 per cent in amaranthus as against 28.82 per cent in sunflower. The maintenance of greater soil moisture content in amaranthus (Fig.1) might have had a bearing on the observed lesser decrease in LAD. Throughout the experimental growth period, prior to watering soil moisture content was always more in amaranthus grown pots. This might have facilitated higher tissue water status which favoured leaf expansion (Clough and Milthrope, 1975; Palta, 1984; Chu and McPherson, 1977; Masle and Passioura, 1987). As a result of higher tissue water status, the reduction in prewatering assimilation rate was also low in amaranthus which also explains the higher DM produced in amaranthus under stress.

## Physiological traits associated with WUE in plants maintained at 100 and 60 per cent field capacity

Of the two physiological parameters, assimilation (DM/LAD) and transpiration (CWT/LAD), TQ seems to be more dependent on variation in rate of water loss, since among the two, in species which showed low TQ, the rate of water loss was significantly less compared to the species with high TQ. Hence, a highly positive relation was seen between rate of water loss and TQ ($r = 0.954$), emphasising that between species, the differences in TQ seems to be more by a higher assimilation per unit conductance ($A/g_s$) in $C_4$ species.

## Relation between 'A' and '$g_s$' determined by gas exchange technique, and growth characteristics associated with WUE

The two gas exchange parameters associated with TQ are A and $g_s$. A ratio of A to $g_s$ ($A/g_s$) is itself called intrinsic water use efficiency. Water use efficiency can be increased either by maintenance of $g_s$ and increasing A or a lowering of $g_s$ while A is maintained. Osmond et al., (1980) and Slatyer (1970) have observed a two and half to three times higher $A/g_s$ ratios in $C_4$ species than those found in $C_3$ species under similar environmental conditions.

An attempt was made in this experiment to determine intrinsic WUE from the data on A and $g_s$ by using a portable IRGA (LI-6000). A significant negative relationship (r = -0.965) was seen between $A/g_s$ and TW. Significant positive relations were also found between $g_s$ and CWT/LAD (r = 0.964) and between 'A' and DM/LAD (r = 0.750). Further, $g_s$ showed a positive relation with TW indicating the importance of stomatal regulation of water loss for achieving higher WUE. Since, DM/LAD differences were not much, differences in $g_s$ alone contributed for differences in water use efficiency. The results indicate that, if the gas exchange parameters are carefully recorded, intrinsic WUE differences between species can be studied.

It can be concluded that in crops distinctly differing in WUE like a $C_3$ sunflower and a $C_4$ amaranthus an instantaneous measurement on $g_s$ and $A/g_s$ could be a good measure of mean rate of water loss and WUE based on CWT and $\Delta$DM.

### Differences in WUE in selected $C_3$ and $C_4$ species at a given input of water

The second experiment was conducted principally with two specific objectives. Firstly in a rainfed situation the amount of water available will be same for different species. So, WUE differences were studied in a situation close to the environmental condition by providing uniform quantities of water to all the species. In the nonstressed plants input of water was such that the pots were brought back to near field capacity twice a day and 60 per cent of the water applied to nonstressed ones was supplied to the stressed plants. Secondly, it is well established that $C_4$ species have a higher WUE than $C_3$ species (Teare et al., 1973; Hattendorf et al., 1988). Here, it was aimed to study how different species of $C_3$ and $C_4$ perform at a given water regime where CWA was same for all species.

TQ differences were studied at two water regimes. In the first regime adequate (near to field capacity) water was given and in the second only 60 per cent of the first regime was given. Here, the root DM has also been accounted for computation of TQ. Thus it is a true reflection of WUE. Also, the DM associated parameters like NAR and DM/LAD have been computed based on TDM.

Under nonstressed conditions generally $C_4$ species recorded a higher WUE than $C_3$ species. This was due to greater biomass produced in the $C_4$ species. Mean DM accumulated in $C_4$ species was 86.03 per cent greater than $C_3$ species. The net assimilation rate differences between the $C_3$ and $C_4$ were not much when compared to LAD differences. The $C_4$ species recorded 28.23 per cent higher mean NAR and 52.89 per cent higher LAD than the $C_3$ species. So, the main factor contributing for differences in DM observed was differences in functional leaf area (LAD) and only to a lesser extent, the differences in NAR.

Amongst the three $C_4$ species, sorghum recorded the lowest TQ value of 251.13 as compared to 263.16 in amaranthus and 277.60 in ragi. The observed NAR differences did not vary much among the three species. But the transpirational water loss (CWR/NAR) was the lowest in sorghum and LAD, the highest. Therefore lower rate of water loss has contributed to the greater WUE observed in sorghum.

Among the three $C_3$ species the highest WUE was observed in sunflower. Though the rate of water loss was high in sunflower, very high NAR contributed for a greater DM production and thus a lower TQ in sunflower.

In Fig.2 growth and physiological characteristics of amaranthus and sunflower in the first and the second experiments are compared. When the input of water is high but not maintained at field capacity, $C_4$ species, because of their higher WUE produce more DM or yield. But when maintained at field capacity the differences in DM production as well as functional leaf area putforth tend to be marginal between the two species (Table-1). As such no differences exist in NAR between the species. So, at a given level of water input, intrinsically high WUE types ($C_4$ species) are advantageous as they maintain better tissue water status aiding in higher leaf expansion rates

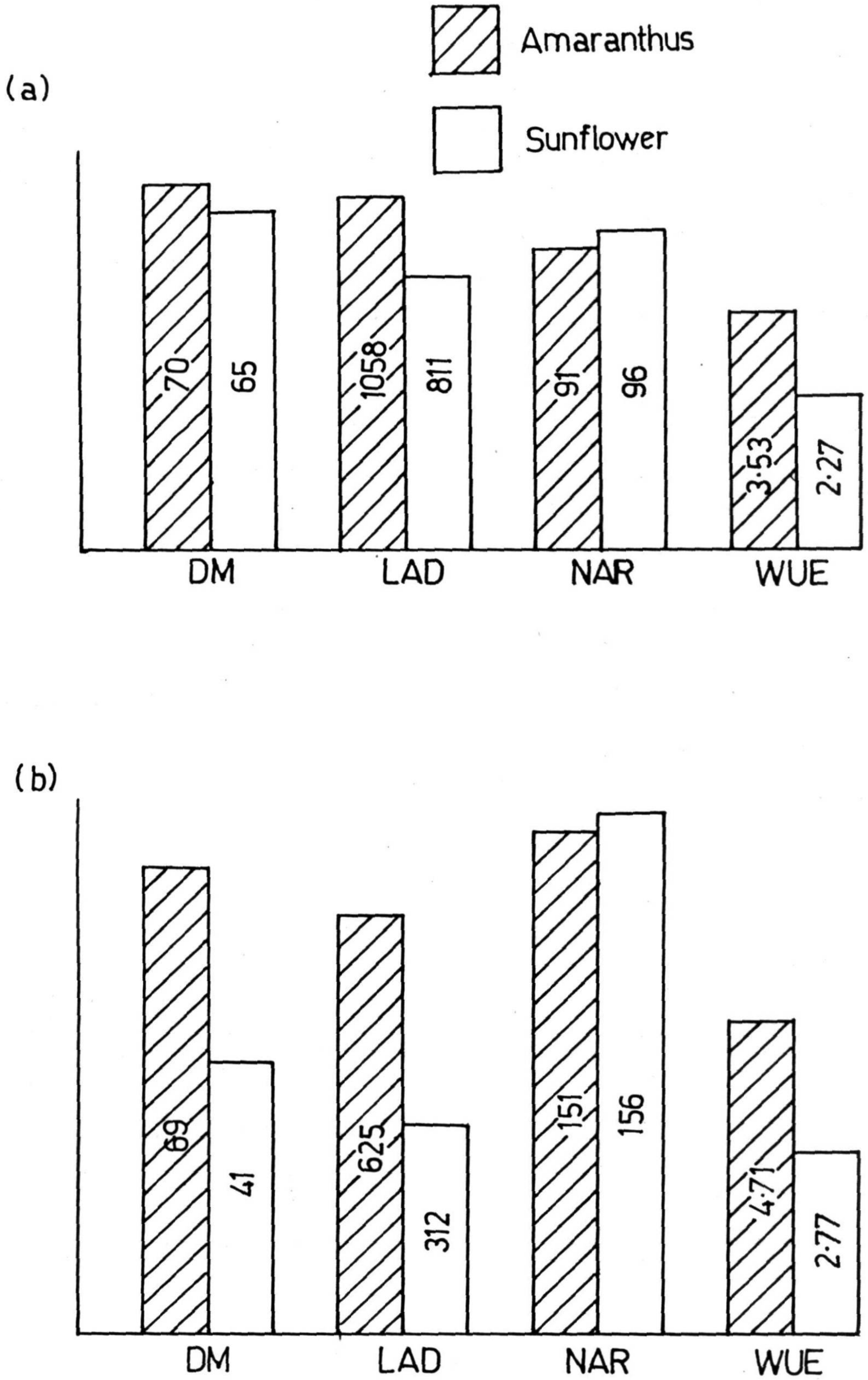

FIG. 2 DIFFERENCES IN DM, LAD, NAR AND WUE IN AMARANTHUS AND SUNFLOWER PLANTS MAINTAINED AT (a) NEAR FC AND (b) WHEN ADEQUATE BUT UNIFORM INPUT OF WATER WAS GIVEN.

as evidenced by higher LAD values recorded in $C_4$ species. Only one $C_3$ species (cowpea) had higher LAD than other two $C_3$ species and close to a $C_4$ species (amaranthus). This was possibly due to maintenance of higher leaf water status through lower conductances in cowpea compared to sunflower. Mean rate of water loss in cowpea was 24.4 as against 47.41 in sunflower whereas LAD was higher in cowpea (606.42) than in sunflower (312.50).

The results of this experiment further emphasise that higher WUE could be one factor which determines the biomass production when the input of water is limited as it often occurs under rainfed conditions.

In both the experiments where WUE was basically derived based on CWT (or CWA) and $\Delta$ DM, $C_4$ plants had significantly lower TQ. This was mainly due to higher assimilation per unit conductance as evidenced by data on A, $g_s$, DM/LAD and CWA/LAD. Though the differences in DM/LAD and A were not marked low $g_s$ was responsible for higher A/$g_s$ values and thus higher WUE. Though A/$g_s$ reflects assimilation per unit conductance indirectly it is a reflection of mesophyll conductance and Ci. Since the Ci is the sum effect of factors associated with substomatal $CO_2$ translocation and its assimilation, a higher WUE in $C_4$ species is predominantly due to higher mesophyll conductances.

The results obtained further confirm the importance of mesophyll conductances (Ci) for differences in the intrinsic WUE observed in $C_3$ and $C_4$ species. It has been well established that Ci in $C_4$ plants is less compared to $C_3$ and it was attributed to higher mesophyll factors. 'A'/Ci value which is a reflection of carboxylation efficiency is always high in $C_4$ plants whereas it is low in $C_3$ indicating that a higher Ci is required to maintain a known 'A' in $C_3$ plants. In general $g_s$ Vs Ci relationship has been shown in $C_3$ plants. So 'A' can be enhanced by enhancing the Ci which in turn can be enhanced by a higher $g_s$. However, such a stomatal behaviour enhances the rate of water loss. So one of the prerequisites to enhance the WUE in $C_3$ type of plants is to identify types with higher A/Ci ratios.

Amongst the $C_3$ species the data on gas exchange indicates that in cowpea $A/g_s$ values are more and the values were in between sunflower, a $C_3$ and amaranthus, a $C_4$ species. However, the DM/LAD and CWA/LAD data did not show a similar trend. Some more experiments are needed to confirm the data obtained in this experiment.

### Effect of low (but uniform) input of water on WUE in different species

Under stress, when input of water was low, total biomass produced was less. However, biomass gain per unit water transpired increased under stress. The mean WUE in these six species under nonstressed conditions was 3.5766, and under stressed conditions it was 3.9455, thus recording a 10.31 per cent increase over control.

A number of field studies conducted have shown that under stress conditions, WUE decreased (Sinclair _et_ _al_., 1975; Sivakumar _et_ _al_., 1979; Garrity _et_ _al_., 1982). A decrease in WUE under stress is attributed to the effect of increased VPD and temperature associated with water stress, on the rate of water loss. A higher VPD increases the transpiration without altering the carbon gain much and thus decreases WUE (Rawson _et_ _al_., 1977; Zur and Jones, 1984; Mabrouk and Jones, 1984). But in several other studies an increase in WUE under stress was shown (Sivakumar _et_ _al_., 1979; Cavagnavo and Jain, 1985). Though the reports on the effect of stress on WUE are conflicting it is possible that WUE increases under stress because often root biomass increases under stress which was not included for field estimations of WUE.

In the present study, micrometeorological parameters like VPD and temperature were similar for all crops grown under two moisture regimes. Even root DM was recorded in the second experiment. However, even in the first experiment where root DM was not included, an increase in WUE under stress was noticed emphasising further that WUE, under mild stress generally increases.

Under stress when tissue water potential decreases both assimilation rate and conductance decrease. Although the carbon exchange rate (CER) and transpiration rates are affected due to increased $r_s$, the transpiration is affected more than CER because, the stomatal resistances are relatively larger for water vapour diffusion than for $CO_2$ (Gaastra, 1959; Bierhuzien, 1976). However, high WUE can be achieved only when mesophyll resistances are low and when it is not increased much under stress (Jones, 1976; Turner, 1986). In other words, the inevitable increase in stomatal resistance ($r_s$) as a result of decrease in leaf turgor in plants subjected to stress should not be associated with a concomitant increase in mesophyll resistance. Therefore depending on the relative effect of moisture stress on mesophyll conductance and stomatal conductance, the WUE either increases or decreases (Farquhar and Richards, 1984; Guy and Reid, 1986).

Therefore, a greater decrease in stomatal conductance than mesophyll conductance in plants subjected to water stress might have resulted in an increased water use efficiency (Farquhar and Richards, 1984; Guy and Reid, 1986). But when mesophyll conductance was decreased to a greater extent a decrease in WUE was reported (Sinclair, 1975). The stomatal regulation of water loss by decreasing the stomatal conductances in plants subjected to moisture stress will result in an increase in WUE when concomitant decrease in mesophyll factors is not observed.

Among the six species used for the second experiment, the mean reduction in assimilation (DM/LAD) was very marginal (3.97%) in cowpea, amaranthus and ragi. But they recorded a mean increase of 13.05 per cent in WUE under stress. The mean transpiration rate (CWA/LAD) decreased by 14.91 per cent under stress. This indicates that in the above three species the effect of stress was more pronounced on stomatal factors while the mesophyll stability was maintained. This resulted in a higher increase in WUE under stress. In the remaining three species (sunflower, soybean and sorghum) mean DM/LAD decreased by 15.63 per cent, CWT/LAD by 17.20 per cent and WUE increased only by 7.09 per cent meaning both the stomatal and nonstomatal factors were affected to almost similar extent resulting in a lesser increase in WUE under stress.

In both the experiments, in general WUE increased in all the species under stress. Results from the second experiment in Fig.3 indicate that $g_s$ (or CWA/LAD) decreased

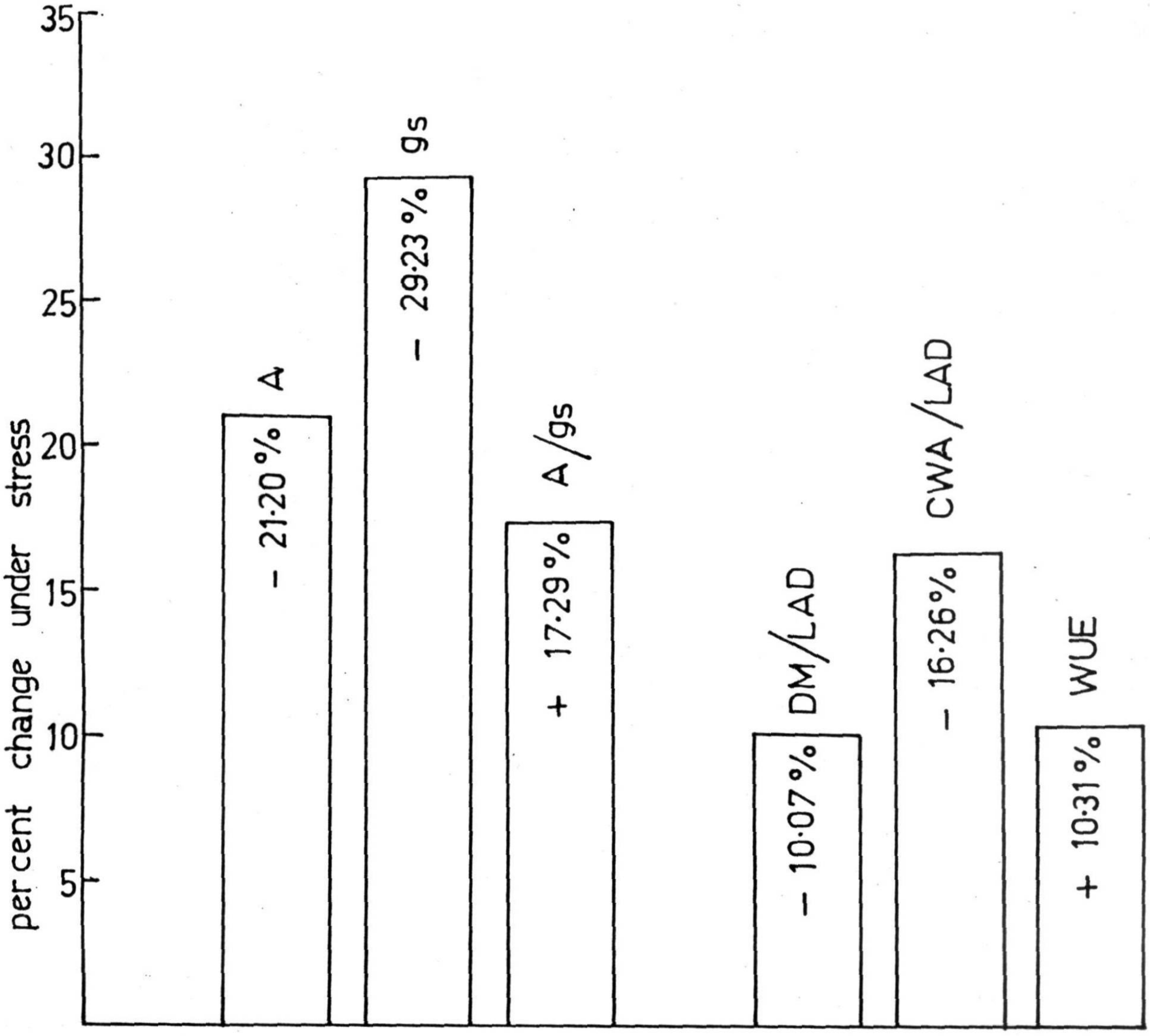

FIG. 3 CHANGE IN PHYSIOLOGICAL AND GAS EXCHANGE TRAITS ASSOCIATED WITH WUE, UNDER STRESS. (Values are means of all six species)

to a greater extent under stress than 'A' (or DM/LAD). The mean increase in the two physiological traits. DM/LAD and CWA/LAD was 10.07 and 16.26 per cent respectively. Data on gas exchange parameters also shows a similar trend. In other words mesophyll factors were maintained to a greater extent under stress in this type of study than stomatal factors. Therefore a greater decrease in stomatal conductances seems to be the major contributing factor for an increase in WUE under stress. Keeping this in view, an attempt was made to study the stomatal sensitivity of the six species included in the second experiment.

Amaranthus and soybean were the two species which recorded similar decreases in DM/LAD under stress. In other words, in these two species effect of stress on mesophyll was to the same degree. But the transpiration decreased to a greater extent in amaranthus (21.94 per cent) than in soybean (11.75 per cent). This greater decrease in water loss in amaranthus was also associated with a greater increase in WUE (13.70 per cent) under stress. It can be concluded here that, under similar intensities of stress effect on the nonstomatal factors, the species (amaranthus) with better control of water loss can achieve higher WUE under stress.

### Differences in stomatal sensitivity to light in selected $C_3$ and $C_4$ species

Changes in stomatal behaviour in response to changes in environmental factors is an adaptation to optimize carbon assimilation and water loss. The threshold level for an environmental factor for decreasing stomatal conductance or closing the stomata may vary in different species. Often the species which show a higher threshold for a particular environmental factor for a decrease in stomatal conductance are termed as sensitive crops for stomatal behaviour. The stomatal sensitivity can be assessed by altering either ambient $CO_2$ concentration or light intensity or tissue water status. In this experiment an attempt was made to assess the relative sensitivity of these species to light with regards to their stomatal behaviour, stomatal sensitivity was assessed in terms of the change in the stomatal resistance when a plant is transferred from high light intensity to low light.

In general the $C_4$ species showed more sensitivity. Amongst the $C_3$, sunflower was least sensitive,cowpea and soybean also showed greater sensitivity of stomata when transferred from high light to low light intensities. However, the data obtained on stomatal sensitivity did not show any relationship to observed reductions in rates of water loss in plants subjected to stress. For example, in sunflower, the decrease in rate of water loss (CWA/LAD) under stress was 15.25 per cent whereas decrease in stomatal conductance when transferred to low light was 21.75 per cent. The corresponding values for soybean were 11.75 per cent for CWA/LAD and 97.48 per cent for conductance.

There are several evidences to show that changes in Ci due to alterations in environmental factors regulate stomatal conductances. Although any environmental stress like decrease in light intensity, inadequate nutrition, low tissue water potential affect mesophyll factors resulting in an increase in Ci which in turn has a feedback control on $g_s$ (Farquhar and Richards, 1984). However, the feedback control of Ci on $g_s$ may not be the same in all environmental stresses. As reported by James (1988) the relation between Ci and $g_s$ is weak under low light and inadequate nutrition but very strong under moisture stress. Per unit decrease in Ci, $g_s$ is affected to a greater extent under stress. So depending on the environmental factor, feedback controls of stomata may vary. Hence, the data got for sensitivity to light cannot be extrapolated to the data observed under water stress.

The results of this experiment indicate that amongst the three $C_3$ species studied the differences in stomatal sensitivity to light was not related to observed changes in CWA/LAD under stress. Some more experiments need to be conducted to relate the differential sensitivities of the stomata to light stress and other abiotic stresses to understand the Ci versus $g_s$ relationship in different species or varieties.

### Physiological and gas exchange traits associated with WUE in six species at a given input of water

To arrive at the importance of different physiological traits associated with WUE the correlations between TQ and different growth and gas exchange characters were determined.

### The relationship between WUE under stressed and nonstressed conditions

Correlation was done to study the relationship between TQ under stressed and nonstressed conditions.

When all the six species were analysed together, there was a very high relation between control and stress suggesting that intrinsically low TQ types are advantageous.

In $C_3$ crops, TQ under control was related to TQ under stress with a 'r' value of 0.413. However, the relation was very weak in $C_4$ crops. This means that a species with a higher TQ in control can have a lower TQ under stress and vice versa.

Physiological traits associated with TQ

The experiment involved species which had intrinsically greater variation in TQ and certain growth parameters like rate of water loss and NAR. So the relationship between growth and physiological characteristics with TQ has been studied not only in the pooled data from all the six species, but also separately in the $C_3$ and $C_4$ species.

When all the species were studied together a negative relation was seen between TQ and TDM. TQ was significantly related to NAR with a 'r' value of -0.551. A positive relationship was seen between rate of loss (CWA/LAD) and TQ meaning lesser the rate of transpirational water loss, higher is the WUE.

In the $C_3$ species TQ had a negative relation with CWA/LAD too. This meant that it is not only the rate of water loss but also the NAR that contributes for higher WUE. This is evident in sunflower which recorded the highest CWA/LAD but simultaneously it also recorded the highest NAR.

Unlike the $C_3$ species, the $C_4$ crops showed a positive relation between TQ and NAR and also between TQ and CWA/LAD.

The correlation data indicate that both NAR and rate of water loss contribute for the differences in WUE. Such a thing emerged when the correlations were made for all the six species. However, when the $C_4$ species alone were compared the differences in CWA/LAD seems to be an important physiological parameter contributing for variations in WUE. Similarly in $C_3$ species the NAR differences seems to be the major factor contributing for differences in WUE.

## Relationship between gas exchange characteristics and growth characters associated with TQ

The two gas exchange parameters, assimilation (A) and conductance ($g_s$) were recorded using a LICOR-1600 portable photosynthesis system and $A/g_s$, a reflection of intrinsic WUE was computed. These instantaneous measurements were related to the growth characters which reflect these values.

When the relations were studied in all the six species together, 'A' showed a significant positive relation with NAR, $g_s$ showed a positive relation with rate of water loss (CWA/LAD) and $A/g_s$, a reflection of instantaneous measurement of WUE showed a significant negative relation with TQ. Similar relations were seen between A and NAR, $g_s$ and CWA/LAD and $A/g_s$ and TQ in the three $C_3$ crops. However, in the $C_4$ species the relation between $A/g_s$ and TW was not significant. Within each group of either $C_3$ or $C_4$ 'A' was positively related with $g_s$. This meant that as $g_s$ increased, Ci increased promoting higher assimilation rates.

In recent years WUE and TW are being assessed by determining the assimilation and stomatal conductance in a single leaf. By using this technique, differences in WUE among the species and the effect of environmental factors were studied (Fischer and Turner, 1978; Rawson et al., 1977; 1978; Sinclair et al., 1975; Osmond, 1980).

Osmond (1980) suggested the term intrinsic WUE for $A/g_s$ ratio to compare the species differences in WUE. He observed that under favourable environmental conditions, the $C_4$ plants have WUE two and half to three times to those of $C_3$ plants. This was correlated with DM produced between the two crop species. In the same study he highlighted the importance of $A/g_s$ ratio under moisture stress conditions when partial closure of stomata occurs.

Even in the present study the $C_4$ species exhibited a higher WUE and their higher WUE was significantly related to higher $A/g_s$ ratios recorded by the $C_4$ crop species.

Thus, the significant relationship observed in these experiments between instantaneous gas exchange characteristics and the growth characters associated with TQ in the six species again emphasises that instantaneous gas exchange measurements are related to similar physiological traits determined over a specific growth period. This suggests that TQ is controlled strongly even at the whole plant level due to variations in assimilation to conductance ratios.

## Conclusion

The experiments conducted in this study further confirm that WUE is distinctly more in $C_4$ species like amaranthus, sorghum and ragi compared to $C_3$ species like sunflower, cowpea and soybean.

By using containers and determining CWT (or CWA) and DM for a specific growth period the differences in WUE amongst the species can be precisely determined. WUE, mean rate of water loss ($\frac{\text{CWA}}{\text{LAD}}$ or CWT/LAD) and mean assimilation rate for the entire growth period (DM/LAD) determined at the whole plant level in container studies showed a significant relationship with the instantaneous gas exchange characters like $A/g_s$, A and $g_s$.

When the plants were grown at near field capacity the difference in biomass between amaranthus and sunflower was marginal though WUE was distinctly different. However, at a given input of water the biomass production was significantly higher in amaranthus. Similar trend which was noticed in other $C_4$ species compared to $C_3$ species suggests that under rainfed conditions when input of water is same for all crops, species or genotypes with high WUE may have a specific advantage.

The leaf area (LAD) the rate of water loss (CWT/LAD or $g_s$) and assimilation rate (NAR or A) determine the DM produced and differences in TQ. Intrinsically low $g_s$ facilitates higher moisture conservation which helps in greater leaf expansion rates resulting in high LAD and thus DM at a given input of water. At all water regimes, high WUE in $C_4$ is predominantly due to low rate of water loss and marginally due to higher assimilation rate (DM/LAD).

WUE increased under stress in all species, such a thing was seen either when plants were grown at a lower water regime (60 per cent field capacity) or when all plants were given a reduced uniform input of water. Increase in WUE in stressed plants is due to higher assimilation per unit amount of water lost. However, the extent of increase in WUE differed among the species. Highest increase was seen in cowpea followed by amaranthus, sorghum, ragi, soybean and sunflower. In cowpea, amaranthus and ragi the greater increase in WUE was due to a greater decrease in water loss and a concomitant lesser decrease in assimilation rate. Whereas the lesser increase in WUE under stress in the remaining three species (sunflower, soybean and sorghum) was associated with a substantially greater decrease in assimilation rate also as compared to cowpea, amaranthus and ragi. In general increased WUE under stress was associated with a greater decrease in rate of water loss compared to the assimilation rate. Stability of the mesophyll factors and greater sensitivities of the stomata seem to be important to achieve higher carbon gain per unit amount of water lost under stress.

An attempt made to assess relative sensitivity of species based on stomatal sensitivity to light did not show any relationship between light sensitivities and extent of decrease in rate of water loss under stress.

The results indicate that species or genotypes with higher WUE may have a specific advantage under limited input of water which is a characteristic feature of kharif rainfed conditions. It is worthwhile to identify amongst the $C_3$ species, the ones with higher A/Ci ratios which results in higher assimilation rates per unit conductances. Under stress greater stability of mesophyll factor and higher sensitivity of stomatal factors are desirable.

# SUMMARY

# VI. SUMMARY

In the present investigation pot culture experiments were conducted to study the differences in WUE and the different physiological and gas exchange traits contributing to WUE in selected $C_3$ (sunflower, cowpea and soybean) and $C_4$ (amaranthus, sorghum and ragi) species. The biomass accumulated and cumulative water transpired (or added), for a given experimental period were determined. From the data on DM and CWT (or CWA) and LAD, the TQ, WUE, rate of water loss, NAR and DM/LAD ratios were computed. Using a Portable IRGA (LI-6000) gas exchange parameters like 'A' and $g_s$ were determined and $A/g_s$ ratios were computed and related to similar physiological traits.

The first experiment was conducted with the objective of determining the differences in WUE in sunflower and amaranthus and also to identify the growth and physiological, and gas exchange traits associated with TQ. The experiment was done employing a gravimetric technique and the WUE differences were studied at two moisture regimes (FC and 60 per cent FC). A parallel experiment was conducted to assess the intrinsic differences if any, in 'A' and $g_s$ in sunflower and amaranthus subjected to different degrees of moisture stress.

Based on the results obtained in these two experiments, the conclusions are;

1. The TQ was less in amaranthus than in sunflower when plants were grown either at field capacity or at 60 per cent FC.
2. CWT recorded by the two species at any given moisture regime differed markedly but the $\Delta$ DM differences were not significant.
3. In plants grown at 60 per cent field capacity, TQ decreased in both the species. Per cent reduction was more in amaranthus (9.12%) compared to sunflower (3.57%).
4. Though the DM and LAD decreased in both the species under stress, the decrease was to a lesser extent in amaranthus. Per cent decrease in NAR was similar in both the species. But the intrinsic low CWT/LAD and its greater decrease at 60 per cent FC was responsible for better soil moisture conservation which possibly facilitated a greater LAD and its metabolic activity leading to higher DM production, at 60 per cent FC in amaranthus.

5. Gas exchange measurement of $g_s$ was related to TW with a 'r' value of 0.967, and the rate of water loss (CWT/LAD) which reflects the mean transpiration rate was related to TW with an 'r' value of 0.954.

6. In amaranthus the assimilation per unit conductance was higher than in sunflower.

7. Stomatal conductance ($g_s$) measured by porometry showed a significant positive relation with rate of water loss (r = 0.964). A negative significant relation was seen between $A/g_s$ and TW (r = -0.965).

8. Sunflower showed a higher 'A' as well as $g_s$ values than amaranthus at any given leaf water potential but the effect of a given degree of stress on 'A' was same in both the species indicating no superiority in terms of greater mesophyll stability in any one species over the other, under stress.

In the second experiment the WUE differences in three $C_3$ species and three $C_4$ species were studied. The objective here was to simulate the rainfed field situation by providing same input of water to all the crops. WUE differences were studied at two given inputs of water (one near field capacity and the other 60 per cent of the first). So, unlike in the first experiment where a gravimetric technique was adopted, here WUE was computed based on CWA and $\Delta$ DM during a specific growth period. Other growth and gas exchange traits were recorded as in the first experiment. Here, for WUE computation even root DM was included. Parallelly, to see if the observed reductions in TW under low input of water was related to stomatal sensitivity of these species, another experiment was conducted on stomatal sensitivity to light. Based on the results from the two experiments, the conclusions are:

1. In this experiment also the TW in $C_4$ species was significantly less. When the input of water was relatively adequate the TW values for different species were 213 (amaranthus), 210 (sorghum), 223 (ragi), 361 (sunflower), 430 (cowpea) and 428 (soybean) and when it was relatively inadequate the corresponding TW values were 187, 184, 205, 360, 351 and 418.

2. At any given water input the $C_4$ species recorded a higher DM owing to their higher WUE indicating the potentiality of $C_4$ species in DM production, under water lilited conditions.

3. WUE increased under stress (at low water input) in all the six species. However, the extent of increase

in WUE differed among the species. Highest increase was seen in cowpea (18.96%) followed by amaranthus, sorghum, ragi, soybean and sunflower. In general increased water use efficiency under stress was associated with a greater decrease in rate of water loss compared to the assimilation rate. The mean increase in WUE under low water input was 10.31 per cent; DM/LAD and CWA/LAD decreased by 10.07 per cent and 16.26 per cent respectively.

4. Stomatal sensitivity to light was in general high in $C_4$ species but even soybean and cowpea showed relatively higher sensitivity than sunflower. The relative sensitivity of the species based on stomatal sensitivity to light did not show any relationship between light sensitivity and extent of decrease in rate of water loss under moisture stress.

5. When correlations were made for all the six species, both NAR and CWA/LAD seemed to contribute for differences in WUE. However, in $C_4$ species, differences in rate of water loss seemed to be an important physiological parameter contributing for variations in WUE. Similarly in $C_3$ species the NAR

differences seemed to be the one contributing for variations in WUE.

6. Instantaneous gas exchange measurements showed a significant relationship with similar physiological traits determined for the entire growth period. Thus 'A' was related to NAR (r = 0.871), $g_s$ with CWA/LAD (r = 0.914) and TQ with $A/g_s$ (r = -0.916) Also within each group of either $C_3$ or $C_4$ species 'A' was positively related with $g_s$ indicating that as $g_s$ increased, Ci increased promoting higher assimilation rates.

From the first and the second experiment the following four conclusions can be drawn;

1. Both by maintaining plants at a particular soil moisture level by adopting gravimetric technique and also when a known input of water was given for a specific growth period, distinct differences in WUE can be precisely determined by assessing the CWT (or CWA) and $\Delta$ DM. The WUE determined by gas exchange parameters using IRGA also showed a relationship with TQ, indicating that even at the whole plant level TQ is controlled by ratio of assimilation to water loss.

2. When soil moisture is not a limiting factor (when plants are grown near field capacity) the biomass differences between sunflower and amaranthus were not marked, though WUE differed. However, at a given input of water (high or low) the DM produced was significantly higher in amaranthus. Similar trend which was noticed in other $C_4$ species compared to $C_3$ species suggests that under rainfed conditions when input of water is same for all the crops, species or genotypes with high WUE may have a specific advantage in producing high biomass.

3. The $C_4$ plants have a high A/Ci ratios which facilitate high WUE. It is worthwhile to identify from among the $C_3$ species the ones with high A/Ci ratio which results in higher assimilation per unit conductance .

4. Under water limited conditions in all species there is a tendency to increase assimilation per unit conductance resulting in a decrease in TQ. To achieve a high WUE under stress a greater stability of mesophyll factors and a higher sensitivity of stomatal factors are desirable.

# REFERENCES

# VII. REFERENCES

Arun, M.B., 1985, Genotypic differences in physiological characteristics associated with water use efficiency in soybean, M.Sc.(Agri.) thesis, Univ. Agric. Sci., Bangalore.

Baker, N.D. and Robert, B. Musgrave, 1964, The effect of low level moisture stresse on the rate of apparent photosynthesis in corn, Crop Sci., 4: 249-253.

Bierhuzien, J.F., 1976, Irrigation and water use efficiency. In: Water and plant life. Eds. O.L. Lange, L. Kappen and E.D. Schulze, Springer - Verlag, Berlin, pp.421-431.

Blake, T.J., Tschaplinski, T.J. and Eastham, A., 1984, Stomatal control of water use efficiency in poplar clones and hybrids, Can. J. Bot., 62: 1344-1351.

Blum, A. and Sullivan, C.Y., 1986, The comparative drought resistance of land races of sorghum and millet from dry and humid regions, Ann. Bot., 57: 835-846.

Briggs, L.J. and Shantz, H.L., 1914, J. Agric. Res., 3: 1-64.

Brouwer, R., 1981, Effect of environmental conditions on root functioning, Acta. Hortic., 119: 91-101.

Brown, R.H. and Simmons, R.L., 1979, Photosynthesis of grass species differing in $CO_2$ fixation pathways. I.. Water use efficiency, *Crop Sci.*, **19**: 375-379.

Caldwell, M.M., White, R.S., Moore, R.T. and Camp, L.B., 1977, Carbon balance, productivity and water use of cold winter desert shrub communities dominated by $C_3$ and $C_4$ species, *Oecologia*, **29**: 275-300.

Cavagnavo, J.B. and Jain, S.K., 1985, Effect of water stress in four amaranthus species, *Amaranth*, News Letter No.3.

Chatterson, N.J., Hanna, W.W., Power, J.B. and Lee, D.R., 1975, Photosynthesis and transpiration of bloom and bloomless sorghum, *Can. J. Plant Sci.*, **55**: 641-643.

Chu, A.C.P. and McPhe son, H.G., 1977, Sensitivity to desiccation of leaf extension in prairie grass, *Aust.. J. Plant Physiol.*, **4**: 381-387.

Constable, G.A. and Rawson, H.M., 1980, Effect of leaf position, expansion and age on photosynthesis, transpiration and WUE of cotton, *Aust . J. Plant Physiol.*, **7**: 89-100.

Clough, B.F. and Milthrope, F.L., 1975, Effects of water deficit in leaf development in tobacco, *Aust. J. Plant Physiol.*, **2**: 291-300.

Downes, R.W., 1970, Effect of light intensity and leaf temperature on photosynthesis and transpiration in wheat and sorghum, *Aust. J. Biol. Sci.*, **23**: 775-782.

Das, V.S.R. and Shantakumari, M., 1977, Stomatal characteristics of some dicotyledonous plants in relation to the $C_4$ and $C_3$ pathways of photosynthesis, *Plant and Cell Physiol.*, **18**: 935-938.

Eastin, J.D., Sullivan, C.Y. and Francis, C.A., 1982, Variation in crop physiological and morphological characteristics controlling water use efficiency and grain yield, Report of water research and technology, U.S. Department of the Interior, Washington, D.C.

Evans, J.R., Sharkey, T.D., Berry, J.A. and Farquhar, G.D., 1986, Carbon isotope discrimination measured concurrently with gas exchange to investigate $CO_2$ diffusion in leaves of higher plants, *Aust. J. Plant Physiol.*, **13**: 281-292.

Evenari, M., Lange, O.L., Schulze, E.-D., Kappen, L. and Buschbom, U., 1977, Net photosynthesis, dry matter production and phenological development of apricot trees (*Prunus armeniaca* L.)cultivated in Negev Highlands (Israel). *Flora*, **166**: 383-414.

Farquhar, G.D. and Richards, R.A., 1984, Isotope composition of plant carbon correlates with water use efficiency of wheat genotypes, Aust. J. Plant Physiol., 11: 539-552.

Fischer, R.A. and Turner, N.C., 1978, Plant productivity in the arid and semi-arid zones. Ann. Rev. Plant Physiol., 29: 277-317.

French, R.J. and Schultz, J.E., 1984, Aust. J. Agri. Res., 35: 743-775.

Gaastra, P., 1959, Photosynthesis of crop plants as influenced by light, carbon dioxide, temperature and stomatal diffusion resistances, Meded Landbuwhogesch, Wageningen, 59: 1-68.

Garrity, D.P., Darrel, G.W., Sullivan, C.Y. and James, R., 1982, Moisture deficits and grain sorghum performance. Evapotranspiration yield relationships, Agron. J., 75: 815-820.

Gifford, R.M., 1979, Growth and yield of $CO_2$ enriched wheat under water limited conditions, Aust. J. Plant Physiol., 6: 367-378.

Gifford, R.M. and Morison, J.L., 1985, Photosynth. Res., 7: 69-76.

Goudriaan, J. and Larr, H.H. Van., 1978, Relations between leaf resistance, $CO_2$ concentration and $CO_2$ assimilation in maize, beans, lalang grass and sunflower, Photosynthetica, 12: 241-249.

Gregory, F.G., 1926, The effect of climatic conditions on the growth of barley, Ann. Bot., 40: 1-26.

Guy, R.D. and Reid, D.M., 1986, Photosynthesis and the influence of $CO_2$ enrichment on $\delta^{13}C$ values in $C_3$ halophyte, Plant, Cell and Enrivonment, 9: 65-72.

Hasegawa, S., 1977, Agroclimatological studies in $C_3$ plants and $C_4$ plants, "Transpiration rates and leaf temperatures", J. Agri. Meteorology, 33: 129-136.

Hattendorf, M.J., Redelfs, M.S., Amos, B., Stone, L.R. and Gwin, R.E., 1988, Comparative water use characteristics of six row crops, Agron. J., 80: 80-85.

Hitoshi Oguro, Kokichi Hin ata and Shigesaburo Tsunoda, 1985, Comparative anatomy and morphology of leaves between $C_3$ and $C_4$ species in Panicum, Annals of Botany, 55: 859-862.

Huibick, K.T., Farquhar, G.D. and Shorter, R., 1986, Correlation between water use efficiency and carbon isotope discrimination in diverse peanut (Arachis) germplasm, Aust. J. Plant Physiol., 13: 803-816.

Hunt, R., 1983, In: Plant growth curves: The functional approach to plant growth analysis, Ed. R. Hunt, Edward Arnold, London.

James Jacob, 1988, Quantification of stomatal and mesophyll limitations of photosynthesis under stress - A mathematical approach, Ph.D. thesis, Univ Agri. Sci., Bangalore.

Johnson, D.A., Richards, R.A. and Turner, N.C., 1983, Yield water relations, gas exchange and surface reflectances of near isogenic wheat lines differing in glaucousness, Crop Sci., 23: 318-325.

Jones, H.G., 1976, J. Applied Ecology, 13: 605-622.

Jones, P., Allen, L.H. Jr. and Jones, J.W., 1985, Responses of soybean canopy photosynthesis and transpiration to whole day temperature changes in different $CO_2$ environments, Agron. J., 77: 242-248.

Khairi, M.M.A. and Hall, A.E., 1976, Temperature and humidity effects on net photosynthesis and transpiration of citrus, Physiol.Plant, 36: 29-34.

Kimball, B.A. and Idso, S.B., 1983, Increasing $CO_2$: Effects on crop yield, water use and climate, Agric. Water Manage, 7: 55-72.

Kramer, P.J., 1980, Drought stress, and the origin of adaptations,. In: Adaptation of plants to water and high temperature stress. Eds. N.C. Turner and P.J. Kramer, Wiley, New York, pp.7-20.

Ku, S.B. and Edward, G.E., 1978, Photosynthetic efficiency of Panicum hians and P. milioides in relation to $C_3$ and $C_4$ plants, Plant and Cell Physiol., 19: 665-675.

Lawes, J.B., 1850, J. Hortic. Soc., London, 5: 38-63.

Ludlow, M.M. and Wilson, G.L., 1972, Photosynthesis of tropical pasture plants. IV. Basis and consequences of differences between grasses and legumes, Aust. J. Biol. Sci., 25: 1133-1145.

Mabrouk, A. El-Sharkawy and James, H. Cock, 1984, Water use efficiency of cassava. I. Effect of air humidity and water stress on stomatal conductance and gas exchange, Crop Sci., 24: 497-502.

Marion, H. O'Leary, 1981, Carbon isotope fractionation in plants, Phytochemistry, 20: 553-567.

Masle, J. and Farquhar, G.D., 1988, Effects of soil strength on the relation of WUE and growth to carbon isotope discrimination in wheat seedlings, Plant Physiol., 86: 32-38.

Masle, J. and Passioura, J.B., 1987, The effect of soil strength on the growth of young wheat plants, Aust. J. Plant Physiol., 14: 643-656.

Morgan, J.A., 1986, The effects of N nutrition on the water relations and gas exchange characteristics of wheat (*Triticum aestivum* L.), Plant Physiol., 80: 52-58.

Morison, J.L. and Gifford, R.M., 1984, Plant growth and water use with limited water supply in high $CO_2$ concentrations, II. Plant dry weight, partitioning and water use efficiency, Aust.J. Plant Physiol., 11: 375-384.

Nagamani, N., 1985, Genotypic variation in plant conductances, transpiration and their relationship to water use efficiency in cowpea (*Vigna ung iculata* (L.) Walp.), M.Sc.(Agri.) thesis, Univ. Agri. Sci., Bangalore.

Nobel, P.S., 1980, Leaf anatomy and water use efficiency. In: Adaptation of plants to water and high temperature stress, Eds. N.C. Turner and P.J. Kramer, Wiley, New York, pp.43-55.

Olson, R.A., Hanway, D.G. and Dreier, A.F., 1960, Nebraska Agr. Expt. Sta. Quart., 7: 7-9.

Osmond, C.B., Bjorkman, O. and Anderson, D.J., 1980, "Physiological processes in plant ecology". Springer-Verlag, Berlin and New York.

Paleg, L.G. and Aspinall, D., 1981, The physiology and biochemistry of drought resistance in plants, Academic Press, New York.

Palta, J.A., 1984, Influence of water deficits on gas exchange and the leaf area development of cassava cultivars, *J. Expt. Bot.*, **35**: 1444-1449.

Pandey, S.L., Sinha, A.K., Alimchandani, K.C. and Krishna Murari, 1977, Effect of levels of nitrogen and phosphorous on the yield and water use efficiency of wheat (Sonalika) under shallow water table conditions, *Indian J. Agron.*, **22**: 12-15.

Passioura, J.B., 1983, Roots and drought resistance, *Agric. Water Manage.*, **7**: 265-280.

Patterson, D.J., 1986, Responses of soybean (*Glycine max*) and three $C_4$ grass weeds to $CO_2$ enrichment during drought, *Weed Sci.*, **34**: 203-210.

Rathore, S.S. and Singh, R.M., 1977, Water use efficiency and water use pattern as influenced by moisture regimes, N and P fertilization by dwarf wheat, *Indian J. Agron.*, **22**: 181-182.

Rawson, H.M., 1979, Vertical wilting and photosynthesis, transpiration and water use efficiency of sunflower leaves, Aust. J. Plant Physiol., 6: 109-120.

Rawson, H.M. and Begg, J.E., 1977, The effect of atmospheric humidity on photosynthesis, transpiration and WUE of leaves of several plant species, Planta, 134: 5-10.

Rawson, H.M., Begg, J.E. and Woodward, R.G., 1977, The effect of atmospheric humidity on photosynthesis, transpiration and water use efficiency of leaves of several plant species, Planta, 134: 10-15.

Rawson, H.M., Constable, G.A. and Howe, G.M., 1980, Carbon production of sunflower cultivars in field and controlled environments, II. Leaf growth, Aust. J. Plant Physiol., 7: 575-586.

Rawson, H.M., Turner, N.C. and Begg, J.L., 1978, Agronomic and physiological responses of soybean and sorghum crops to water deficits. V. Photosynthesis, transpiration and water use efficiency of leaves, Aust. J. Plant Physiol., 5: 195-209.

Sashidhar, V.R., 1987, Physiological characteristics associated with water use efficiency in six small millets and in genotypes of finger millet (*Eleusine coracana* Gaertn.) Ph.D. thesis, Univ. Agric. Sci., Bangalore.

Schultz, J.E., 1974, Root development of wheat at the flowering stage under different cultural practices, Agric. Res., 1: 12-17.

Schulze, E. -D., Lange, O.L., Kappen, L., Evenari, M. and Buschbom, U., 1975, The role of air humidity and temperature in controlling stomatal resistance of *Prunus armeniaca* L. under desert conditions. III. The effect on water use efficiency, Oecologia, 19: 303-314.

Schulze, E.-D., Lange, O.L., Kappen, L., Buschbom, U. and Evenari, M., 1973, Stomatal responses to changes in temperature at increasing water stress, Planta, 110: 29-42.

Shackel, K.A. and Hall, A.E., 1979, Reversible leaflet movements in relation to drought adaptation of cowpea *Vigna ung iculata* (L.) Walp. Aust. J. Plant Physioll, 6: 265-276.

Shantz, H.L. and Piemeisel, L.N., 1927, The water requirement of plants at Akron, Colorado, J. Agric. Res., 34: 1093-1190.

Slatyer, R.O., 1973, The effect of internal water status on plant growth, development and yield, In: Plant responses to climatic factors. Ed. R.O. Slatyer, Proc. Uppsala Symp., UNESCO., Paris, pp. 177-188.

Sinclair, T.R., Bingham, G.E., Lemon, E.R. and Allen, L.H. Jr., 1975, Water use efficiency of field grown maize during moisture stress. Plant, Physiol., 56: 245-249.

Singh, B.P. and Whitson, E.N., 1976, Evapotranspiration and water use efficiency of soybean lines differing in growth habit, Agron. J., 68: 834-845.

Sivakumar, M.V.K., Seetharama, N., Sodor Singh and Bidinger, F.R., 1979, Water relations, growth and dry matter accumulation of sorghum under post-rainy season conditions, Agron. J., 71: 843-847.

Slatyer, R.O., 1970, Comparitive photosynthesis, growth and transpiration of two species of Atriplex, Planta, 93: 175-189.

Steven, C. Huber, Hugo, H. Rogers and Fred, L. Mowry, 1984, Effect of water stress on photosynthesis and carbon partitioning in soybean (Glycine max) plants grown in the field at different $CO_2$ levels, Plant Physiol., 76: 244-249.

Struik, G.T. and Bray, J.R., 1970, Root-shoot ratios of native forest herbs and Zea mays at different soil moisture levels, Ecology, 51: 892-893.

Teare, I.D., Kanemasu, E.T., Powers, W.L. and Jacobs, H.S., 1973, Water use efficiency and its relation to crop canopy area, stomatal regulation and root distribution, Agron. J., 65: 207-211.

Troughton, J.H., 1969, Plant water status and carbon dioxide exchange of cotton leaves, Aust. J. Biol. Sci., 22: 289-302.

Troughton, J.H., Cord, K.A. and Hendy, C.H., 1974, Carnegie Inst. Washington, Year b. 73, 768.

Turner, N.C., 1979, Drought resistance and adaptation to water deficits in crop plants, In: Stress Physiology in Crop Plants, Eds. H.Mussel and R.C. Staples, Wiley, New York, pp.343-372.

Turner, N.C., 1986, Adaptations to water deficits: A changing perspective, Aust. J. Plant Physiol., 13: 175-190.

Turner, N.C., 1986, Crop water deficits: A decade of progress, Adv. Agron., 39: 1-43.

Turner, N.C. and Begg, J.E., 1981, Plant water relations and adaptations to stress, Plant Soil, 58: 97-131.

Turner, N.C., Schulze, E.-D. and Gollan, T., 1984, The responses of stomata and leaf gas exchange to vapour pressure deficits and soil water content. I. Species comparison at high soil water contents, Oecologia, 63: 338-342.

Udaya Kumar, M., Sashidhar, V.R. and Prasad, T.G., 1986, Physiological approaches for improving productivity of small millets under rainfed conditions. Paper presented at the International Workshop on Small Millets (ICAR, UAS, IDRC), Oct.28-Nov.4, Bangalore.

Uma, S., 1987, Transpiration quotient (TQ) and water use efficiency (WUE) in different $C_3$ and $C_4$ species and its relationship with biomass and productivity under moisture stress conditions, M.Sc.(Agri.) thesis, Univ. Agric. Sci., Bangalore.

Walker, G.K. and Richards, J.E., 1985, Agron. J., 77: 263-269.

Wong, S.C. and Dunin, F.X., 1987, Photosynthesis and transpiration of trees in a Eucalyptus forest stand: $CO_2$ light and humidity responses, Aust. J. Plant Physiol., 14: 619-632.

Zadi, M.S., 1983, Drought stress influence on some physiological processes in winter wheat (*Triticum aestivum*). *Dissertation Abstract International*, B., **44**: 670.

Zur, B. and Jones, J.W., 1984, Diurnal changes in the instantaneous water use efficiency of a soybean crop. *Agricultural and Forest Meteorology,* **33**: 45-51.

# APPNDICES

Appendix-1. Soil moisture content in sunflower and amaranthus during the experimental period prior to bringing the pots to respective soil moisture regimes.

| | | | 31-34 DAS | 35-38 DAS | 39-42 DAS | 43-46 DAS | 47-50 DAS | 51-53 DAS | 54-56 DAS | 31-56 DAS |
|---|---|---|---|---|---|---|---|---|---|---|
| SUNFLOWER | Control | Amount(ml) | 1151 | 861 | 655 | 626 | 648 | 698 | 802 | 777 |
| | | %FC | 63.95 | 47.85 | 36.39 | 34.79 | 35.97 | 38.80 | 44.54 | 43.17 |
| | | %SMC | 14.39 | 10.77 | 8.18 | 7.83 | 8.09 | 8.73 | 10.02 | 9.71 |
| | Stress | Amount(ml) | 636 | 563 | 460 | 366 | 350 | 402 | 432 | 462 |
| | | %FC | 35.33 | 31.27 | 25.55 | 20.33 | 19.44 | 22.33 | 24.00 | 25.67 |
| | | %SMC | 7.95 | 7.03 | 5.75 | 4.58 | 4.38 | 5.03 | 5.40 | 5.78 |
| AMARANTHUS | Control | Amount(ml) | 1361 | 1200 | 1013 | 1061 | 953 | 1142 | 1102 | 1118 |
| | | %FC | 75.61 | 66.67 | 56.28 | 58.94 | 52.94 | 63.44 | 61.22 | 62.14 |
| | | %SMC | 17.01 | 15.00 | 12.66 | 13.26 | 11.92 | 14.28 | 13.78 | 13.98 |
| | Stress | Amount(ml) | 740 | 643 | 553 | 559 | 551 | 683 | 670 | 625 |
| | | %FC | 41.11 | 35.72 | 30.72 | 31.55 | 30.61 | 37.94 | 37.22 | 34.70 |
| | | %SMC | 99.25 | 8.04 | 6.91 | 6.99 | 6.89 | 8.54 | 8.38 | 7.81 |

Appendix-2(a). Relationship among growth and physiological characteristics in the six species grown at two different inputs of water.

| | NAR | LAD | DM | DM/LAD | CWA/LAD |
|---|---|---|---|---|---|
| TQ | -0.551** | -0.554** | -0.741** | -0.297** | 0.575 |
| NAR | - | 0.118 | 0.667** | 0.906** | 0.297** |
| LAD | - | - | 0.787** | -0.286** | -0.710** |
| △ DM | - | - | - | 0.334** | -0.379** |
| DM/LAD | - | - | - | - | 0.588** |

Appendix-2(b). Relationship among growth and physiological characteristics in the six species grown at moisture regime-1.

| | NAR | LAD | DM | DM/LAD | CWA/LAD |
|---|---|---|---|---|---|
| TQ | -0.672 | -0.632** | -0.989** | -0.388** | 0.481** |
| NAR | - | -0.105 | 0.606** | 0.925** | 0.299* |
| LAD | - | - | 0.697** | -0.453** | -0.563** |
| Δ DM | - | - | - | 0.453** | -0.563** |
| DM/LAD | - | - | - | - | 0.606** |

*Significant at 5 per cent
**Significant at 1 per cent

Appendix-2(c). Relationship among growth and physiological characteristics in the six species grown at moisture regime-2.

| | NAR | LAD | DM | DM/LAD | CWA/LAD |
|---|---|---|---|---|---|
| TQ | -0.637** | -0.744** | -0.991** | -0.263 | 0.666** |
| NAR | - | 0.023 | 0.589** | 0.897** | 0.140 |
| LAD | - | - | - | -0.401** | -0.916** |
| △ DM | - | - | - | - | -0.703** |
| DM/LAD | - | - | - | - | 0.526** |

Appendix-2(d). Relationship among growth and physiological characteristics in the group of three $C_3$ species grown at two different inputs of water.

| | NAR | LAD | DM_ | DM/LAD | CWA/LAD |
|---|---|---|---|---|---|
| TQ | -0.576** | 0.610** | 0.040 | -0.569** | -0.418** |
| NAR | - | -0.533** | 0.542** | 0.972** | 0.948** |
| LAD | - | - | 0.362* | -0.702** | -0.640** |
| △ DM | - | - | - | 0.387** | 0.440** |
| DM/LAD | - | - | - | - | 0.982** |

Appendix-2(e). Relationship among growth and physiological characteristics in the group of three $C_3$ species grown at moisture regime-1.

| | NAR | LAD | DM | DM/LAD | CWA/LAD |
|---|---|---|---|---|---|
| TC | -0.893** | 0.744** | -0.916** | -0.873** | -0.829** |
| NAR | - | -0.935** | 0.907** | 0.987** | 0.969** |
| LAD | - | - | -0.788** | -0.976** | -0.988** |
| Δ DM | - | - | - | 0.879** | 0.827** |
| DM/LAD | - | - | - | - | 0.993** |

Appendix-2(f). Relationship among growth and physiological characteristics in the group of three $C_3$ species grown at moisture regime-2.

| | NAR | LAD | DM | DM/LAD | CWA/LAD |
|---|---|---|---|---|---|
| TΩ | -0.594** | 0.093 | -0.991** | -0.403 | -0.220 |
| NAR | - | -0.850** | 0.541**. | 0.972** | -0.987** |
| LAD | - | - | -0.037 | -0.947** | -0.987** |
| Δ DM | - | - | - | 0.348 | 0.162 |
| DM/LAD | - | - | - | - | 0.979** |

Appendix-2(g). Relationship among growth and physiological characteristics in the group of three $C_4$ species grown at two different inputs of water.

| | NAR | LAD | DM | DM/LAD | CWA/LAD |
|---|---|---|---|---|---|
| TΩ | 0.305* | 0.409** | 0.569** | 0.160 | 0.518** |
| NAR | - | 0.088 | 0.769** | 0.842** | 0.845** |
| LAD | - | - | 0.679** | -0.410** | -0.201 |
| $\Delta$ DM | - | - | - | - | 0.541** |
| DM/LAD | - | - | - | - | 0.912** |

**Appendix-2(h).** Relationship among growth and physiological characteristics in the group of three $C_4$ species grown at moisture regime-1.

| | NAR | LAD | DM | DM/LAD | CWA/LAD |
|---|---|---|---|---|---|
| TQ | -0.503* | -0.198 | -0.993** | -0.130 | 0.157 |
| NAR | - | -0.680** | 0.486** | 0.850** | 0.716** |
| LAD | - | - | 0.205 | -0.918** | -0.987** |
| △ DM | - | - | - | 0.123 | -0.165 |
| DM/LAD | - | - | - | - | 0.927** |

Appendix-2(i). Relationship among growth and physiological characteristics in the group of three $C_4$ species grown at moisture regime-2.

| | NAR | LAD | DM | DM/LAD | CWA/LAD |
|---|---|---|---|---|---|
| TΩ | -0.535** | -0.125 | -0.943** | -0.244 | 0.090 |
| NAR | - | -0.761** | 0.303 | 0.941** | 0.767** |
| LAD | - | - | 0.347 | -0.928** | -0.987** |
| △ DM | - | - | - | 0.012 | -0.336 |
| DM/LAD | - | - | - | - | 0.933** |

Appendix-3(a). Relationship between gas exchange and growth paramerers associated with transpiration quotient (TQ) in six species grown at two different inputs of water.

| | TQ | NAR | $\Delta$ DM | DM/LAD | CWA/LAD |
|---|---|---|---|---|---|
| A | -0.631** | 0.871** | 0.798** | 0.669** | 0.028 |
| $g_s$ | 0.551** | 0.285** | -0.241* | 0.522** | 0.914** |
| $A/g_s$ | -0.916** | 0.297** | 0.632** | 0.018 | -0.742** |

A V/s $g_s$ = 0.167

A V/s $A/g_s$ = 0.380**

$g_s$ V/s $A/g_s$ = -0.776**

Appendix-3(b). Relationship between gas exchange and growth parameters associated with TQ in six species grown at moisture regime-1.

| | TQ | NAR | $\Delta$ DM | DM/LAD | CWA/LAD |
|---|---|---|---|---|---|
| A | -0.902** | 0.888** | 0.872** | 0.667** | -0.138 |
| $g_s$ | 0.519** | 0.185 | -0.584** | 0.498** | 0.936** |
| $A/g_s$ | -0.903** | 0.370** | 0.922** | 0.037 | -0.736** |

A V/s $g_s$ = 0.191

A V/s $A/g_s$ = 0.691**

$g_s$ V/s $A/g_s$ = -0.817**

Appendix-3(c). Relationship between gas exchange and growth parameters associated with TQ in six species grown at moisture regime-2.

| | TQ | NAR | △ DM | DM/LAD | CWA/LAD |
|---|---|---|---|---|---|
| A | -0.661** | 0.858** | 0.648** | 0.681** | -0.021 |
| $g_s$ | 0.573** | 0.150 | -0.570** | 0.491** | 0.899** |
| $A/g_s$ | -0.939** | 0.459** | 0.923** | 0.076 | -0.754** |

A V/s $g_s$ = 0.187

A V/s $A/g_s$ = 0.400**

$g_s$ V/s $A/g_s$ = -0.766**

**Appendix-3(d).** Relationship between gas exchange and growth parameters associated with TQ in the group of three $C_3$ species grown at two different inputs of water.

| | TQ | NAR | Δ DM | DM/LAD | CWA/LAD |
|---|---|---|---|---|---|
| A | -0.343* | 0.915** | 0.601** | 0.884** | 0.901** |
| $g_s$ | -0.123 | 0.779** | 0.509** | 0.839** | 0.909** |
| $A/g_s$ | -0.365 | -0.120 | -0.052 | -0.270 | -0.387** |

A V/s $g_s$ = 0.826**

A V/s $A/g_s$ = 0.176

$g_s$ V/s $A/g_s$ = -0.654**

Appendix-3(e). Relationship between gas exchange and growth parameters associated with TQ in the group of three $C_3$ species grown at moisture regime-1.

| | TQ | NAR | Δ DM | DM/LAD | CWA/LAD |
|---|---|---|---|---|---|
| A | -0.836** | 0.970** | 0.845** | 0.949** | 0.937** |
| $g_s$ | -0.726** | 0.817** | 0.700** | 0.898** | 0.916** |
| $A/g_s$ | 0.245 | -0.260 | -0.191 | 0.411* | -0.456* |

A V/s $g_s$ = 0.722**

A V/s $A/g_s$ = -0.191

$g_s$ V/s $A/g_s$ = -0.764**

Appendix-3(f). Relationship between gas exchange and growth parameters associated with TQ in the group of three $C_3$ species grown at moisture regime-2.

| | TQ | NAR | Δ DM | DM/LAD | CWA/LAD |
|---|---|---|---|---|---|
| A | -0.452* | 0.971** | 0.399 | 0.983** | 0.946** |
| $g_s$ | 0.090 | 0.708** | -0.140 | 0.852** | 0.928** |
| $A/g_s$ | -0.753** | 0.069 | 0.763** | -0.155 | -0.330 |

A V/s $g_s$ = 0.817**

A V/s $A/g_s$ = -0.089

$g_s$ V/s $A/g_s$ = -0.614**

**Appendix-3(g).** Relationship between gas exchange and growth parameters associated with TΩ in the group of three $C_4$ species grown at two different inputs of water.

| | TΩ | NAR | Δ DM | DM/LAD | CWA/LAD |
|---|---|---|---|---|---|
| A | 0.070 | 0.702** | 0.696** | 0.406** | 0.394** |
| $g_s$ | 0.142 | 0.829** | 0.748** | 0.567** | 0.561** |
| $A/g_s$ | -0.208** | -0.841** | -0.785** | -0.582** | -0.607** |

A V/s $g_s$ = 0.968**

A V/s $A/g_s$ = 0.932**

$g_s$ V/s $A/g_s$ = 0.981**

Appendix-3(h). Relationship between gas exchange and growth parameters associated with TQ in the group of three $C_4$ species grown at moisture regime-1.

| | TQ | NAR | $\Delta$ DM | DM/LAD | CWT/LAD |
|---|---|---|---|---|---|
| A | -0.549** | 0.751** | 0.541** | 0.424* | 0.251 |
| $g_s$ | -0.496* | 0.876** | 0.489* | 0.656** | 0.502* |
| $A/g_s$ | 0.460* | -0.905** | -0.454* | -0.738** | -0.596** |

A V/s $g_s$ = 0.955**

A V/s $A/g_s$ = -0.910**

$g_s$ V/s $A/g_s$ = -0.992**

Appendix-3(i). Relationship between gas exchange and growth parameters associated with TQ in the group of three $C_4$ species grown at moisture regime-2.

| | TQ | NAR | $\Delta$ DM | DM/LAD | CWT/LAD |
|---|---|---|---|---|---|
| A | -0.855** | 0.452* | 0.705** | 0.209 | -0.023 |
| $g_s$ | -0.836** | 0.576** | 0.649** | 0.366 | 0.144 |
| $A/g_s$ | 0.833** | -0.587** | -0.642** | -0.381 | -0.161 |

A V/s $g_s$ = +0.985**

A V/s $A/g_s$ = -0.982**

$g_s$ V/s $A/g_s$ = -1.00**