QUANTIFICATION OF STOMATAL AND MESOPHYLL LIMITATIONS OF PHOTOSYNTHESIS UNDER STRESS – A MATHEMATICAL APPROACH

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Dedicated to the Memory of Late
Shri. Krishnan Nair who was the
first guru to we Brothers and Sisters

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ABBREVIATIONS USED

A	Photosynthetic rate
ABA	Abscisic acid
ATP	Adenosine triphosphate
ANOVA	Analysis of variance
CA	Carbonic anhydrase
Ca	Ambient CO_2 concentration
CD	Critical difference
CER	Carbon exchange rate
CF	Coupling factor
Ci	Internal CO_2 concentration
CRD	Completely randomised design
E	Transpiration rate
ET	Evapo-transpiration
ETC	Electron transport chain
EXP	Exponential
g_m	Mesophyll conductance
g_s	Stomatal conductance
LAI	Leaf area index
lm	Mesophyll limitation of photosynthesis
ln	Natural logarithm
ls	Stomatal limitation of photsynthesis
m	Meter
ME	Malic enzyme
MLR	Multiple linear regression model
NS	Not significant
PAR	Photsynthetically active radiation
PSII	Pigment system II

$p(CO_2)$	Partial pressure of CO_2
PEPcase	Phospho enolpyruvate carboxylase
Q.Y.	Quantum yield
R^2	Coefficient of determination
Rubisco	Ribulose bisphosphate carboxylase/oxygenase
r_a	Boundary layer resistance
r_m	Mesophyll resistance
r_s	Stomatal resistance
RH	Relative humidity
s	Second
TDM	Total dry matter
VPD	Vapour pressure deficit
WUE	Water use efficiency
ψ	Water potential
X	Independent variable
Y	Dependent variable
μE	Micro Einsteins

PARAMETERS AND THEIR UNITS

In all the tables and figures given in this thesis the various parameters and their units are as given below:

Parameter	Unit
A	$\mu\ \mathrm{mol.m^{-2}.s^{-1}}$
PAR	$\mu\ \mathrm{E.m^{-2}.s^{-1}}$
Ci	$\mathrm{\mu l.l^{-1}}$
A/Ci	$\mathrm{x10^{-2}}\ \mu\ \mathrm{mol.m^{-2}.s^{-1}.\ \mu l^{-1}.l.}$
E	$\mathrm{mmol.m^{-2}.s^{-1}}$
A/E	$\mu\ \mathrm{mol.mmol.^{-1}}$
g_s	$\mathrm{mol.m^{-2}.s^{-1}}$
A/g_s	$\mu\ \mathrm{mol.mol^{-1}}$
dA/dCi	$\mathrm{x10^{-3}mol.m^{-2}.s^{-1}.\ \mu l^{-1}.l.}$
dg/dCi	$\mathrm{mol.m^{-2}.s^{-1}.\ \mu l^{-1}.l.}$
∂ Ci/∂A	$\mathrm{\mu l.l^{-1}.mol^{-1}.m^{-2}.s^{1}}$
∂Ci/∂g	$\mathrm{\mu l.l^{-1}.mol^{-1}.m^{2}.s^{1}}$

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INTRODUCTION

I INTRODUCTION

Photosynthesis is a function of a large number of independant and inter-dependant variables. These variables could be plant variables like the stomatal frequency, aperture size, efficiency of the various enzyme systems, translocation, sink capacity etc.; the environmental variables include light intensity, atmospheric temperature, relative humidity, wind velocity, ambient CO_2 concentrations, soil water status, etc. All these are interacting variables. Many of these variables will have a direct as well as an indirect effect on photosynthesis. For instance light has a direct effect on photosynthesis since it is the source of the energy required for the process. At the same time stomata respond to light intensity. Thus, light influences photosynthesis indirectly by causing stomatal opening.

Though essentially a biochemical process, photosynthesis can be considered as a diffusion process also. The diffusion pathway of CO_2 into the chloroplast includes boundary air layer, stomatal pore, substomatal and intercellular spaces, cell wall, cytoplasm and the various membrane systems. All along this path CO_2 experiences a resistance for diffusion and broadly they can be divided into boundary air layer resistance (ra), stomatal resistance (rs) and mesophyll resistance (rm). Boundary air layer resistance is a function of the micro habitat prevailing around the stomatal opening. Stomatal resistance is a function of stomatal frequency and aperture size. Mesophyll resistance is a function of various subcomponents of the mesophyll namely the efficiency of various reactions including the photochemical reactions, occurence of photorespiration, mesophyll water potential etc. The reciprocal of resistance is defined as conductance.

Stomatal conductance (g_s) and mesophyll conductance (g_m) are the two physical components of CO_2 diffusion into the leaf and through the leaf. Hence, photosynthesis (A) is a function of these two components.

$$A = f\ (g_s, g_m)$$

It is relatively easy to measure 'g_s' by using porometers. However, there is no direct method to determine 'g_m'. Hence, it is difficult to estimate the extent of individual contributions of these components.

Variations in environmental factors affect the above function. It is easy to quantify the environmental variability if it is imposed under controlled conditions. But it is extremely difficult to assess the intensity as well as the duration of a stress under natural conditions. The situation becomes more complex when more than one variable change.

A variety of abiotic stress exists in the agroclimatic zones of the world. They include drought stress, low light or high light stress, nutrient stress, salinity stress etc. Drought stress is probably the most important among these abiotic stresses.

Plant growth and productivity are very strongly correlated with moisture availability. Drought is known to be the most important single variable which reduces productivity in many parts of the world. Drought and famine are recurring phenomena in many third world countries.

It has been the interest of many workers to understand how abiotic stresses affect photosynthesis. Water stress reduces 'g_s', 'g_m' and 'A' (Udaya Kumar et al., 1988; Jacob and Udaya Kumar, 1988). Stabilizing photosynthesis during any abiotic stress will help in stabilising yield also. For any attempt in this direction, it is necessary to understand the various limitations of photosynthesis. In this context quantification of the relative stomatal and mesophyll limitations of photosynthesis under stress assumes importance. Fqrquhar and Sharkey (1982) and Krieg and Huttmacher (1986) reported that mesophyll factors impart more limitation on photosynthesis during drought stress. However, they did not quantify the mesophyll limitations.

Experiments were conducted in this study with the following objectives.

i) To study the effect of abiotic stresses like low N, low light and drought stress on 'A' 'g_s' and associated parameters in sunflower, amaranthus and sorghum.

ii) To study the inter-relationship among the various gas exchange parameteres in these species as affected by the above mentioned abiotic stresses.

iii) To quantify the relative stomatal and mesophyll limitations of 'A' under drought stress conditions in sunflower and amaranthus.

iv) To estimate the stomatal and assimilation loop gains with an idea to understand the adaptation strategies of sunflower and amaranthus plants to drought stress conditions.

REVIEW OF LITERATURE

II REVIEW OF LITERATURE

Importance of photosynthesis needs no mentioning. It occupies such a unique position in biology that all the living organisms depend on this process for their energy requirement either directly or indirectly. There are many environmental and plant factors affecting photosynthesis. The environmental factors include light, atmospheric CO_2 concentration, H_2O, temperature, humidity etc. The plant factors include chlorophyll content, levels of various enzymes, sink capacity, stomatal frequency and aperture size of stomata.

Though a complicated biochemical process photosynthesis is essentially a diffusion process also (Kridemann and Downton, 1981). Carbondioxide diffuses into the leaf through the stomata and the cells into the chloroplast stroma where it is reduced into hexoses. Oxygen which is a byproduct of photosynthesis diffuses out of the leaf into the atmosphere through the same path. The leaf offers a number of resistances for the diffusion of CO_2. They are mainly classified into stomatal resistances and non-stomatal resistances. The non-stomatal resistances include liquid phase resistance, carboxylation resistance, photochemical resistance, photorespiratory resistance and other residual resistances including biochemical resistances (Kridemann and Downton, 1981). Photosynthesis is affected by changes in any of these parameters. All these factors are subjected to natural changes. Hence, it is necessary to understand the relationship between these factors and photosynthesis. An attempt is made here to review some of these aspects.

2.1 Relationship between stomatal conductances, photosynthesis and transpiration under varying environment

Stomatal density and geometry decide conductance (Korner et al., 1970). A linear relationship exists between 'A' and 'g_s' and the way in which they are related has great ecological significance (Schulze and Hall, 1982). If during any variations in environmental conditions, 'A' and 'g_s' change proportionally in a straight line passing through the origin, Ci remains constant. If the leaf temperature and VPD also had remained unchanged WUE also would remain constant. If 'A' and 'g_s' relation is non-linear, it indicates that either 'A' or 'g_s' responds more strongly to the changes in the environment. In this case, Ci and WUE would not be constant. However, information regarding the simultaneous responses of stomata, water loss and CER has not been thoroughly reviewed.

Comprehensive investigations of correlation between 'g_s' and 'A' were conducted by Wong (1979) and Wong et al., (1979). They show a linear relation between 'g' at Amax and Amax for a large number of species under varying environmental conditions. The slope of the 'g' at Amax Vs. 'A' regression lines varies considerably between C_3 and C_4

species (Wong, 1979; Holngren, 1968 and Downes, 1971). This is corollary of the fact that Ci of C_4 species was lesser than that of C_4 species. Small slopes of 'g' (Amax) Vs. 'A' indicate higher WUE.

Wong (1979) reported linear regression passing through origin between 'g' (at Amax) and Amax for C_3 and C_4 species subjected to different N_2 and P levels. However, Goudriaan and Van Keulen (1979) did not find any strong correlation between 'g' at Amax and Amax in *Helianthus annuus* subjected to nitrogen stress. Medina (1972) observed that in *Atriplex patula* there was no association between 'g' at Amax and Amax at two levels of nitrogen. For C_3 species the response of 'A' to independent variations in 'g' are strongly curvilinear.

During leaf expansion photosynthetic capacity increases, after which Amax remains relatively constant and then steadily decreases as leaf senesceses (Ludlow and Wilson, 1971; Woodward and Rawson, 1976). In this case also there existed a linear relation between 'g_s' at Amax and Amax in different species; with C_4 species having lower slopes.

Farquhar (1978) demonstrated that a linear association between 'g' and 'A' is consistent with the concept of optimal stomatal function as proposed by Cowan and Farquhar (1977). The slopes of 'g' (at Amax) Vs. Amax curves varied substantially among C_3 species although they may not reach as low a slope as that of C_4 species. Similar slopes as exhibited by some C_3 and most C_4 species signify higher intrinsic WUE and lower Ci and improved adaptation to arid conditions. It is hypothesised that this slope may not vary within a genotype.

Plants adapted to shade have low photosynthetic capacity than those adapted to high light. Atriplex patula normally grows in exposed habitats, but shows shade type responses when grown in shade i.e. a reduction in Amax and 'g' at Amax (in low PAR). It is apparent that low Amax was not solely due to low leaf conductance (Bjorkman, 1981).

PAR for 95 per cent of 'g' max to be obtained is 100-2000 μ mol.m^{-2}. d^{-1}. On many occasions C_4 species need greater PAR for full conductance than C_3 species. Sunflower in the studies of Goudriaan and Van Laar (1978) showed only small responses of leaf conductance to light but not in the studies of Ramos (1981). These variations in stomatal sensitivity have not been explained.

Close coupling of stomatal aperture with leaf ψ was reported during stomatal oscillations (Cowan, 1972). Insensitivity to changes in leaf ψ occurs to certain threshold levels beyond which stomata abruptly close (Turner, 1974; Ludlow, 1980). In Glycine max no clear relationship between 'g_s' and ψ was observed as 'g_s' increased in the afternoons even in stressed plants were ψ was far below any threshold value (Turner et al., 1984). Leaf conductance and leaf ψ followed a nonlinear relationship in sorghum bicolor (Jones and Rawson, 1979).

Paired measurement of 'A' and 'g_s' for two C_3 species under long term drought exhibited curvilinear relations (Nobel, 1978; Hall and Schulze, 1980) unlike the linear relations observed in the C_4 species Zea mays (Wong, 1979), Sorghum bicolor (Jones, 1979) and Asterbla lappacea (Doley and Trivett, 1974). It is clear from these studies that drought affected the stomata of the C_3 species to a relatively greater extent than it affected photosynthetic metabolism. This could probably be the main reason for improved WUE by the C_3 species under drought stress conditions.

2.2 Stomatal control by carbondioxide

In general absence of CO_2 enhances stomatal opening. Increase in $p(CO_2)$ causes stomata to close (Meidner and Mansfield, 1968; Raschke, 1975a) and the magnitude of response varies with species.

Raschke (1979) proposes a scheme for stomatal sensitivity to CO_2 based on the continuous acid metabolism in the guard cells. Malate formation is necessary to meet the anion required during stomatal opening as well as for stomatal closure (Raschke, 1975 a,b). Malate level in cytoplasm of guard cells is a reflection of CO_2 in the environment of these cells and that increased malate levels cause leakage from the vacuoles by affecting the stomatal permeability of tonoplast and plasmalemma. The malate level in cytoplasms is in balance between malate formation, removal into vacuole and deacidification. Rates of malate formation and deacidification are affected by the pH dependencies of PEP-case and malic enzyme (ME). High malate and low pH inhibit PEP-case while ME activity increases as pH decreases. Thus PEP-case-ME can cause cytoplasmic malate and H^+ levels to fluctuate in response to changes in Ci.

Stomatal responses to Ci is substantiated in some C_4 species whereas stomatal response to light by some C_3 species cannot be explained by their response to Ci.

2.3 Stomatal control by abscisic acid

ABA causes stomatal closure within a few minutes of application (Mittelhouser and VAN Steveninck, 1969; Cummins et al., 1971; Kridemann et al., 1972) and is reversible (Cummins et al., 1971). ABA may not be the only compound produced during stress which can act as a messenger of stress to stomata. Wellburn et al., (1974) found all-trans-farnesol to increase with H_2O stress. This is a sequiterpenoid.

Stomatal aperture is proportional to the guard cell volume (Raschke, 1979). The increase in guard cell volume due to decrease in ψ alone can not explain the decrease in the 'g_s', however. This means that guard cells release solutes to close stomata. There is evidence that solute loss from guard cells begins when ABA produced in the mesophyll arrives at the stomata if the mesophyll has sensed the stress (Hsiao, 1973; 1976 and Raschke, 1975a).

The mechanism of ABA action is not very clear. But ABA sensitizes stomata to CO_2 and CO_2 enhances stomatal response to ABA (Raschke, 1975b). ABA facilitate acidification of guard cell either by blocking expulsion of H^+ or by activating H^+ pump directed into the cytoplasm.

Insensitivity of guard cell to changes in atmospheric humidity or leaf ψ is mainly due to high solute content and modulus of elasticity of guard cells (Raschke, 1979). Hence, loss of solutes from guard cell is necessary to achieve effective stomatal closure. ABA serves as a messenger in a feed back loop controlling water loss, though the exact mechanism involved in the nature of action of ABA is highly debated based on observations of Hiron and Wright (1973), Beardsell and Cohen (1975), Walton et al., (1977) etc.

The threshold ψ below which ABA synthesis occurs varies with species and growth conditions. It is believed that rather than ψ, it is turgor that determines stomatal closure (Turner, 1974; Cowan, 1977) and ABA synthesis (Bearsfell and Coehen, 1975). Feed back loop gain analysis involving E, turgor, ABA and 'g_s' has not been done yet.

2.4 Stomatal control by Light

Presence of a blue light receptor in guard cell is now widely accepted (Mediner and Mansfield, 1968; Raschke, 1975b). Blue light stimulates stomatal opening and uptake of Rb as tracer of K (Hsiao *et al.*, 1973) and induces swelling of guard cell protoplast of onion in the presence of K (Zeiger and Hapler, 1977).

Responses of guard cell to CO_2, ABA and light thus play a key role in the regulation of gas exchange levels.

2.5 Stomatal responses to water stress and humidity

Generally 'A' is not sensitive to decrease in ψ up to a certain threshold ψ value. This varied from -5 to -25 bars (Boyer, 1976). 'A' and E follow the same diffusion path and derive energy from light.

There are two conflicting demands - Maximising 'A' while preventing the loss of water to levels damaging to tissues. The threshold ψ for decreasing 'g_s' can vary with a previous history of stress and osmoregulation adjustment (Begg and Turner, 1976; Ludlow, 1980).

Stomata may close when RH is decreased and evaporation demand is high even if ψ is greater than threshold level. This is called "Pessimistic" (Jones, 1980) behaviour since stomata anticipates a continued dry spell and tries to save water at the cost of 'A'. Crop plants do not show this character as much as trees and wild herbs show. Stomatal movements are "hydroactive" and not just "hydropassive" (Stalfelt, 1955) since there is solute loss from guard cells during stress followed by changes in volume .

Though Stalfelt could not identify the ion responsible for hydroactive stomatal movements it was found to be K^+ (Hsiao, 1973; Ehret and Boyer, 1979). Stomatal closure during stress correlates with K^+ loss from guard cells.

ABA is involved in K^+ efflux from guard cells (Rashke, 1979). Discrepancies in the lack of correlation between time lag in ABA synthesis or degradation and stomatal movements are mostly due to the preferential localization of ABA in or around guard cells. Since guard cells constitute only 10 per cent of leaf volume (Raschke, 1979) ABA content of bulk leaf is a poor indicator of its concentration at stomatal site. Though mesophyll produces large quantities of ABA which is transported to guard cells (Loveys, 1977) guard cells also are capable of ABA synthesis (Weiler *et al.*, 1982).

2.6 Non-stomatal effects of water stress on photosynthesis

Mesophyll resistances may remain constant upto certain ψ and later may shoot up (Hsiao, 1978) and the reason for different behaviour of r_s is not clear (Pearcy, 1982). Rapid stress caused rapid increase in r_s (Mooney, *et al.*, 1977), whereas in the natural environment r_s stayed nearly constant upto a leaf ψ of -49 bar in the case of the perennial desert shrub, *Larrea divaricata*. Jones and Rawson (1979) found similar effects in sorghum. In both cases slow stress resulted in osmotic adjustment leading to maintenance of turgor. Maintenance of turgor need not always give lower leaf ψ (Ackerson and Hebert, 1981;).

Rubisco activity was lowered due to stress developed in several days in C_3 species (Jones, 1973; Johnson _et_ _al_., 1974;). The other enzymes whose activities are reduced during slow stress are carbonic anhydrase in cotton (Jones, 1973) and Ribulose 5-Pkinase and PEP-case in barley (Huffaker _et_ _al_., 1970). RuBP regeneration also could be limiting under condition of stress (Farquhar&Shankey 1982).

Boyer and Bowen (1970) reported even moderate stress inhibited PS-II activity in chloroplast fragments isolated from stressed sunflower plants, but needed a still lower ψ to inhibit PS-II in peas. Cotton chloroplast fragments from severeally stressed plants showed inhibited Hill activity (Fry, 1970). Mohanty and Boyer (1976) found that PS-II activity and quantum yield were decreased due to stress.

Govindjee _et_ _al_. (1981) reported reduced ratio of maximum to minimum florescence (P/O) in intact leaves under water stress due to blockage of electrons on the water side of PS-II.

In sunflower various features of ETC and phostophosphorylation were found to be inhibited by rapid stress in the range affecting 'A'. (Keck and Boyer, 1974) and uncoupling of photophosphorylation from ETC (Santarins and Ernst, 1967). CF_1 extracted from stressed leaves showed less

ATPase activity compared to that from control leaves (Youmis et al., 1979). Stress altered the conformation of CF protein probably in the random coil region. Chloroplast membrane also would undergo changes, particularly at ψ of -16 to -18 bars and more so at still ψ. These changes were reversible in vivo and not in vitro which is a puzzling aspect of stress effects on chloroplasts.

2.7 Carbon exchange, transpiration and water use efficiency

Photosynthesis and transpiration share a common pathway namely stomata. The crux of the problem of water relations vis-a-vis the carbon budget of the plant has in the fact that nature, during the course of evolution, did not favour the development of a biomembrane which is permeable to CO_2 but impermeable to water (Hall, 1982). Under normal conditions.

$$(Wi - Wa) > (Ca - Ci); \quad E > 'A' \text{ and}$$

$$\text{Molar evaporation ratio } \frac{E}{'A'} = \frac{(Wi - Wa)\, g^W}{(Ci - Ca)\, g^C}$$

Where E and 'A' are molar fluxes of water and CO_2; (Wi - Wa) and (Ci - Ca) differences in the molar fractions of H_2O and CO_2 between intercellular spaces (i) and atmosphere (a) and g^W and g^C are conductances of diffusion paths for water vapour and CO_2, respectively (Sharkey, 1985).

$\frac{gw}{gc}$ = Ratio of diffusion co-efficients of water and CO_2

in air $\frac{D^w}{D^c} = 1.56$

E exerts a negative feed back on E itself through transpirational cooling and 'A' exerts a more negative feed back on 'A' by decreasing Ci.

Extending Ohms Law Analogy,

$Ca - Ci = A / g^c$ and rearranging

$'A' = (Ca - Ci)\ g^c$ (Sharkey, 1985)

In C_4 species a reduction in 'g_s' does not reduce 'A' appreciably. This lack of dependance of 'A' on 'g_s' occurs when 'A' is saturated with respect to CO_2, "Break point" is that point on 'A'. Ci curve beyond which there is no increase in 'A' with respect to increase in Ci (Cowan, 1977). In C_3 plants the break point is never achieved in natural conditions. Hence, under natural conditions, stomata of C_3 species should be at their maximal aperture if maximal 'A' is the goal. In C_4 species this goal is reached once the break point is exceeded.

Raschke (1979) observes that "transpiration ratio" E/A declines when stomata close, since decrease in 'g_s' affects E and 'A' to different degrees. In Xanthium strumarium when 'g_s' is 0.4 mol $m^{-2} s^{-2}$, 336 moles of water was lost per mol of CO_2 fixed. When 'g_s was 0.1 mol m^{-2} s^{-1} it declined to 156 mols of water per mol of CO_2. This clearly indicates that the instantaneous WUE measured as A/E ratio would increase as water stress progresses.

From a physiological point of view WUE can be defined as the amount of carbon dioxide fixed (A) per unit amount of water transpired (E) from a given area of leaf in a given time. Under field conditions, a more agronomic definition takes into account the total dry matter produced (TDM) and the total amount of water lost by evapotranspiration (ET) from a unit land area in a given period of time.

Since, WUE is closely associated with 'A' and E, any plant or environmental factors affecting 'A' or E or both would certainly affect WUE.

According to the optimization theory proposed by Farquhar (1973), 'g_s' changes in such a way as to keep $dA / dg_s = dE / dg_s$. Hence, dA / dE is a constant. The rate of change in 'A' with respect to E has been found to be constant in different crop species under different environmental conditions (Farquhar et al., 1980; Hall and Schulze, 1980).

However, this has been contradicted recently when Sashidhar (1987) did not observe any linear relationship between 'A' and E with a constant slope. This is explained on the basis of differential effects of various environmental parameters on 'A' and E.

Considering the resistances operating in the diffusive pathways of CO_2 influx and water vapour efflux, we find that

$$A = f\ ('r_s'\ 'r_m'\ 'ra')$$

$$A = f\ ('r_s'\ 'r_a')$$

This clearly indicates that any factor that is going to alter the resistances would have different impacts on the diffusion rates of CO_2 and water vapour. Because the total resistance (Σ R) offered for CO_2 flux is more than that for water vapour at any given conditions.

$$\Sigma R\ (A) > \Sigma R\ (E)$$

This is particularly so because of the more biochemical linkage of CO_2 than H_2O inside the leaf which is a function of various intrinsic metabolic processes. Still it is possible that any external variable can cause constant changes in dA /dE provided the variable under question influences r_s and r_m to the same extent. However, considering the more physical nature of r_s and the more biochemical nature of r_m it is very unlikely that an external variable can cause equal effects on them. Drought stress was found to increase r_s more than reduce 'A' during early periods of drying (Krieg, 1988).

Even at given constant levels of resistances and other conditions, CO_2 influx rate would be lesser than H_2O efflux rate (Fischer and Turner, 1978) since the molecular weight of the former is higher. Hence, r_s for water vapour is multiplied with 1.6 to get the r_s for CO_2 where 1.6 is the ratio of the binary diffussivities of water vapour/air and CO_2 air (Jarvis, 1971).

By extending ohm's Law, 'A' and E can be expressed as follows (Sharkey, 1985):

$$'A' = \frac{Ca - Ci}{\Sigma R} = \frac{\Delta CO_2}{\Sigma R}$$

$$E = \frac{VPD}{\Sigma R}$$

Any environmental variable which can alter ΣR (for instance drought which increases ΣR) usually enhances VPD through direct or indirect effect. Drought is usually associated with high temperature and light intensity which increase the evaporative demand of the atmosphere Fischer (1978) and Turner (1987) have shown that an increase in the VPD increases E and not 'A', and thus reduces WUE. Rawson (1977) found that under well watered conditions WUE decreased as VPD increased and vice verse during stress in several C_3 and C_4 species.

Turner (1984) observed a decrease in WUE as VPD increased. He highlighted the importance of intrinsic photosynthetic characteristics for higher WUE even at high VPD. It may be observed that as VPD increases 'g_s' decreases (Mooney _et al_, 1977). This reduction in 'g_s' could have an adverse effect on 'A'. Hence, maintaining 'A' at reducing

'g_s' levels assumes significance. This is particularly so during water stress when 'A' and 'g_s' are decreasing and evaporative demand of the atmosphere is increasing. Kramer (1983) suggests that r_s and r_m are affected to different extents depending on the degree and magnitude of stress. Air temperature also influences WUE through VPD. Schulze et al (1976) and Khairi and Hall (1976) observed a reduction in WUE as air temperature increased.

Boundary layer resistance also assumes importance as a parameter indirectly determining WUE particularly in dry areas where there is good amount of air circulation. When the air is static, there is a build up of water in the microhabitat of the canopy which increases the r_a for E. However, when there is a constant and gentle breeze, vapour pressure goes down. This is not as much as a variable as far as 'A' is concerned since CO_2 is fairly uniformly present in the lower layers of the atmosphere than water vapour. It could be of only a minor advantage for photosynthesis since a mild breeze can cause better aeration of the canopy where the CO_2 concentration is gradually getting depleted because of continuous uptake by leaf.

Thus any environmental variation triggers a number of complexly intereacting processes which ultimately decide 'A' 'g_s' E and thus, WUE. Though single parameter models describing the changes in 'A', 'g_s' E etc have been developed extensively a comprehensive picture of their interactions, particularly under field conditions has not yet come out.

Johnson et al. (1974) observed that A/E ratio remained unchanged as leaf ψ declined in barley and wheat. However, Johnson et at. (1975) found an increase in A/E with reducing leaf ψ in four arid and alpine tundra plant species.

WUE as measured from A/E ratios was found to increase in sorghum and pearl millet under moisture stress (Blum and Sullivan, 1986). This was because of more reduction in E and 'A'. This is possible only if 'g_m' is not affected as much as 'g_s' is affected. They also observed that races adapated to low rainfall areas had higher WUE under stress conditions.

Wheat cultivars under reduced leaf ψ showed an increase in WUE which was associated with greater decrease in 'g_s' than 'A' and with positive shifts in 13_C values (Farquhar and Richards, 1984). Similar results were reported by Guy and Reid (1986) in Puccinellia, a C_3 halophyte subjected to salinity stress.

Fischer (1981) found that in some field crops wherever WUE was decreased in response to decline in leaf ψ levels, there was a decrease in 'g_m' also. A similar result has been reported by Sinclair et al., (1975). Hence, maintenance of mesophyll factors assumes prime importance for higher WUE during stress. This inevitably leads to higher dA / dg_s ratios and thus a reduction in Ci as stress progresses (UdayaKumar et al., 1988; Sashidhar 1987; Jacob and Udaya-

Kumar, 1988; Kreig, 1988; Farquhar and Richards, 1984).

Osmond <u>et al</u>., (1980) defined dA / dg_s ratio as "intrinsic" water use efficiency. They also proposed the importance of high 'A'/ 'g_s' ratios under conditions of moisture stress. This ratio was 2 to 3 times more in C_4 species than C_3 species under optimum conditions. Wong (1979) however, observed a linear change in 'A' and 'g_s' in response to light intensity and N_2 levels leading to constant 'A'/ 'g_s' ratios and Ci.

Importance of 'g_m' was highlighted as back as in 1959 by Gaastra. Slayter (1973) reported species differences in 'g_m' sensitivity towater stress. He found that drought adapted species and races, particularly C_4 species showed lesser reduction in 'g_m' as stress progressed. Similar opinion has been raised by Bunce (1981). However, Boyer (1970) found that both 'g_s' and 'g_m' were decreased in corn and soybean as leaf ψ declined. Beyond a leaf ψ of -1.6 MPa. 'g_m' also decreased due to damage to photosynthetic apparatus.

There could be a number of parameters contributing to the maintenance of 'g_m' under stress. Carbonic anhydrase activity which increases with light intensity (Reyssand Prioul, 1975) was found to be directly related to 'g_m' (Raven, 1976). Regeneration of RuBP could be yet another biochemical limitation (Krieg and Hutmacher, 1986).

However, water stress is known to cause photo-oxidation of chlorophyll and photo inhibition of photosynthetic ETC-particularly under very low tissue ψ levels and low Ci conditions and under high light intensity.

2.8 Comparative water economy of C_3 and C_4 species

The mesophyll Ci is about 100 u bar and b.s. cell Ci = 200-3000 u bar in C_4 species (Hatch and Osmond, 1976; Wong, et al., 1979). Hence, in C_4 plants 'A' is CO_2 saturated under natural atmosphere unlike in C_3 species where 'A' can still increase if Ci is increased. In C_3 species under normal conditions Ci = 220 u bar (Wong et al., 1979). The substantial lower 'g_s' in C_4 species leads to lower E. Due to CO_2 concentration mechanism C_4 plants oxygenase of Rubisco is prevented and thus net 'A' is high. Hence, 'A'/E is high in C_4 species (Osmond et al., 1982).

C_3 species function at only about half CO_2 saturation. C_3 plants with elevated Rubisco activity as a result of increased N nutrition do not saturate until full sunlight like C_4 species (Osmond et al., 1982).

Though dA'/ dE is higher in C_4 species, the energy costs of biochemical reactions in C_4 species (5 ATP + 2 NADPH mol^{-1} CO_2 fixed) is greater than those in C_3 species (3 ATP + 2 NADPH mol^{-1} CO_2 fixed). The intrinsic Q.Y. of C_4 plants is 0.056 mol CO_2 $Einstein^{-1}$ in air and is independent of temperature and O_2 concentration. In C_3 plants the intrinsic Q.Y. is 0.080 in low O_2 level, but is a function of temperature and O_2 (Ehlerenger & Bjorkman, 1977). The biochemistry of C_4 pathway permits increased potential 'A' with increase in PAR and temperature compared to C_3 pathway. However, cool temperatures during wet seasons may reduce the potential advantage in productivity of C_4 plants in many desert environments (Mooney et al., 1974).

Since, the special photosynthetic pathways are seen in relatively less number of terrestrial plant families, it is assumed that this is one of the factors which contributes to ecological success of plants. Out of the 538 known families of flowering plants and ferns (Willis, 1973), only 17 contain C_4 texa and 20 contain CAM taxa. Genetic limitations may primarily determine the expression of these metabolic processes in many habitats.

2.9 Increase in atmospheric CO_2 levels and the possible changes in photosynthesis, transpiration and WUE

Apart from water, which is abundant on earth and many a times a scarse input in agricultural systems, carbon dioxide is the other 'substrate' for photosynthesis. Global CO_2 level is increasing at a fast rate and would double during next century (Baes et al., 1977). This would certainly have very striking effects - direct and indirect - on 'A' 'g_s', E and WUE. CO_2 enrichment studies have been conducted both in green house and field conditions to understand these effects.

Stomata is known to be sensitive to CO_2 (Dubbe _et al_., 1978). High levels of CO_2 in the ambient air will lead to an enhancement in Ci. It has been reported that Ci=0.7 Ca for C_3 species and Ci=0.4 Ca for C_4 species. Thus an increase in Ca results in increasing Ci which would now reduce E and enhance 'A' if other conditions, particularly temperature, are not unfavourable.

An increase in WUE was reported in wheat cultivars (Gifford, 1979) and Paspalum (Gifford and Morison, 1985) at higher levels of ambient CO_2 levels.

Jones _et al_. (1985) found that 'A' increased with Ca at a constant temperature of 28°C though 'g_s was reduced markedly. This resulted in reducing E and thus enhancing WUE. They also reported that irrespective of the CO_2 levels WUE decreased if temperature was increased from 28 to 35°C since it increased the evaporative demand of the atmosphere.

Photorespiration which is more predominant in C_3 species is mainly due to the dual functioning of Rubisco as a carboxylase or oxygenase depending upon the partial pressures of CO_2 and O_2 inside the leaf Rubisco has a higher km value for CO_2 compared to PEP-case. Hence, enhancing the Ci would probably reduce photorespiration and thus increase the net 'A'. Since this is coupled with a reduction in 'g_s' there is scope for improving the WUE of C_3 species under high ambient CO_2 levels.

During periods of water stress, wheat plants grown at high CO_2 levels (1000 ppm) produced equally high TDM compared to the non-stressed plants grown at 350 ppm CO_2 (Sionit *et al.*, 1980 a). Hart and Wright (1976) demonstrated that drought tolerance increased as CO_2 level increased. 'A' increased linearly as Ca was increased from 350-500 (Nevins and Loomis, 1970) in sugar beets. Wong (1979) reported increase in WUE and dry weight of cotton and maize grown under increased CO_2 levels. Many investigators believe (Wittwer, 1979) that CO_2 has all of the characteristics of a fertilizer and the agricultural production should increase as global CO_2 increase.

2.10 Mathematical models of plant water loss and plant water relations

Mathematical models of plant water loss and plant water relations represents attempts to quantify functioning of individual organs, organisms or populations in response to the environment. Guideline for development, evaluation and use of mathematical models in ecosystems are only just begining to emerge (Hall., 1982).

Mathematical models of biological systems are works of art combining both fact and fiction, and they only occasionally and partially simulate reality (Passioura, 1973). It would be prudent to assume that one could develop mathematical models which can perfectly mimic the complex biosystems. However, specific models can be developed for specific purposes by constructing subsystems within plants and ecosystems (Loomis _et al_., 1979).

Stomatal mechanisms have been subjected to considerable analysis using mathematical models (Cooke _et al_., 1976; DeMichele and Sharpe, 1973; Sharpe and Wu, 1978; Shoemaker and Srivastava, 1973). Farquhar _et al_., (1978) modelled environmental effects on stomata by applying differential calculus and classical feedback theory. This approach has been further extended to study the 'g_s' Ci interactions (Wong _et al_., 1978) and contribution of 'g_s' to E (Farquhar, 1978).

Models are developed to explore the interactions between plant water loss and plant water status (Cowan and Mithorpe, 1968, Cowan, 1972). Stomatal regulation of E and 'A' has been extensively modelled by Cowan a Farquhar (1977). Ultimately predictions of water and carbon balance provide information regarding plant adaptation and competition (Ehleringer and Mooney 1978; Jones, 1980; Mooney and Ehlermger, 1978). It is impossible to develop a single model to accomplish the above said objectives. But any model developed today could well be used as a submodel for a more comprehensive and exhaustive one (Hall 1982).

All models will have deficiencies. Like interpretations of experimental studies, models should be recognised as only occupying some position on a continuum between the states of general ignorance and partial understanding (Hall., 1982).

It is useful to include submodels of CER in models of E, since 'A' and E are intensively dependent. Stomatal aperture of some species are strongly dependent upon the Ci (Rashke, 1976). It has been proposed that 'g_s' may be influenced by a product from photosynthesis in the mesophyll cells (Wong _et al._, 1979) or by the energy status of the mesophyll (Wong, 1979). Hence, a comprehensive model of 'A', E, Ci and respiration is needed for better understanding of stomatal adjustments to external and internal environment.

2.11 Transpiration : A diffusive process

Considering E as a diffusive process, Hall (1982) defines resistance to water vapour (rh)

$$rh = \frac{LK^{*}}{Dh^{o}\ (T/To)^{1.75}\ (Po/P)}$$

where;

L- is the effective length path

K^{*} - is a dimension less coefficient describing the influences of stomatal densities and aperture dimensions in gaseous diffusion.

Dh^{o} - is the diffusion coefficient for water vapour in air at reference levels of absolute temperature (To) and atmospheric pressure (Po)

T - is the ambient temperature

P - is the ambient atmospheric pressure

A method has been described by Cowan (1977) which over comes some of the errors due to temperature and pressure on diffusivities.

$$Jh = \frac{\Delta e}{r^t p}$$

Jh - is the flux of water vapour (mass area^{-1} time^{-1})

Δe - is the difference in vapour pressure

P - is the atmospheric pressure

r^t - is the total resistance to diffusion of water vapour. Hence, r^t has the units 1/ transpirational flux.

By assuming iosthermal conditions and applying ideal gas equation,

$$r^t = \frac{RTo^{1.75}}{MD^o hPoTo^{0.75}} LK^*$$

Here r^t is a function of the geometric properties of the epidermis (L and K^*) and several constants (R, To, M, $D^o h$ and Po). R is the universal gas constant; M is the molecular weight of water. This also has a small dependence on absolute temperature ($1/To^{0.75}$).

Based upon the conventional system

$$Ji = \Delta Ci/ri$$

where Ji is the flux of a specific gas (i) (mass area^{-1}). Δ Ci is the difference in the concentration along the flow path (mass mol^{-1}) and ri (time length^{-1}) is a resistance of diffusion. This resistance ri can be expressed as follows by the application of Fick's law.

ri = L/D^1 where L is the effective length path of diffusion and D^1 is an effective diffusion coefficient. Again D^1 = Di/K* where Di is the conventional diffusion coefficient. Thus, the dimension of conventional resistance (time length^{-1}) and the new one defined by Cowan (1977) (1/mass area^{-1} time $^{-1}$) are different. However, resistance based on the old system can be converted into the new system using the following equation.

$$r^t = rVo \text{------} \frac{PoT}{PTo}$$

Where Vo is the molar volume of an ideal gas at To and Po (Vo = 0.0224 m^3 mol^{-1} at To = 273 K and Po = 1.01 bar).

Gaseous transport equations are also useful to estimate Ci as described by Jarvis (1971). Since at low concentration volume fractions are equivalent to relative partial pressure, it is more appropriate to use concentrations that are on a volume basis. In the case of resistances, it is more appropriate to use conductances (reciprocal of resistances) as the latter shows a more linear relationship with E and 'A'.

Resistances to the diffusion of one gas are commonly converted to apply to another gas by using the ratios of the diffusion coefficients as described by Jarvis (1971). However, when CO_2 is diffusing into and water vapour is diffusing out of the leaves through the same pathway the interaction due to molecular collisions is not taken into account.

2.12 Steady state models of stomatal function

Hall (1982) has reviewed three types of models of stomatal functioning namely multiple correlation models, phenomenological models and models based upon hypothetical systems of stomatal regulation.

Multiple regression equations relating leaf resistance to solar irradiance, air temperature, VPD and xylem potential have been developed by Hinckley et al., (1975) but their predictability was very poor. The intrinsically linear, logarthamic multiple regression analysis by Pallardy and Kozlowski (1979) of the empirical relationship between leaf resistance and solar radiation, VPD, temperature and leaf water potential shows more stomatal opening with greater leaf water deficits.

Emperical models have to be used cautiously. They may be used for predictions and interpolation rather than for analysing the mechanisms involved or for extrapolating outside the range of values used in developing then (Hall 1982). Emperical models can further be strengthened by developing phenomenological modes.

Models based only upon stomatal responses to Ci would not completely explain the stomatal responses to light of many species (Wong _et_ _al_., 1978), (Wong, 1979; Ramos, 1981). For some species blue light has a special effect on guard cells and the action spectrum differs from that of 'A' (Raschke, 1979).

The photon flux required for 35 per cent of gmax varies from 100 to 2000 μ mol. $m^{-2}s^{-1}$ depending upon spp, age and environment (Turner, 1974; Burrows and Milthorpe, 1976). The responses of 'g_s' to photon flux follows a rectangular hyperbola and some times different functions are more appropriate (Burrows and Milthorpe, 1976). Stomatal responses to light may vary during morning for opening and evening for closing (Hinckley _et_ _al_., 1975; Ramos, 1981). Because large 'g_s' was observed in the morning than in the evening for the same PAR. Hysteresis may also be observed in opposite direction if stomatal responses to photon flux are determined over a short period of time (Jarvis, 1980).

Changes in atmospheric humidity can alter 'g_s' irrespective of the bulk leaf water status (Hall _et_ _al_., 1976; Hall and Hoffman, 1976; Schulze and Kuippers 1979; Losch, 1979)

Stomatal responses to humidity and

VPD have been proposed by Farquhar (1978) and Thorpe et al., (1980).

Stomatal responses to temperature at fixed levels of VPD gave the optimum type response (Jarvis, 1980) usually observed in the responses of biosystem to temperature and progressive increase in 'g_s' over a substantial range of temperature (Hall et al., 1976). The shape of 'g_s' temperature curve may change with drought (Schulze et al, 1976) or may remain the same. Plants subjected to very extremes of temperature for about one hour showed very low 'g_s' for several hours or days (Bauer, 1978; Neilson and Jarvis, 1975) suggesting the irreversibility in stomatal responses of temperature.

Differences in the stomatal responses to Ci have been reported in many species by Burrows and Milthorpe (1976) Hall et al., (1976) Jarvis (1980) Raschke et al., (1978) Wong, 1979; Ramos, 1981) Raschke (1976) has hypothesized that stress may sensitize stomata to Ci.

Turner and Begg (1978) observed that stomata do not respond to changes in leaf ψ untill a threshold level, below which it decreases sharply. But Jones and Rawson (1979) found a continuous decline in 'g_s' over a range of ψ from -15 to -30 bars. It may be noted that stomatal apertures are related to the turgor potential of guard cells and adjacent cells, and the osmotic potential of these cells need not be constant or uniquely related to the bulk leaf osmotic potential (Raschke, 1979; Beadle et al., 1978). A rapid reduction in leaf ψ lead 'g_s' to exhibit threshold responses to bulk leaf ψ (Ehlig and Gardner, 1964; Hall and Hoffman, 1976) resembling the threshold level of Turner and Begg (1978). But a slow depletion of water in the root zone did not fit the threshold model and resulted in gradual decrease in relative transpiration rates (Ehlig andGardner, 1964). Under field conditions the interaction of E factors and plant water status makes it difficult to assess the effects on stomata of leaf ψ. Stomatal conductance and leaf ψ integrated over time would give a better picture of stress effects on them.

Among the models based on hypothetical systems of stomatal regulation, the theory of Cowan and Farquhar (1977) provides a basis for modelling stomatal function in relation to optimization of water use which states that dA'/ dE would tend to remain constant. Variations in atmosphere humidity at fixed temperature resulted in constant dA'/ dE in Nicotiana glaca and Corylus avellana (Farquhar et at., 1980) and with variation in both temperature and humidity for Vigna unguiculata (Hall and Schulze, 1980). The Hall (1982) says dA'/ dE was not a constant when PAR was increased though 'A' 'g_s' showed a linear relation. Linear association between 'A' and 'g_s' with short term changes in photon flux has been reported for several spp (Goudriaan and Vanlaar, 1978;

Louwerse and Zweerde, 1977; Wong et al., 1978; 1979; Wong, 1979).

Hall and Schulze (1980) found an increase in dA'/ dE and decrease in 'g_s' as stress progressed in Vigna unguiculata. However, for corylus avellana, dA'/ dE showed a decreasing tendency with stress (Farquhar, et al., 1980).

2.13 Dynamic models of stomatal function

A dynamic model is one where time is an independant variable and the phenomena under question vary with it (Hall 1982). All biological processes being dynamic, a dynamic model is more powerful than a steady-state or hybrid model. The dynamic model of Cowan (1972) analysing the oscillations in 'g_s' is probably the most significant in this category. Development of comprehensive dynamic models of stomatal function require a better understanding of the system that is available at this time.

Controversy and lack of understanding of water transport in the soil plant system is a serious contraint in developing models. Even the basis of evalating component of ψ has been questioned (Spanner, 1973; Passioura, 1980). The complexity is evident from the facts that liquid water transport may be intimately linked with solute transport (Dalton et al., 1975) Fiscns and Kramer, 1975), interface resistance may occur between roots and soil (Cowan and Millthope, 1968, Faiz, 1973; Herkelrate, 1975), the liquid water pathway between soil and shoots may exhibit irreversibility (Hall 1982) and there need not be observed an equilibrium between plant and soil ψ (Schulze et al., 1980 . Syversten et al., 1975).

Cowan and Millthorpe (1968) extended the analogy of electrical circuits to explain the low water loss during certain seasons maintaining sufficient water in the tissues to sustain this for several days or weeks. A dynamic model of water transport in plants has been developed by Cowan (1972) which incorporates effects due to capacitance. A detailed discussion of the quantitative treatment of the capacitance of various plant tissues has been presented by Jarvis (1975). However, development of realistic and dynamic models of plant water loss and water relations will require more complete understanding of the water transport and water relations of soil plant system that is currently available.

2.14 Integrated models of canopy water loss

Jury (1979) has presented a review of integrated models of canopy water loss. Modelling canopies based on a unit leaf area could be eroneous because of the complexity of structural and E characteristics of the canopies. Canopies have been modelled as discrete strata containing leaves having specific leaf area index (Duncan et al., 1967; Jacob, 1984) and average angle (Duncan et al., 1967). For developing phenological models of canopy water, evaporation from ground has also to be taken into account. This depends mainly on the evaporative demand until soil dries and then upon the water movement properties of the soil (Ritchie, 1972). In the field studies canopy conductances have been estimated by summing product of leaf conductance and LAI for different strate in the canopy (Biscoe *et al*., 1976; Jacob, 1984).

MATERIAL AND METHODS

III MATERIAL AND METHODS

The experiments conducted in this study were carried out in the Department of Crop Physiology, University of Agricultural Sciences, Bangalore, India during 1984-88. Experiments were conducted to study the effect of abiotic stresses like low N, low light and drought stresses on the photosynthetic and stomatal behaviour of three crop species. They were sunflower (Helianthus annuus) hybrid BSH-1, Amaranthus species (Var.R-104) and Jowar (Sorghum bicolor) hybrid CSH-5. These species have very distinct photosynthetic pathways; sunflower being a C_3 species and the other two being C_4 species.

The various experiments conducted can be broadly divided into the following categories.

1. Effect of different levels of fertilizer N on gas exchange and associated characteristics in sunflower, amaranthus and sorghum.
2. Effect of different light intensities on gas exchange and associated characteristic in sunflower, amaranthus and sorghum.
3. Effect of moisture stress on gas exchange and associated characteristics in sunflower, amaranthus and sorghum.

4. Studies to quantify stomatal and mesophyll limitations of photosynthesis under moisture stress condition in amaranthus and sunflower.

5. Stuides to quantify feed back loop gains involving stomata and CO_2 in sunflower and amaranthus at different levels of moisture stress.

6. Studies on chlorophyll quenching as affected by water status in sunflower, amaranthus and sorghum.

The details of the procedure adopted for each of the above mentioned experiments are given below.

2.1 Effect of different levels of fertiliser nitrogen on gas exchange rates and associated characteristics in sunflower, amaranthus and sorghum

This experiment was conducted during summer 1985. Sunflower, amaranthus and sorghum plants were raised in battery containers in green house.

2.1.1 Preparation of pots

Battery containers made of carbonised rubber with handle grips, were used to raise the crops. Each container was filled with 8 kg of red loamy soil containing sufficient farm yard manure. Each species was sown in 15 pots.

2.1.2 Sowing

About 15-20 seeds were sown in each pot. Thinning was done at regular intervals and finally 2 plants were maintained per plot. The pots were watered regularly.

2.1.3 Nitrogen treatments

Three levels of fertilizer nitrogen were employed for each species. They were;

(a) Recommended level (optimum)

(b) 50 per cent more than recommended level (high)

(c) 50 per cent less than recommended level (low).

P_2O_5 and K_2O were applied as per the standard package of practices.

2.1.4 Measurement of gas exchange rates

When the plants were about 50 days old photosynthetic rate '(A)' stomatal conductance '(g_s)', photosynthetically active radiation (PAR), atmospheric CO_2 concentration (Ca) and transpiration rate (E) were determined by using portable Photosynthesis System (LT-6000). These measurements were made on the top fully expanded leaves of each species under full sunlight between 10 a.m. and 12 noon. The light intensity during this period was more than 1800 $\mu E.m^{-2}.s^{-1}$. The abreviations used for the various gas exchange parameters and their respective dimensions are given below.

Item	Abbreviation	Units
Photosynthetic rate	'A'	μ mole.$m^{-2}s^{-1}$
Stomatal conductance	'g_s'	mole.$m^{-2}s^{-1}$
Photosynthetically active radiation	PAR	μE.$m^{-2}s^{-1}$
Atmospheric CO_2 concentration	Ca	μl.1^{-1}
Transpiration rate	E	m.mole.$m^{-2}s^{-1}$.

These parameters were directly measured by the instrument (LI-6000).

2.1.4.1 Portable photosynthesis system (LI-6000)

LI-6000 consists of a system console, a CO_2 analyser and a leaf chamber with humidity and PAR sensors. Measurements are made of leaf temperature, relative humidity, CO_2 concentration and photosynthetically active reduction (PAR). The LI-6000 is designed to measure simultaneously and rapidly, the instantaneous CO_2 and water vapour exchange rates.

The LI-6000 incorporates a transient measurement technique, whereby an actively photosynthesising and transpiring leaf, when enclosed in a container causes the humidity of the air in the container to increase and the CO_2 to decrease. The rate at which the humidity and CO_2 change, depends directly upon the stomatal conductance of the leaf and its apparent photosynthetic rate. The rate of change of humidity and CO_2 are obtained by making measurements seperated by intervals of time. The CO_2 measurement is made by the IRGA, which is the part of the whole system. The LICOR-6000 is thus a rapid, computer aided, gas exchange measurement system.

2.1.5 Derived parameters from gas exchange data

Various parameters associated with photosynthesis and transpiration were calculated from the primary gas exchange data as follows.

(i) A/g_s ratio

This ratio was calculated directly from the observed values of 'A' and 'g_s'.

$$'A'/'g_s' = \frac{'A'\ (\mu.mole.m^{-2}s^{-1})}{'g_s\ (mole.m^{-2}s^{-1})}$$

This ratio is a measure of the amount of carbon fixed per unit conductance.

(ii) Internal CO_2 concentration (Ci)

This is calculated from the following formula:

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

(iii) A/Ci ratio

A/Ci ratio was calculated directly from the values of 'A' and Ci.

$$\text{A/Ci ratio} = \frac{\text{A } (\mu\text{.moles.m}^{-2}\text{.s}^{-1})}{\text{Ci } (\mu\text{l.l}^{-1})}$$

This ratio is an indirect measure of the carboxylation efficiency.

(iv) A/E ratio

A/E ratios were calculated directly from the values of A and E. This ratio is a measure of the instantaneous water use efficiency.

$$\text{A/E ratios} = \frac{\text{A } (\mu\text{.mole.m}^{-2}\text{.s}^{-1})}{\text{E } (\text{m.mole.m}^{-2}\text{.s}^{-1})}$$

All the values were subjected to standard analysis of variance. The design was completely sandomised design (CRD).

Regression models for various gas exchange parameters were developed by the help of a personnel computer. The first order derivative of each function was then obtained by applying simple differentiation.

2.2 Effect of different light intensities on gas exchange and associated characteristics in sunflower, amaranthus and sorghum

Pot grown plants of sunflower, amaranthus and sorghum were subjected different light levels for varying durations and gas exchange rates were monitored. The details of the procedure are given below.

2.2.1 Effect of moderate light intensities

2.2.1.1 Experiment-1: Sunflower, amaranthus and sorghum plants were raised in battery containers as described above. When the plants were about 50 days old they were shifted from the open sunlight (2000 $\mu E.m^{-2}.s^{-1}$) to a moderate light intensity (400-600 $\mu E.m^{-2}.s^{-1}$). The plants were kept in that light regime for 90 minutes. Gas exchange rates were monitored at regular intervals in the top well expand levels of each species by using a portable Photosynthesis System (LI-6000). The various observations recorded were 'A', 'g_s' PAR and E. Associated parameters like A/'g_s', Ci, A/Ci and A/E were calculated as described earlier. The data was subjected to ANOVA test in CRD.

2.2.1.2 Experiment-2

A similar experiment was repeated for a second time. In this case 50 days old potted plants of sunflower, amaranthus and sorghum were shifted from high light (2000 $\mu E.m^{-2}.s^{-1}$) to a moderate light (400-600 $\mu E.M^{-2}.s.^{-1}$) and kept in that light regime for 24 hours. Afterwards they were shifted back to hight (2000 $\mu E.m^{-2}.s.^{-1}$). Gas exchange rates were monitored during this period by using a Portable Photosynthesis System (LI-6000). Associated parameters mentioned above were also calculated and the data were analysed in CRD.

2.2.2 Effect of low light intensities

2.2.2.1 Experiment-1: Sunflower, amaranthus and sorghum plants were raised in battery containers as described above, when the plants were about 50 days old, they were shifted from open sun (2000 $\mu E.m^{-2}s^{-1}$) to very low light (100 $\mu E.m^{-2}s^{-1}$) and gas exchange rates measured for 210 minutes by using a Portable Photosynthesis System (LI-6000). Afterwards some plants were brought back to high light and some plants were kept at the low light for 10 days. Gas exchange rates were measured in these plants also.

2.2.2.2 Experiment-2

A similar experiment was conducted for a second time using sunflower and amaranthus. The gas exchange rates and associated parameters calculated from the above two experiments were analysed following CRD design.

2.2.3 Effect of sequential transferring to moderate and low light intensities

In this experiment also 50 days old potted plants of sunflower and amaranthus were used. They were shifted from high light to a moderate light (500 $\mu E.m^{-2}s^{-1}$) first and kept there for 15 minutes. Afterwards they were shifted to a low light (100 $\mu E.m^{-2}s^{-1}$) and kept there for 15 minutes. Gas exchange rates and other parameters were calculated and analysed as described earlier.

2.2.4 Effect of continuous mutual shading

Field grown plants of sunflower, sorghum and amaranthus were used in this study. When the crops were about 50 days old well exposed and shaded (due to mutual shading) leaves were tagged in the top, middle and bottom levels of the canopy. No shaded young leaves (top canopy) were available since there was no mutual shading in the top canopy. Gas exchange rates were measured by using a Portable Photosynthesis System (LI-6000) and associated parameters calculated. The data was analysed in CRD as described above.

2.3 Effect of moisture stress on gas exchange rates and associated characteristics in sunflower, amaranthus and sorghum

A series of experiments were conducted to study the effect of low lead ψ on gas exchange characteristics. They included pot grown as well as field grown plants apart from excised leaves. The details are as follows.

2.3.1 Pot culture experiment (Summer, 1987)

Sunflower, amaranthus and sorghum plants were raised in battery containers as mentioned above under optimum conditions. When the plants were about 45 days old irrigation was stopped for two days and then rewatered. Gas exchange rates and associated parameters were calculated by using a Portable Photosynthesis System (LI-6000). The leaf ψ was determined by using a Water Potential Data System (WESCOR). The data was analysed in CRD.

A multiple linear regression (MLR) analysis was done for 'A' and A/E on a personal computer similarly an inter-correlation matrix for the various gas exchange parameters was also developed on the same computer.

2.3.2 Field experiments

2.3.2.1 Experiment-1 (summer, 1986)

Sunflower and amaranthus plants were raised in the field following the standard package of practices. The plot size was 3.5 x 3 m. There were these replications.

When the crops were about twentyfive days old irrigation was stopped and gas exchange rates were measured for the next twenty days by using a Portable Photosynthesis System (LI-6000). Associated parameters were also calculated and analysed.

An inter-correlation matrix for the various gas exchange parameters was developed with the aid of a personal computer. An MLR analysis was also done for 'A' and A/E for both the species. The same computer was used to develop regression models for the various parameters. The first order derivative was then calculated by applying differential calculus.

2.3.2.2 Experiment-2 (summer, 1987)

A second field experiment was conducted in the above lines using sunflower, amaranthus and sorghum when the crops were forty five days old irrigation was stopped and gas exchange rates measured for the next seven days using a Portable Photosynthesis System (LI-6000). The associated parameters were also calculated and analysed as described earlier.

The various gas exchange parameters were used to develop an inter-correlation matrix using a personal computer. Separate MLR models were also developed for 'A' and A/E for all the three species studied.

2.3.3 Effect of moisture stress on gas exchange in excised leaves

Fully grown mature leaves were excised from field grown plants of sunflower and amaranthus and allowed to live wilt under the field conditions for sixteen minutes in the case of amaranthus. The reductions in the leaf ψ was monitored by a Water Potential Data System (WESCOR). Observations on gas exchange rates were also made during the course of live wilting by using a Portable Photosynthesis System (LI-6000). The derived parameters were calculated and the data were analysed in CRD.

Regression models for the various parameters were then developed on a personal computer.

2.3.4 Experiments with ABA

2.3.4.1 Experiment-1

Fully grown matured leaves of sunflower and amaranthus were removed from potted plants. The petiole was again cut under water. Two different concentrations of ABA (10^{-6}M and 10^{-4}M) were fed to these leaves through the transpiration stream (by dipping the petiole in ABA solution). The feeding was done for twenty minutes in glass house conditions (1000 μ.E.m.$^{-2}$s.$^{-1}$) and at the end of the feeding period gas exchange rates were determined by using a PPS (LI-6000). The related parameters were then calculated and analysed in CRD. Leaves kept in water for the same period served as controls.

An inter-correlation matrix for the various parameters was developed on a personal computer. MLR model was also developed for 'A' for both the species.

2.3.4.2 Experiment-2

The experimental procedure remained similar to the above. Here, only one concentration of ABA (10^{-4}M) was used. Apart from ABA treatment and water control there were other four treatments. They were EGTA (1 μM), ruthenium red (RR), (1 μM), EGTA (1uM) + ABA (10^{-4}M) and RR (1 μM) + ABA (10^{-4}M) were also fed through the transpiration stream. The leaves were fed with these chemicals under a moderate light (500 μE.m.$^{-2}$s.$^{-1}$).

2.3.4.3 Experiment-3

This experiment was a repetition of the above one. However, RR treatment was not given. In all other respects it was a repetition of experiment 2.

2.4 Estimation of stomatal and mesophyll limitations of photosynthesis

The method proposed by Farquhar and Sharkey (1982) has been used here to quantify the stomatal limitation of photosynthesis. Their method has been further modified to estimate mesophyll limitations of photosynthesis also.

The first step in this analysis involves the development of 'A'.Ci curves. Potted and field grown plants experiencing different intensities of drought stress were exposed to different levels of ambient CO_2 levels for about 10 minutes by enclosing the plants in a polythene structure of size 5M x 3M x 6M. The light intensity available inside the structure was above 1600 $\mu E.m^{-2}s^{-1}$. Carbondioxide was released inside the chamber by treating $CaCO_3$ with dilute HCl by means of a Kip's Apparatus.

$$CaCO_3 + 2HCl \dashrightarrow CaCl_2 + H_2O + CO_2$$

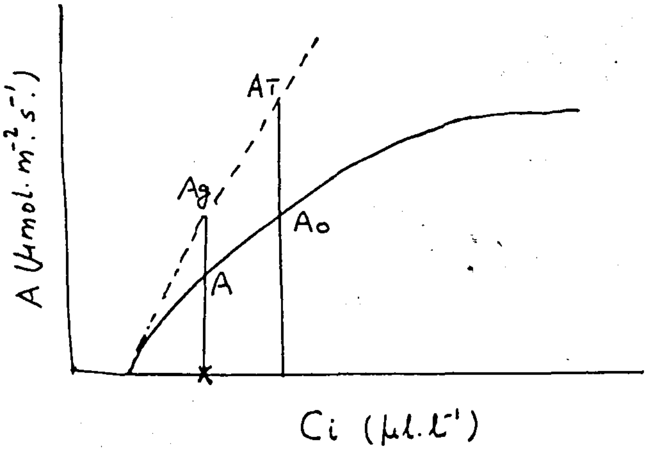
When the ambient CO_2 level inside the structure was increased to a known level, release of CO_2 gas was stopped and sufficient time was given for uniform spreading of CO_2 inside which was monitored by an ADC CO_2 analyser at different points inside the structure. Then the gas exchange rates were determined with a Portable Photosynthesis System (LICOR-6000).

The intercellular CO_2 concentration (Ci) was then calculated from the formula.

$$Ci = Ca - \frac{1.6A}{'g_s'} \quad \text{(Farquhar and Sharkey, 1982)}$$

where, Ca is the ambient CO_2 level ($\mu l.l^{-1}$), 'A' is the photosynthetic rate (μ mol.$m^{-2}.s^{-1}$) and 'g_s' is the stomatal conductance (mol.$m^{-2}.s^{-1}$).

Then 'A'.Ci curves were plotted based on the principle of least squares with the help of a personal computer. On the 'A'.Ci curve the following points were marked.



(i) A - Observed photosynthetic rate at any given time.

(ii) Ao - Potential photosynthetic rate when stomatal factors are not limiting and mesophyll factors are limiting.

(iii) Ag - Potential photosynthetic rate when mesophyll factors are not limiting and stomatal factors are limiting .

(iv) AT - Potential maximum photosynthetic rate when neither mesophyll nor stomatal factors are limiting.

Farquhar and Sarkey (1982) give the following formula to estimate the relative stomatal limitations (ls)

$$ls = \frac{Ao - 'A'}{Ao} \times 100$$

We define relative mesophyll limitations of observed photosynthesis (lm) as follows.

$$lm = \frac{Ag - 'A'}{A_g} \times 100$$

We further define the mesophyll limitation to the potential photosynthesis (AT) as follows:

$$ML = \frac{AT - Ao}{AT} \times 100$$

These limitations were estimated for sunflower and amaranthus plants raised in pots and field, under control and moisture stress conditions.

2.5 Estimation of stomatal and assimilation loop gains

The stomatal and assimilation loop gains were calculated as per the procedure laid out by Farquhar *et al.*, (1978).

The following steps were followed to estimate these loop gains.

Definitions and procedure

(i) The physical gain of, stomatal feedback loop is defined as the partial derivative of Ci with respect to 'g_s'

i.e., $\frac{\partial Ci}{\partial g_s}$

This is obtained by the following partial deferentation.

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

$$\frac{\partial Ci}{\partial g_s} = \frac{1.6A}{g_s^2}$$

This partial derivative gives the rate of change of Ci when 'g_s' is changed keeping 'A' and Ca constant. Since Ci is bound to increase with an increase in 'g_s' when 'A' and Ca are constant, $\frac{\partial Ci}{\partial g_s}$ is positive.

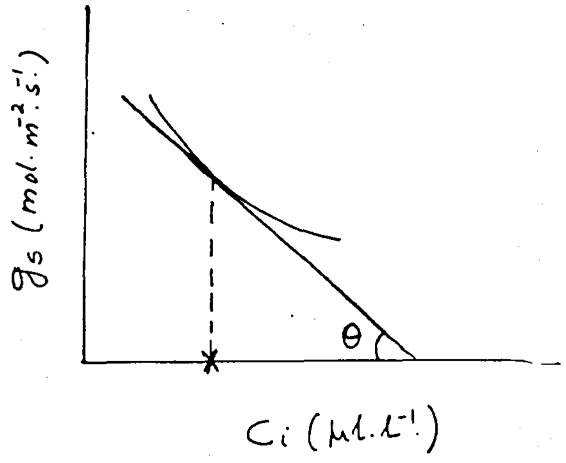
(ii) The physical gain of assimilation feed back loop is defined as the partial derivative of Ci with respect to 'A' i.e., $\partial Ci/\partial A$. This is obtained by the following partial differentiation.

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

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

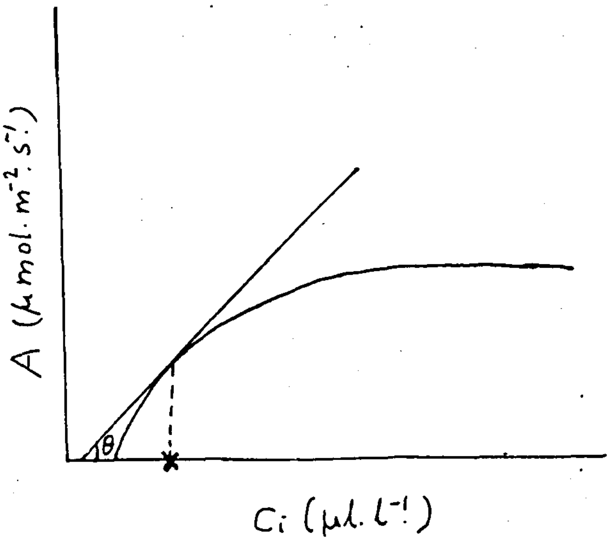
This partial derivative gives the rate of change in Ci when 'A' is changed keeping Ca and 'g_s' constant. Under such a condition Ci will decrease if 'A' increases and so $\frac{\partial Ci}{\partial A}$ is negative.

(iii) Physiological gain of stomatal conductance is defined as the differential co-efficient $\frac{dgs}{d\bar{C}i}$. It is the of change in g_s with respect to Ci. Since g_s will decrease as Ci increases $dg_s/\ dCi$ takes a negative significance. This is obtained from a curve fitted between g_s and Ci. The slope of the curve at any given Ci gives $dg_s/\ dCi$.



Physiological gain of conductance at the operating Ci (marked X) is $\frac{d\, g_s}{d\mathrm{Ci}} = -(\tan\theta)$

(iv) Physiological gain of assimilation loop is defined as the differential co-efficient $dA/d\mathrm{Ci}$ in the rate of change of 'A' with respect to Ci. This derivative is the slope of the A.Ci curves at any operating Ci. Since 'A' increases as Ci increases $dA/d\mathrm{Ci}$ is positive.



The physiological gain of assimilation ($dA/d\mathrm{Ci}$) at the operating Ci (marked X) is given by $\frac{dA}{d\mathrm{Ci}} = \tan\theta$

(v) <u>Operating points of Ca and Ci</u>

From the above definition it is clear that the absolute values of the physiological gains of assimilation and stomal conductance vary depend upon the Ci at which the slopes $dg_s/d\mathrm{Ci}$ and $dA/d\mathrm{Ci}$ are determined. Also the physical gains

$\partial Ci/\partial g_s$ and $\partial Ci/\partial A$ are defined at a constant Ca. Hence it is necessary to choose an operating point of Ca. The corresponding Ci is termed as operating Ci. The operating Ca was taken in our studies as 350 ml l^{-1} since it represents the usual Ca levels in our conditions. The operating Ci levels varied according to the specimen and the treatments in question and was calculated from the formula mentioned above.

(vi) Stomatal loop gain (Gg) is defined as the products of the physical and physiological loops of stomatal conductance. Therefore, $Gg = \frac{dg_s}{dCi} \cdot \frac{\partial Ci}{\partial g_s}$

(vii) Similar assimilation loop gain (GA) is defined as the product of the physical and physiological loops of assimilation. Therefore,

$$GA = \frac{dA}{dCi} \cdot \frac{\partial Ci}{\partial A}$$

(It may be noted that both Gg and GA will take negative signs as one of the components is negative in both the cases).

(viii) Under a given set of conditions if Ca is increased Ci will increase by 1/(1-Gg-GA) times Ca. It may be noted that if Gg = 0, then Ci will increase to the same extent as the increase in Ca. But because of the feed back loops operating ($Gg \neq 0$, $GA \neq 0$)

if Ca is increased by one unit Ci will increase by a fraction of it, namely 1/(1-Gg-GA). This ratio is closer to the slope of a linear curve fitted between Ci and Ca.

This procedure has been successfully used by Dubbe _et al_ (1978) to estimate 'Gg' and 'GA' in a number of species.

2.6 Studies on chlorophyll fluorescence quenching as affected by leaf water status in sunflower, amaranthus and sorghum

Two studies were conducted in this set of experiments. In the first experiment leaf discs were floated in PEG solutions to simulate water stress. In the second experiment excised leaves were allowed to live wilt. The details of the procedure are as follows.

2.6.1 Experiment 1

Fully grown leaves were excised from sunflower, sorghum and amaranthus plants and leaf discs prepared. These discs were then floated in PEG solutions of water potentials -6,-12 and -15 bars in the dark. At the end of 1.5 and three hours of incubation the discs were taken and fluorescence emission was studied by using a Plant Productivity Fluoremeter (SF-20). The emission spectrum was recorded for 50 seconds. From the peak and lowest points on the graph, per cent quenching of fluorescence after 25s and 50s was calculated.

2.6.2 Experiment 2

Mature leaves of sunflower, amaranthus and sorghum were excised and allowed to live wilt under dark. The per cent of water loss was determined gravimetrically and then leaf discs were prepared and fluorescence studied as described above.

EXPERIMENTAL RESULTS

IV RESULTS

4.1 Effect of different levels of fertilizer nitrogen on gas exchange and associated characteristics in sunflower, amaranthus and sorghum

Sunflower, amaranthus and sorghum plants were raised in pots under three different levels of fertilizer nitrogen, namely, recommended level (optimum), 50 per cent more than the recommended level (high) and 50 per cent less than the recommended level (low). When the plants were about 50 days old, measurements on gas exchange rates were taken from the top expanded leaves and the data are presented in Table - 1.

Photosynthetic rate showed very drastic reductions in all the three species at lower N levels and the reductions were significant. At high nitrogen level all the three species showed almost similar values of photosynthetic rate. However, at low nitrogen level sunflower exhibited a very high reduction in photosynthetic rate amounting to about 75 per cent compared to the value at high nitrogen level. The corresponding reductions in photosynthetic rates were to the extent of 53 and 47 per cent in amaranthus and sorghum, respectively. Transpiration rate and stomatal conductance showed only very marginal reduction at lower levels of nitrogen in the case of sunflower. Amaranthus and sorghum also showed only insignificant reduction in transpiration rates. However, stomatal conductance was affected significantly, at the lower levels of nitrogen in amaranthus and sorghum.

The ratio of photosynthetic rate to stomatal conductance (A/g_s) which is an indirect reflection of mesophyll conductance showed a significant reduction with reduction in the nitrogen levels. Concomitantly there was statistically significant increase in the internal CO_2 concentration (Ci) under lower nitrogen level suggesting the loss of mesophyll efficiency for CO_2 reduction. "Carboxylation efficiency" has been calculated as the ratio of photosynthetic rate to internal CO_2 level (A/Ci). It may be noted that under any given level of nitrogen fertilizer, amaranthus and sorghum showed higher A/Ci values compared to sunflower suggesting that carboxylation efficiency was high in these C_4 plants. This is also evident from the fact that A/g_s values were also higher in amaranthus and sorghum compared to sunflower. This indicates the high intrinsic 'gm' of C_4 plants.

The short-term water use efficiency (WUE) calculated as the ratio of photosynthetic rate to transpiration rate (A/E) showed very drastic reduction in sunflower at low nitrogen level. Though the reductions in A/E ratios were also significant in amaranthus and sorghum it was only to a lesser extent compared to sunflower. For instance, when sunflower showed values of 6.50 and 1.20 under high and low nitrogen levels, the corresponding values in sorghum were 8.82 and 6.03 and in amaranthus were 7.33 and 4.38 respectively.

These results suggest that under low levels of nitrogen, photosynthetic rate is affected more than stormal conductance

TABLE 1 : Effect of different levels of fertilizer nitrogen on gas exchange and associated characteristics in Sunflower, Amaranthus and Sorghum

Species	N level	PAR	A	g_s	A/g_s	Ci	A/Ci	E	A/E
SUNFLOWER									
	High	1800	40.76	0.64	65.9	235	16.40	6.70	6.50
	Optimum	1650	19.73	0.52	39.1	275	7.04	6.40	3.57
	Low	1700	10.26	0.60	19.2	310	3.39	6.30	1.20
	F test	NS	*	NS	*	*	*	NS	*
	C D 5%	-	8.58	-	14.2	30	2.51	-	2.08
AMARANTHUS									
	High	2000	43.24	0.52	83.2	205	22.40	5.90	7.33
	Optimum	1900	22.84	0.38	64.7	241	11.70	4.90	4.66
	Low	1900	19.70	0.29	66.4	235	8.90	4.50	4.38
	F test	NS	*	NS	*	*	*	NS	*
	C D 5%	-	7.59	-	9.25	15	3.88	-	2.54
SORGHUM									
	High	1800	42.66	0.37	115.3	153	25.2	4.90	8.82
	Optimum	1800	30.86	0.26	120.3	151	20.3	4.10	7.52
	Low	1800	25.53	0.24	98.1	183	14.1	3.90	6.03
	F test	NS	*	NS	*	*	*	NS	*
	C D 5%	-	4.35	-	16.5	12	5.1	-	1.33

and transpiration rate. The absolute values of A/g_s, A/Ci and A/E were higher in amaranthus and sorghum compared to sunflower suggesting that these two C_4 species have intrinsically high 'gm'. Further, the extent of reduction in these parameters was to lesser extent in these species compared to sunflower. Thus, the mesophyll characteristics of sunflower were more affected at low nitrogen levels.

4.1.1 Relationship between gas exchange parameters

The regression models developed to explain the type of relationship between photosynthetic rate (A), transpiration rate (E) and stomatal conductance (g_s) as influenced by changes in nitrogen levels are given in Table 2.

In none of the three species studied, there existed a linear relationship between 'A' and 'g_s'. The best curve explaining the relationship between 'A' and 'g_s' in sunflower was a positive exponental logarithmic one, but it was with very low co-efficient of determination (about 20%). However, in the case of amaranthus and sorghum a positive semi-logarithmic curve was found to be the best fit with R^2 values of 83 per cent and 57 and 'g_s was non-linear, the rate of change of 'A' with respect to 'g_s' i.e., dA/dg_s was never a constant in any of the species (Table 2). But dA/dg_s itself was a function of 'g_s' at any given level of nitrogen. This clearly indicates that the dependance of 'A' on 'g_s' was not constant under conditions of nitrogen deficiency. It may as well be noted that dA/dg_s is different for different species.

TABLE 2: Regression models for A, E and g_s at different nitrogen levels

SPECIES	Y	X	Y = f (x)	dy/dx
SUNFLOWER	A	g_s	$\mathrm{EXP}\ (8.4848 + 1.2062 \ln g_s$	$\mathrm{EXP}\ (8.4848 + 1.2062 \ln g_s)\ (1.2062/g_s)$
	E	g_s	$\mathrm{EXP}\ (1.194 + 1.0813\ g_s)$	$\mathrm{EXP}\ (1.194 + 1.0813\ g_s)\ (1.0813)$
	A	E	$\mathrm{EXP}\ (0.9239 + 1.0352 \ln E)$	$\mathrm{EXP}\ (0.9239 + 1.0352 \ln e)\ (1.0352/E)$
AMARANTHUS	A	g_s	$56.4091 + 29.006 \ln g_s$	$29.006/g_s$
	E	g_s	$2.1788 + 13.7787\ g_s$	13.7787
	A	E	$-50.365 + 39.058 \ln E$	$39.058/E$
SORGHUM	A	g_s	$59.9963 + 18.9703 \ln g_s$	$18.9703/g_s$
	E	g_s	$1.1932 + 17.7018\ g_s$	17.7018
	A	E	$\mathrm{EXP}\ (2.378 + 0.6232 \ln E)$	$\mathrm{EXP}\ (2.378 + 0.6232 \ln E)\ (0.6232/E)$

The regression models developed to explain the type of relationship between photosynthetic rate (A), transpiration rate (E) and stomatal conductance (g_s) as influenced by changes in nitrogen levels are given in Table 2.

There existed a positve linear relationship between 'E' and 'g_s' in amaranthus and sorghum with high R^2 values of 93 per cent and 89 per cent, respectively (Table 2). However, in sunflower there existed a weak positive exponential relationship between 'E' and 'g_s' (R^2 = 40%). Thus in amaranthus and sorghum, the loss of water per unit increase in 'g_s' i.e., dE/dg_s remained constant, whereas in sunflower there was enhanced water loss with increase in 'g_s in a weak exponential fashion.

Contrary to the notion that there exists a positive linear relationship, all the three species studied showed a positive non-linear relationship between 'A'and 'E' (Table 2). The values of dA/dE was thus dependent on the absolute values of 'E'. It is clear from Table 2, that at physiological range of 'E', amaranthus and sorghim will have higher dA/dE values compared to sunflower.

4.2 Effect of different light intensities on gas exchange rates and associated characteristics in sunflower, amaranthus and sorghum

Sunflower, amaranthus and sorghum plants were grown in pots for about 45 days under optimum conditions. Then they were shifted to different regimes and gas exchange rates were monitored. In another experiment the effect of low light due to constant natural shading was studied in natural crop canopies of sunflower and amaranthus. The bottom and middle canopy leaves which were constantly under shade were tagged and their gas exchange parameters were compared with similar leaves receiving full light located at another area of the canopy. The results of these experiments are discussed below.

4.2.1 Effect of moderate light intensities

Experiment 1

Fifty days old potted plants of sunflower, amaranthus, and sorghum were shifted from high light intensities (2000 $\mu Em.^{-2}s^{-1}$, to a moderate intensity (about 500 $\mu Em.^{-2}s^{-1}$) and kept in that light regime for 90 minutes and gas exchange rates were monitored. The light intensity to which the plants were transferred was not the same for all the species. It was 400 $\mu E.m.^{-2}s.^{-1}$ in the case of sunflower and sorghum and 600 $\mu E.\ m.^{-2}s.^{-1}$ in the case of amaranthus. The data are presented in Tables 3 - 5.

There was a time dependant reduction in 'A' in all the three species studied. The reductions were statistically significant. Stomatal conductance also showed significant reduction in all the three species but the reductions were more drastic in amaranthus and sorghum compared to sunflower. Sunflower maintained higher values of 'gs', compared to the

TABLE 3 : Effect of moderate light intensity on gas exchange and associated characteristics in Sunflower

Duration of exposure (min.)	PAR	A	g_s	A/g_s	Ci	A/Ci	E	A/E
0	1600	33.37	0.490	58.43	240	12.00	6.90	4.20
10	400	10.91	0.325	37.68	280	4.00	3.15	3.48
30	400	11.70	0.426	25.17	300	3.60	3.63	2.83
60	400	12.75	0.416	31.32	290	4.30	4.46	9.85
90	400	6.65	0.370	15.60	315	1.80	3.70	1.57
F test	*	*	*	*	*	*	*	*
C D 5%	600	5.12	0.10	8.22	17	2.21	1.51	2.03

TABLE 4 : Effect of moderate light intensity on gas exchange and associated characteristics in Amaranthus

Duration of exposure (min.)	PAR	A	g_s	A/g_s	Ci	A/Ci	E	A/E
0	1600	37.37	0.310	121.71	130	26.00	4.53	8.84
10	600	9.33	0.110	82.33	208	4.47	1.67	5.80
30	600	7.88	0.093	84.73	204	3.86	2.80	2.81
60	600	4.69	0.065	73.35	220	2.13	3.60	1.20
F test	*	*	*	*	*	*	*	*
C D 5%	650	2.57	0.09	12.55	19	1.81	1.53	1.12

TABLE 5 : Effect of moderate light intensity on **gas exchange and** associated characteristics in Sorghum

Duration of exposure (min.)	PAR	A	g_s	A/g_s	Ci	A/Ci	E	A/E
0	1600	41.60	0.337	124.53	140	29.64	4.23	9.84
10	400	7.90	0.077	106.33	170	8.84	2.90	3.70
30	400	6.84	0.100	69.96	230	9.98	3.00	3.23
60	400	7.07	0.103	73.16	230	3.09	2.90	2.50
90	400	6.81	0.095	72.71	220	3.22	2.44	2.50
F test	*	*	*	*	*	*	*	*
C D 5%	600	1.55	0.08	9.28	12	1.56	1.88	1.03

other two species throughout the period of shading. For instance, 10 minutes after shading sunflower showed a 'gs' of 0.325 $mol.m^{-2}s^{-1}$ whereas the corresponding values were 0.110 and 0.077 $mol.m^{-2}s^{-1}$ in amaranthus and sorghim respectively. Even 60 minutes after exposing to the low light sunflower exhibited a high 'g_s' value (0.416 $mol.m^{-2}s^{-1}$) the corresponding values of 'g_s' in Amaranthus and Sorghum were 0.063 and 0.103 $mol\cdot m^{-2}s^{-1}$, respectively. There were significant reduction in 'E' as the time of shading progressed which followed a similar pattern as that of 'g_s'. Reductions in 'E' were very drastic in Amaranthus and Sunflower compared to Sorghum. The ratio of photosynthetic rate to stomatal conductance (A/g_s) is an indirect measure of the intrinsic mesophyll conductance. The A/g_s ratios were drastically affected in all the three species suggesting that the mesophyll conductance was reduced at low light intensities. There were significant increases in Ci in the three species studied.

The ratio of photosynthetic rate to the internal CO_2 level (A/Ci) a reflection of the carboxylation efficiency of the mesophyll, was also found to be affected very remarkably as the time of shading progressed. The short time water use efficiency estimated from A/E ratio revealed a significant reduction in all the three species. However, A/E ratios were high in Amaranthus and Sorghum at any time of shading.

TABLE 6 : Effect of moderate light intensity on gas exchange and associated characteristics in Sunflower

Duration of exposure (min.)	PAR	A	g_s	A/g_s	Ci	A/Ci	E	A/E
After transfering to moderate light								
0	2000	34.53	0.570	60.60	238	14.21	3.3	10.46
5	600	11.40	0.350	32.57	280	4.15	3.1	3.68
10	600	15.44	0.400	38.60	260	5.55	2.8	4.88
30	600	16.57	0.440	37.66	262	5.92	2.7	6.14
60	600	10.04	0.310	32.39	280	3.59	3.0	3.35
After 24 hours of exposure	400	6.48	0.060	108.00	167	3.88	1.70	3.80
After transfer to high light intensity								
5	2000	37.25	0.440	84.66	205	18.17	3.8	9.81
10	2000	39.13	0.460	85.06	204	19.18	3.5	11.18
15	2000	38.80	0.490	79.18	213	17.96	3.9	9.25
F test	*	*	*	*	*	*	*	*
C D 5%	700	15.51	0.09	15.51	17.12	3.51	0.88	6.28

TABLE 7 : Effect of moderate light intensity on gas exchange and associated characteristics in Amaranthus

Duration of exposure (min.)	PAR	A	g_s	A/g_s	Ci	A/Ci	E	A/E
After transferring to moderate light								
0	2000	38.52	0.280	138.00	120	32.10	2.90	11.30
5	400	5.11	0.045	128.10	136	3.76	1.65	3.09
10	400	4.74	0.040	118.50	163	3.14	1.50	3.20
15	400	5.13	0.050	102.60	180	2.93	1.08	4.75
After 24 h	400	6.48	0.060	108.00	167	3.88	1.70	3.80
Aftet transferring back to high light								
3	2000	26.11	0.190	137.40	120	21.76	2.70	9.70
6	2000	33.44	0.230	145.00	107	31.25	2.90	11.50
9	2000	34.52	0.260	133.00	128	26.96	3.25	10.62
F test	*	*	*	*	*	*	*	*
C D 5%	600	12.15	0.11	15.5	17.1	13.38	1.20	8.35

TABLE 8 : Effect of moderate light intensity on gas exchange and associated characteristics in Sorghum

Duration of exposure (min.)	PAR	A	g_s	A/g_s	Ci	A/Ci	E	A/E
After transferring to moderate light								
0	2000	35.19	0.30	117.30	152	23.15	3.10	11.35
5	500	14.53	0.15	96.87	185	7.85	1.79	8.10
10	500	8.53	0.10	85.3	203	4.21	1.40	6.09
15	500	7.26	0.09	81.00	211	3.44	1.40	5.20
After 24 h	500	4.72	0.09	52.44	260	1.82	1.10	4.29
After transferring back to high light								
5	2000	17.36	0.16	109.00	166	10.45	1.50	11.60
10	2000	27.31	0.22	124.00	141	19.37	1.70	16.06
15	2000	40.58	0.28	144.93	108	37.58	2.50	16.23
F test	*	*	*	*	*	*	*	*
C D 5%	600	3.56	0.13	12.22	15	6.68	1.71	3.75

Experiment-2

Fifty days old potted plants of Sunflower, Amaranthus and Sorghum were shifted from (200 $\mu E.m.^{-2}s.^{-1}$) to a moderate light (400-600 $\mu E.m.^{-2}s.^{-1}$) and kept in that light regime for twenty four hours. Afterwards they were shifter to high light conditions and gas exchange rates were monitored during shading and after shifting the plant back to high light. The light intensity to which the plants were transferred was not the same in all the species because of differences in the extent of light transmitted in different parts of the shade shelters. It was 400 $\mu E.m.^{-2}s.^{-1}$ in Amaranthus, 500 $\mu E.m.^{-2}s.^{-1}$ in Sorghum and 600 $\mu E.m.^{-2}s.^{-1}$ in Sunflower. The data are presented in Tables 6 - 8.

The results were similar to those of the previous experiment. A time dependant reduction in 'A' 'g_s' 'E' and derived parameters like A/g_s, A/Ci and A/E were observed in all the three species. The internal CO_2 level showed an increasing trend.

Reduction in photosynthetic rate was almost at a comparable rate in both Sunflower and Sorghum but very drastic in Amaranthus (Fig.Ia). 'g_s' was higher in sunflower at any given time of shading. Reduction in 'E' (Fig.Ib) and 'g_s' (Fig.Ic) were marked and faster in Amaranthus and Sorghum compared to Sunflower. Sunflower showed transpiration rates of 3 and 2.90 $mol.m.^{-2}s.^{-1}$ one and 24 hours, respectively after shading whereas Sorghum and

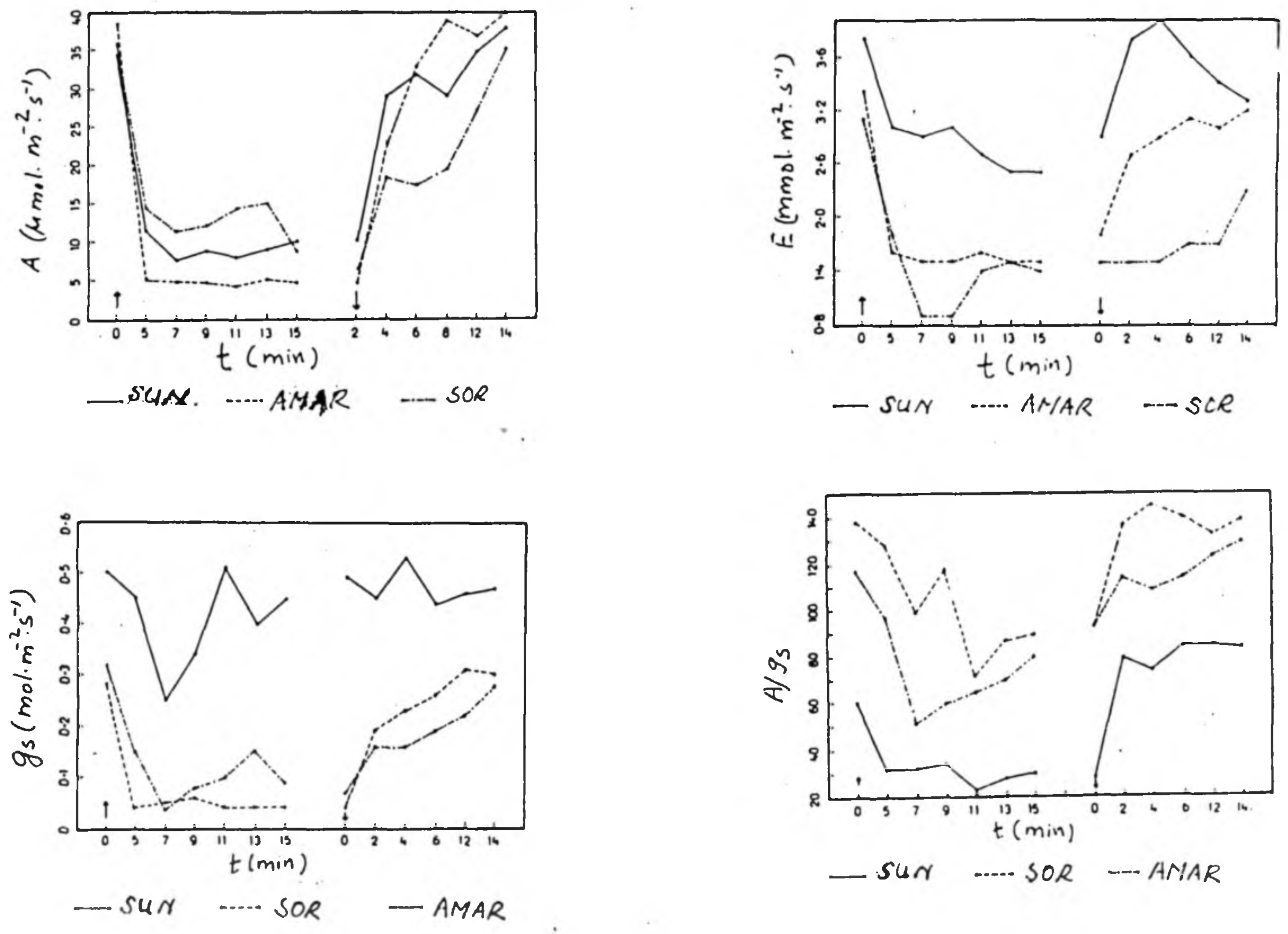


FIG I Effect of moderate light intensity on A, E, g_s and A/g_s ratio in sunflower, sorghum and amaranthus

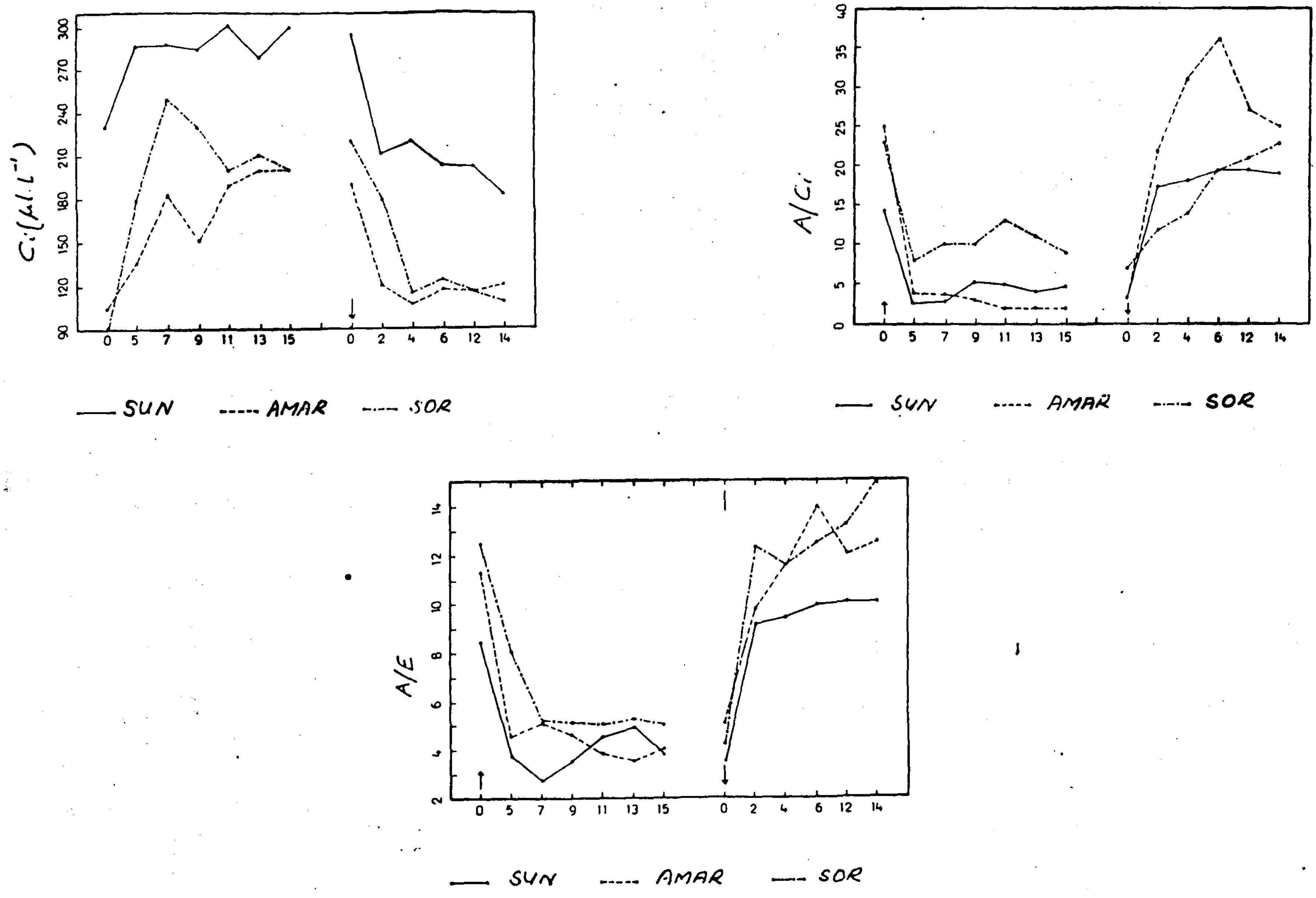


FIG II Effect of moderate light intensity on Ci, A/Ci and A/E ratios in sunflower, sorghum and amaranthus

Amaranthus exhibited as low a transpiration rate of 1.40 and 1.08 mmol.m.$^{-2}$s.$^{-1}$ just 15 minutes after shading. Though there was a time dependant reduction in A/g_s ratios, the values were always higher in Amaranthus and Sorghum compared to Sunflower (Fig.Id). The increase in Ci was more pronounced in Amaranthus and Sorghum (Fig.IIa). Drastic reductions in A/Ci ratios were observed in all the three species but Sorghum showed markedly higher A/Ci values compared to the other two species (Fig.IIb). Reductions in A/E ratios were also very drastic in the species (Fig.IIc).

Transfering these plants from moderate to high light resulted in the recovery of all the gas exchange characteristics to the control levels. During the course of recovery also sunflower maintained higher values of 'E', 'g_s' Ci and to some extent 'A', compared to the other two species. However, A/g_s and A/E values were much higher in Amaranthus and Sorghum. Sunflower and Sorghum showed comparable trends in the recovery of A/Ci ratios whereas in Amaranthus A/Ci ratios showed a very marked jump.

4.2.2 Effect of low light intensities

Potted plants of Sunflower, Amaranthus and Sorghum which were grown in natural sunlight and other optimum conditions were shifted to low light and gas exchange parameters were measured.

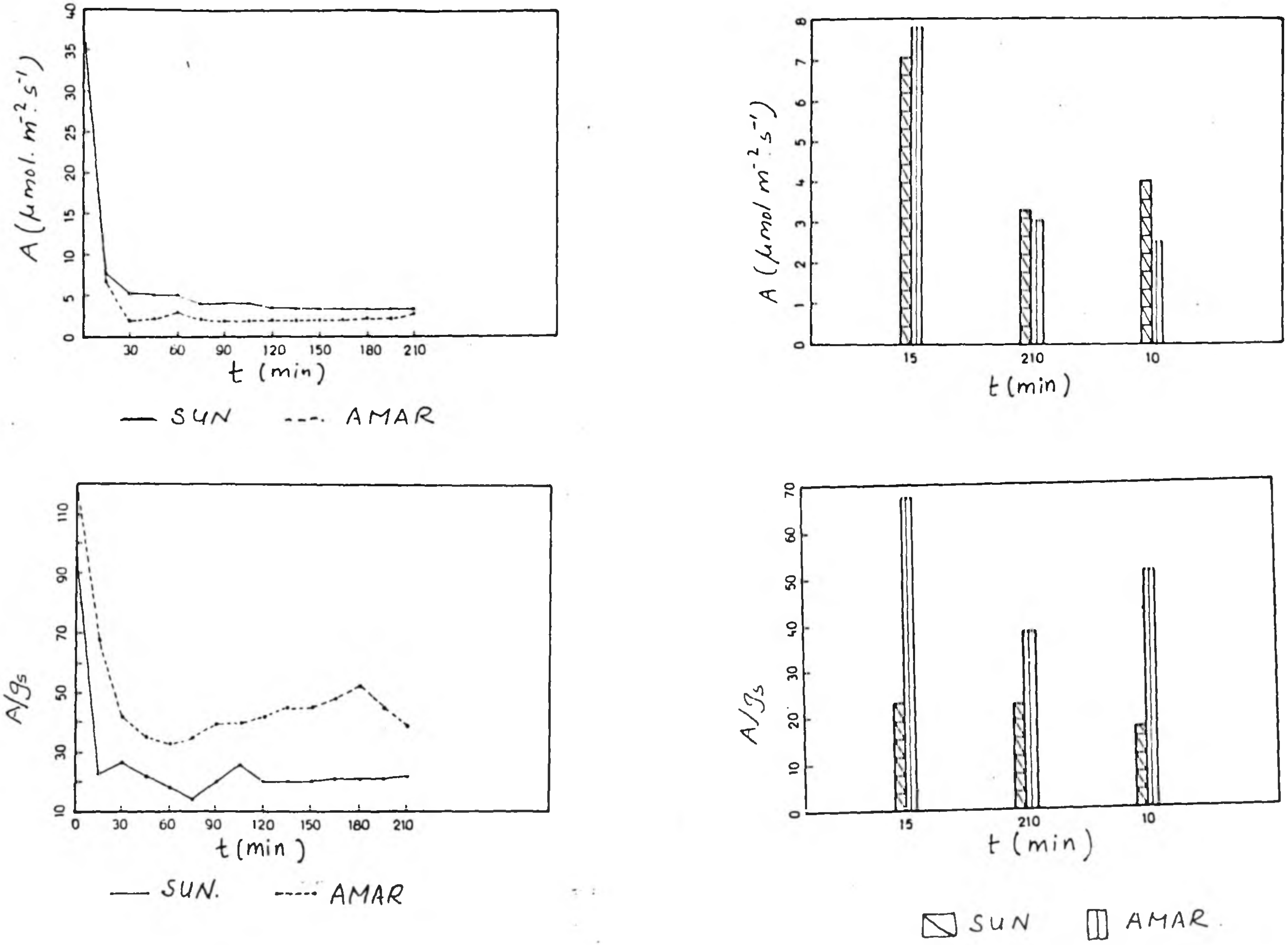


FIG III Effect of low light intensity on A and A/g_s in sunflower and amaranthus

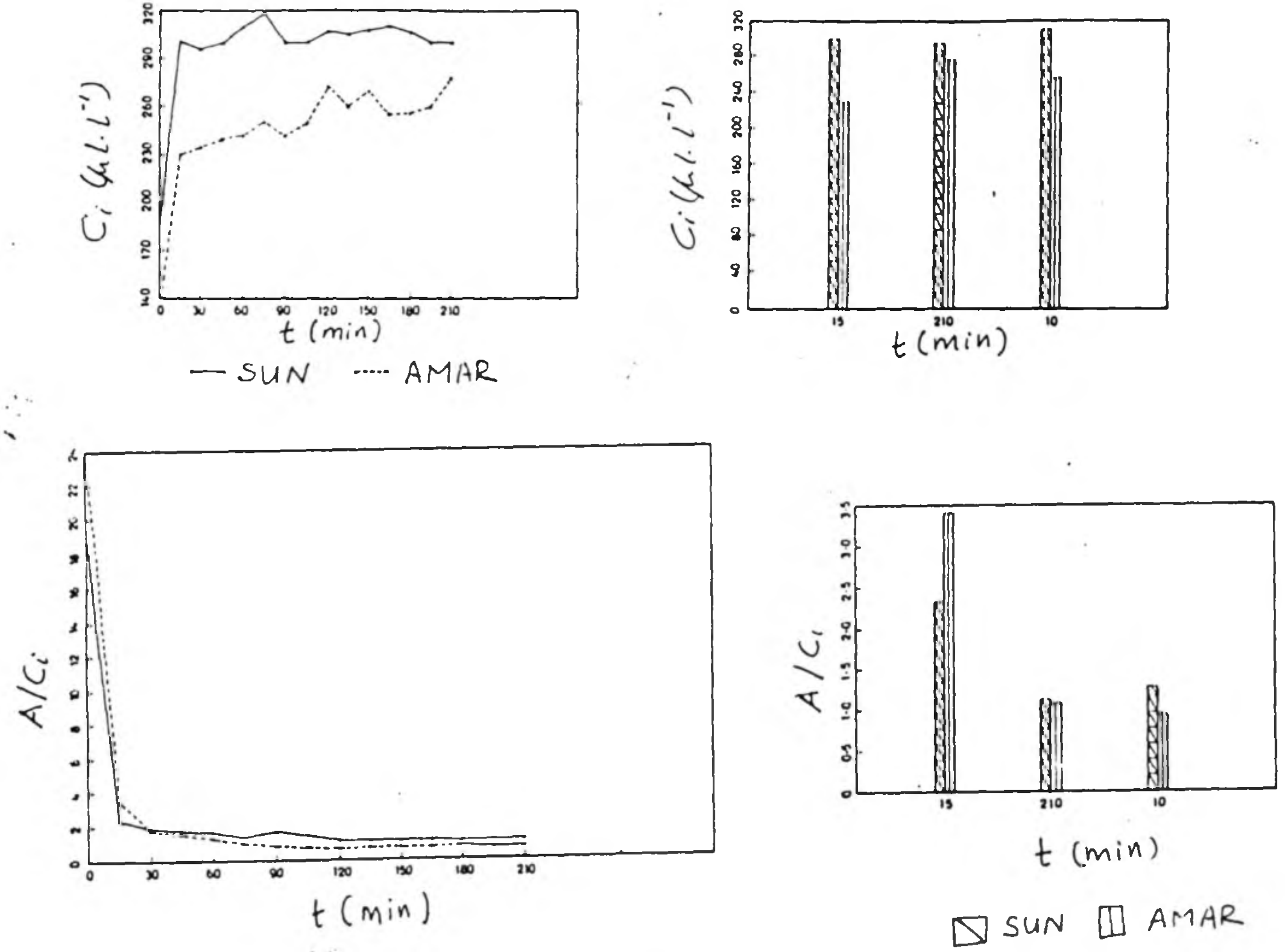


FIG IV Effect of low light intensity on Ci and A/Ci ratio in sunflower and amaranthus

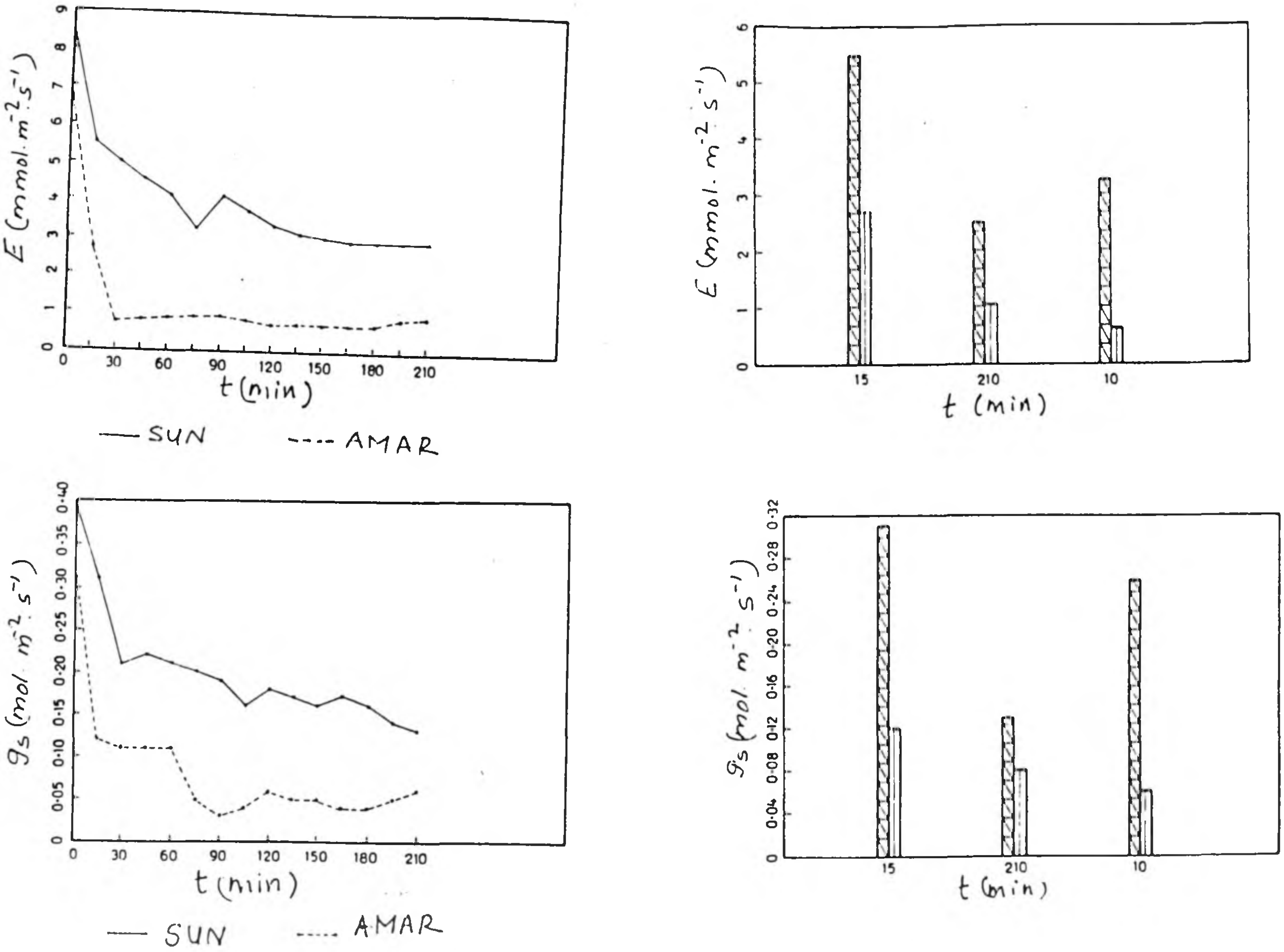


FIG V Effect of low light intensity on E and g_s in sunflower and amaranthus

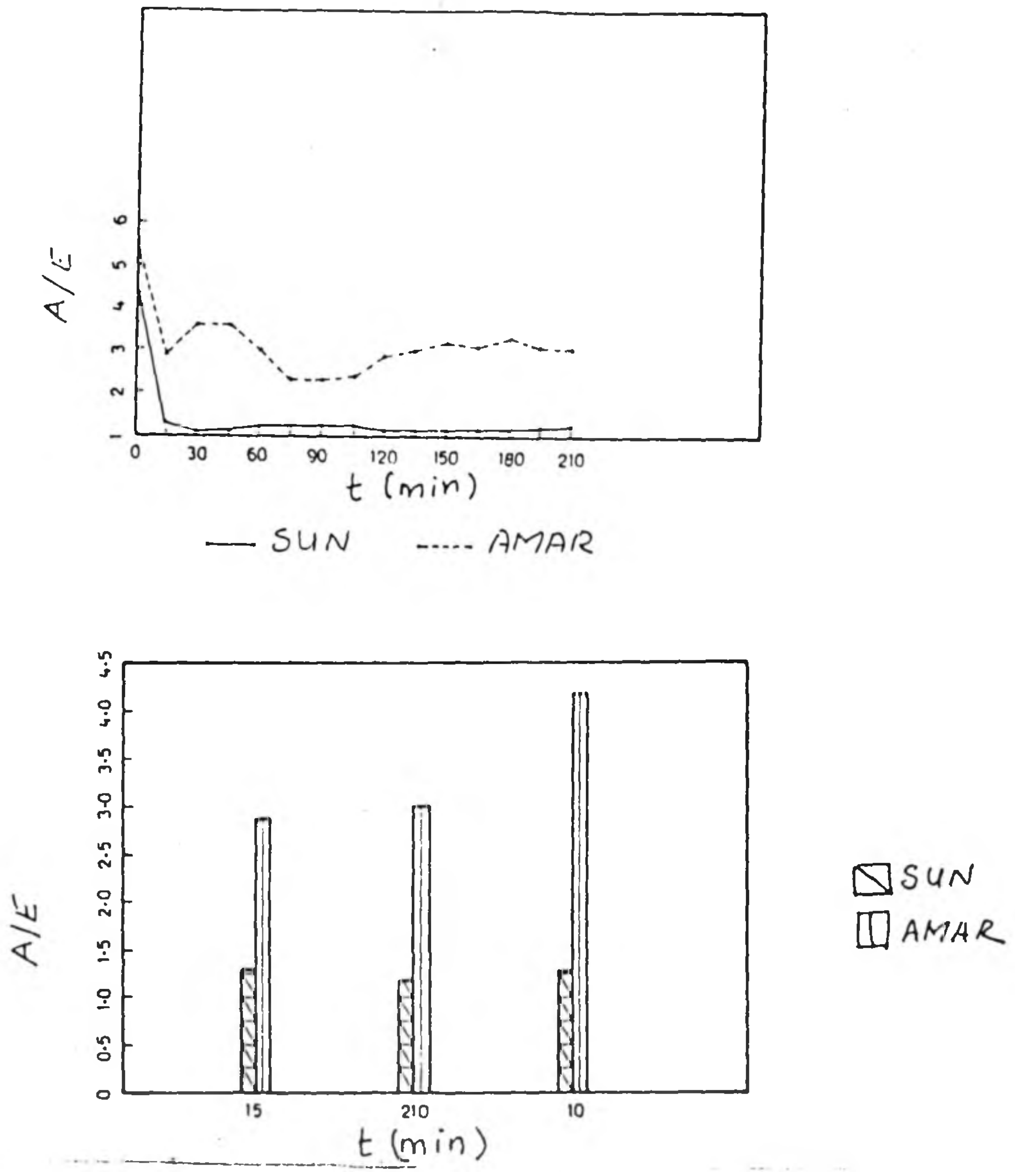


FIG VI Effect of low light intensity on A/E ratio in sunflower and amaranthus

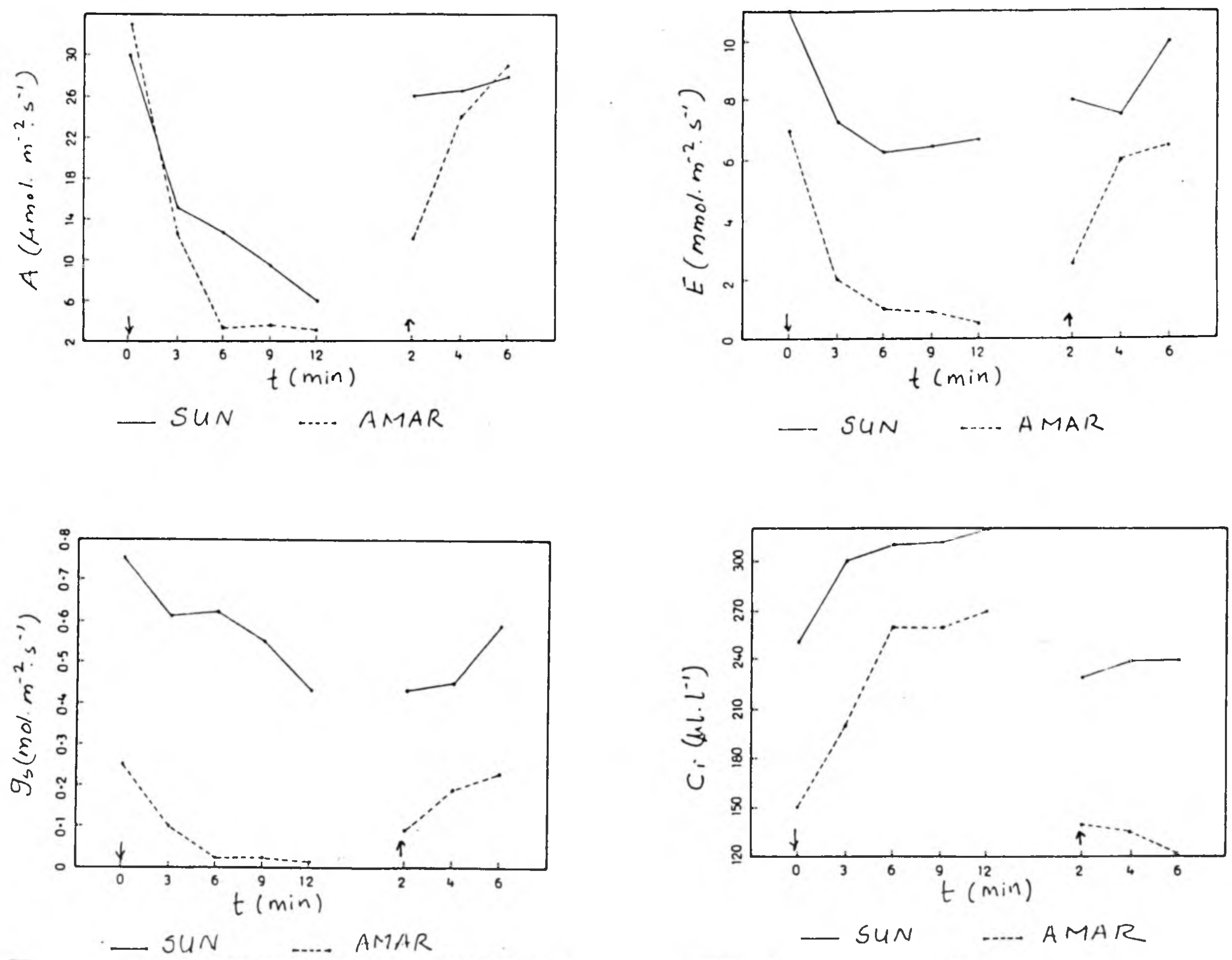


FIG VII Effect of low light intensity on A, E, g_s and Ci in sunflower and amaranthus

Experiment 1

In the first experiment, potted plants which were grown under natural light were shifted to shade where the PAR was less than 100 $\mu E.m^{-2}s^{-1}$ and gas exchange parameters monitored for 210 minutes. Afterwards some plants were brought back to open sun (2000 $\mu E.m^{-2}s^{-1}$) and some were allowed to grow for 10 days under the same shade. The data are presented in Table 9-11.

There was drastic reduction in 'A', 'g_s' and 'E' in all the species when they were shifted to low light. Derived parameters like A/g_s, A/Ci and A/E also showed significant reductions under shade. Internal CO_2 levels were significantly increased under low light conditions.

Reductions in 'A' more or less followed a similar pattern in all the three species (Tables 9-11), however sun flower exhibited slightly higher levels of 'A' throughout the shading (Fig.IIIa and b). A/g_s ratio was higher in Amaranthus and Sorghum compared to sunflower (Fig.IIIc and d). The internal CO_2 level increased under shade and the values were higher in sunflower compared to the other two species (Fig. IVa and b). A/Ci ratios were also decreased drastically in all the three species under shade. But amaranthus and Sorghum showed markedly higher values before shading (Fig.IVc and d).

At any given time of shading sunflower showed higher values of 'E'and 'g_s compared to amaranthus and sorghum (Fig.

TABLE 9 : Effect of low light intensity on gas exchange **rates** and associated characteristics in Sunflower

Duration of exposure (min.)	PAR	A	g_s	A/g_s	Ci	A/Ci	E	A/E
After transferring to low light								
0	2000	35.87	0.388	95.55	187	18.62	8.33	4.33
15	100	7.05	0.305	23.30	301	2.32	5.50	1.30
30	100	5.35	0.205	27.35	296	1.79	5.00	1.05
60	100	5.05	0.275	18.37	310	1.63	4.10	1.20
75	100	4.05	0.305	13.37	319	1.26	3.25	1.25
90	100	5.02	0.190	26.00	298	1.67	4.13	1.23
105	100	4.35	0.155	27.47	296	1.48	3.70	1.20
120	100	3.48	0.176	20.02	307	1.13	3.30	1.06
180	100	3.30	0.160	21.13	306	1.07	2.90	1.16
210	100	3.33	0.130	22.72	292	1.14	2.90	1.20
Back to open sun and 10 min later	2000	25.80	0.360	23.26	222	12.04	6.63	3.90
F test	*	*	*	*	*	*	*	*
C D 5%	100	6.32	0.09	12.81	17	7.92	1.33	1.88

TABLE 10 : Effect of low light intensity on gas exchange and associated characteristics in Amaranthus

Duration of exposure (min.)	PAR	A	g_s	A/g_s	Ci	A/Ci	E	A/E
0	2000	35.87	0.310	117.00	154	23.30	6.70	5.30
15	100	7.80	0.120	68.00	231	3.41	2.70	2.90
30	100	2.08	0.110	43.00		1.69	0.73	6.20
60	100	3.76	0.110	32.80	242	1.32	0.83	4.40
90	100	2.01	0.030	61.22	242	0.81	0.90	2.20
120	100	2.06	0.057	42.00	273	0.67	0.70	2.90
180	100	2.27	0.040	52.08	256	0.86	0.70	3.30
210	100	3.03	0.080	38.48	278	1.09	1.03	3.03
Back to open sun and 10 min later	2000	31.25	0.302	106.30	174	17.90	3.10	10.08
F test	*	*	*	*	*	*	*	*
C D 5%	200	12.28	0.18	23	30	16.1	2.28	1.88

TABLE 11 : Effect of low light intensity on gas exchange and associated characteristics in Sorghum (Pot culture)

Duration of exposure (min.)	PAR	A	g_s	A/g_s	Ci	A/Ci	E	A/E
0	2000	34.9	0.270	155.90	98	38.01	5.2	6.60
15	100	2.50	0.046	42.50	230	1.16	0.63	4.13
60	100	1.28	0.020	64.30	237	0.57	0.30	4.36
210	100	1.35	0.020	67.30	232	0.64	0.40	3.03
Back to open sun and 10 min later	2000	27.50	0.240	132.00	129	18.59	3.56	7.68
F test	*	*	*	*	*	*	*	*
C D 5%	300	16.51	0.12	36.18	18.14	28.12	3.51	3.56

Va-d). Moreover, reductions in 'E'(Fig.Va and 'g_s'(Fig.Vc) were at a slower rate in sunflower. For instance the values of 'E' just before shading were 8.33, 6.70 and 5.2 $m.mol.m^{-2}$ s^{-1} in sunflower, amaranthus and sorghum respectively. At the end of 210 min of shading the respective values were 2.90, 1.03 and 0.40 $m.mol.m^{-2}s^{-1}$. But it may be noted that after 15 min of shading itself, the transpiration rates were as low as 0.63 and 2.70 $m.mol.m^{-2}s^{-1}$ in sorghum and amaranthus respectively and was as high as 5.50 m. $mol.m^{-2}s^{-1}$ in sunflower suggesting the slow rate of reduction in transpiration rate in the latter (Table 9-11). The 'g_s' reductions also followed similar patterns in these species (Fig.V c).

Sorghum and amaranthus showed higher values of A/E ratios at high light as well as shade conditions compared to sunflower (Fig.VIa and b) though all the three species showed drastic reductions in this parameter.

After exposing to severe shade for 210 minutes the plants were shifted back to open sunlight. All the three species showed remarkable recovery in 'A' and 'g_s'. Similarly 'E' also showed appreciable recovery particularly in sorghum. There was reduction in Ci when the plants were shifted back to open sunlight, but the level did not reach as that of control light values. A/Ci and A/E values also showed marked improvements. In amaranthus and sorghim A/E ratios were more than that was observed before shading. This was mainly because of the better recovery in 'A' than 'E'.

TABLE 12 : Effect of low light intensity on gas exchange and associated characteristics in Sunflower (Pot culture)

Duration of exposure (min.)	PAR	A	g_s	A/g_s	Ci	A/Ci	E	A/E
On transfer to low light								
0	1800	33.20	0.750	35.00	260	12.40	11.90	2.79
3	200	15.10	0.610	24.75	300	5.27	7.20	2.10
6	200	12.60	0.620	20.32	310	4.10	6.30	2.00
9	200	9.40	0.550	17.09	312	3.01	6.70	1.40
12	200	3.40	0.430	7.91	325	1.04	4.50	0.60
On transfer to high light								
2	1300	27.80	0.430	64.65	235	11.75	8.00	3.50
4	1300	26.40	0.550	48.00	260	10.15	7.90	3.34
6	1300	26.30	0.590	44.58	250	9.79	7.50	3.51
F test	*	*	*	*	*	*	*	*
C D 5%	300	13.32	0.11	18.13	33	3.88	3.12	0.98

TABLE 13 : Effect of low light **intensity** on gas exchan**ge and associated** characteristics in Amaranthus (Pot culture)

Duration of exposure (min.)	PAR	A	g_s	A/g_s	Ci	A/Ci	E	A/E
On transfer **to** low light								
0	1700	28.3	0.25	113.20	150	17.81	7.00	4.10
3	200	7.15	0.10	73.40	220	3.25	2.20	3.22
6	200	1.13	0.07	17.43	315	0.49	0.50	2.13
Back to open sun								
2	1700	24.10	0.16	151.32	117	22.13	3.79	5.36
4	1700	29.50	0.24	122.91	140	20.58	6.85	4.33
F test	*	*	*	*	*	*	*	*
C D 5%	600	5.52	0.11	15.13	19	12.22	2.15	1.38

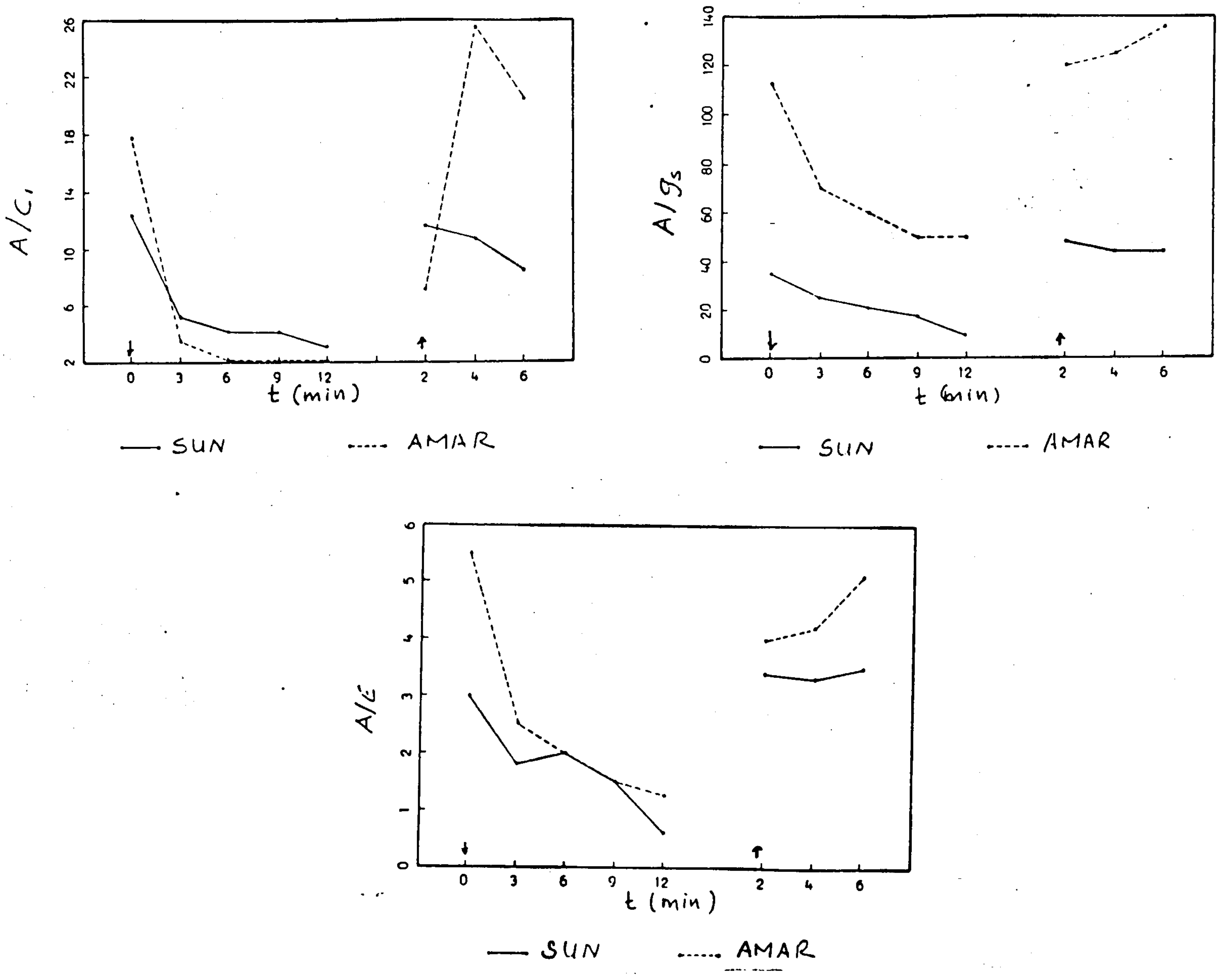


FIG VIII Effect of low light intensity on A/Ci, A/g_s and A/E ratios in sunflower and amaranthus

Continuous exposure to low PAR level for 10 days did not cause any marked changes in the gas exchange characteristics compared to those plants which were exposed to shade for 210 min. However, there was slight improvement in 'g_s' and 'A' in sunflower and sorghum. But these were not significant.

Experiment 2

A second pot culture experiment was also conducted in the similar lines to monitor the time course of changes in gas exchange characteristics of sunflower and amaranthus when these plants were transferred to severe shading (200 $\mu E.m^{-2} s^{-1}$). The data are presented in Tables 12-13. The results are in agreement with those of the previous experiment. A time dependant reduction in the gas exchange rates and associated parameters were observed in both the species. Internal CO_2 level was found to increase. At any given time of shading sunflower maintained higher levels of photosynthetic rate (Fig. VIIa) transpiration rates (Fig. VIIb), stomatal conductance (Fig.VIIc), internal CO_2 level (Fig.VIId), and A/Ci ratio (Fig.VIIIa). However, amaranthus exhibited higher levels of A/g_s ratios (Fig.VIIIb). There were marked reduction in A/E ratios in both the species (Fig.VIIIc).

When the plants were brought back to high light intensity there was an appreciable recovery of gas exchange rate in both the species. The recovery in 'g_s' by two minutes after exposing to high light was very marked in amaranthus.

TABLE 14 : Effect of sequential transfer to moderate and low light intensities on gas exchange and associated characteristics in Sunflower (Pot culture)

Duration of exposure (min.)	PAR	A	g_s	A/g_s	Ci	A/Ci	E	A/E
Top								
0	2000	36.21	0.450	79.95	210	17.06	3.6	8.00
To moderate shade								
15	500	5.83	0.281	20.30	300	1.90	3.4	1.76
To dark shade								
15	100	1.94	0.170	12.00	320	0.61	1.76	1.09
F test	*	*	*	*	*	*	*	*
C D 5%	150	6.82	0.21	19.81	10	8.81	1.53	6.32

TABLE 15: Effect of sequential transfer to moderate and low light intensities on gas exchange and associated characteristics on Amaranthus (Pot culture)

Duration of exposure (min.)	PAR	A	g_s	A/g_s	Ci	A/Ci	E	A/E
Top								
0	1700	36.33	0.280	126.43	138	26.69	3.10	11.71
To moderate light								
15	500	7.98	0.090	82.54	210	3.84	1.53	5.34
To low light								
15	100	1.51	0.030	43.66	270	0.58	0.53	2.91
F test	*	*	*	*	*	*	*	*
C D 5%	200	8.41	0.13	36.8	40	12.12	1.15	3.81

4.2.3. Effect of sequential transfer to moderate and low light intensities

Sunflower and amaranthus plants were grown in pots for 50 days under optimum conditions. Then they were transferred to moderate light intensity (500 $\mu E.m^{-2}s^{-1}$) for 15 minutes and then to low light (100 $\mu E.m^{-2}s^{-1}$) for 15 minutes and gas exchange rates measured. The data are presented in Table 14 and 15. Photosynthetic rates were reduced more or less to similar levels in both the species under both the light levels. Under moderate light, sunflower showed fairly high value of 'g_s' (0.281 $mol.m^{-2}s^{-1}$) compared to that of amaranthus (0.090 $mol.m^{-2}s^{-1}$). Associated parameters like A/g_s, A/Ci, E and A/E also showed a PAR level dependant reduction and Ci showed a PAR dependant increase in both the species. However, under all the PAR levels amaranthus maintained higher A/E, A/g_s and A/Ci ratios and lower 'E' values.

4.2.4 Effect of continuous mutual shading

This experiment was conducted in standing crops of sun flower, amaranthus and sorghum in the fields when they were about 65 days old. The natural canopy of the crop was equally divided into top, middle and bottom levels based on the height of the crops. The main purpose was to see how an exposed leaf behaves vis-a-vis its shaded counterpart of similar age. The data are presented in Tables 16-18.

TABLE 16: Effect of continuous mutual shading or exposure to sun light on gas exchange and associated characteristics in Sunflower

Canopy position	Treatment	PAR	A	g_s	A/g_s	Ci	A/Ci	E	A/E
Top									
	Exposed	2000	38.60	0.400	91.50	182	20.30	9.90	3.94
	Shaded	-	-	-	-	-	-	-	-
Middle									
	Exposed	1800	25.47	0.334	78.80	184	15.70	8.44	3.10
	Shaded	200	5.34	0.226	24.10	301	1.50	4.00	1.20
Bottom									
	Exposed	1500	25.66	0.300	77.00	200	13.52	8.88	3.02
	Shaded	100	5.63	0.127	60.95	242	4.01	2.73	1.62
	F test	*	*	*	*	*	*	*	*
	C D 5%	300	10.28	0.12	37.81	40	7.87	3.81	1.83

TABLE 17: Effect of permanant shading or exposure to sun **light on gas exchange** and associated characteristics in Amaranthus

Canopy position	Treatment	PAR	A	g_s	A/g_s	Ci	A/Ci	E	A/E
Top									
	Exposed	1800	31.24	0.234	135.00	123	33.30	5.40	5.98
	Shaded	-	-	-	-	-	-	-	-
Middle									
	Exposed	1700	39.59	0.250	112.74	120	36.04	5.32	6.45
	Shaded	100	2.79	0.036	107.13	168	1.76	0.74	3.74
Bottom									
	Exposed	1200	17.37	0.170	102.54	164	10.36	3.93	4.41
	Shaded	100	3.79	0.04	94.75	188	2.02	1.70	2.23
	F test	*	*	*	*	*	*	*	*
	C D 5%	300	16.36	0.17	14	22	4.62	4.12	2.63

TABLE 18: Effect of permanant shading **or exposure to sun on gas exchange and** associated characteristics **in Sorghum**

Canopy position	Treatment	PAR	A	g_s	A/g_s	Ci	A/Ci	E	A/E
Top									
	Exposed	1800	29.20	0.250	135.37	158	18.40	6.85	4.80
	Shaded	-	-	-	-	-	-	-	-
Middle									
	Exposed	1800	27.90	0.340	114.89	156	18.00	8.64	3.85
	Shaded	100	1.66	0.070	23.61	302	0.57	2.01	0.83
	F test	*	*	*	*	*	*	*	*
	C D 5%	300	21.81	0.12	17.8	21	4.51	3.61	1.52

Under field conditions the upper young leaves are generally exposed to natural high light intensities since there is not any mutual shading. In the middle canopy as well as in the bottom canopy continuous natural shading caused very marked reductions in the gas exchange rates and related characteristics of the leaves in all the three species. The reductions were more apparant in amaranthus and sorghum.

The permanent shade induced reductions in A (Fig.IXa-c) 'g_s' (Fig.Xa-c), 'E' (Fig.XIa-c) and A/Ci (Fig.XIIa-c) were more drastic in amaranthus and sorghum compared to sunflower. Reduction in A/g_s ratios was more pronounced in sorghum (Fig.XIIIa) compared to sunflower (Fig.XIIIb) and amaranthus (Fig.XIIIc). Hence, increase in Ci values was also more apparant in Sorghum (Fig.XIVa) compared to sunflower (Fig.XIVb) and amaranthus (Fig.XIVc). There were quite appreciable reduct ions in A/E ratios in all the three species studied (Fig.XVa-c) In general, amaranthus and sorghum showed higher values of A/E ratios compared to sunflower in both shaded and exposed leaves at every level.

From the various experiments conducted with low light intensities the following results emerge. Transferring the plants from high light to low light intensities resulted in very remarkable reductions in 'A', 'g_s', A/g_s, A/Ci, 'E' and A/E. The reduction in 'A' were at a faster rate than the reductions in 'g_s' resulting in reduced A/g_s ratios and

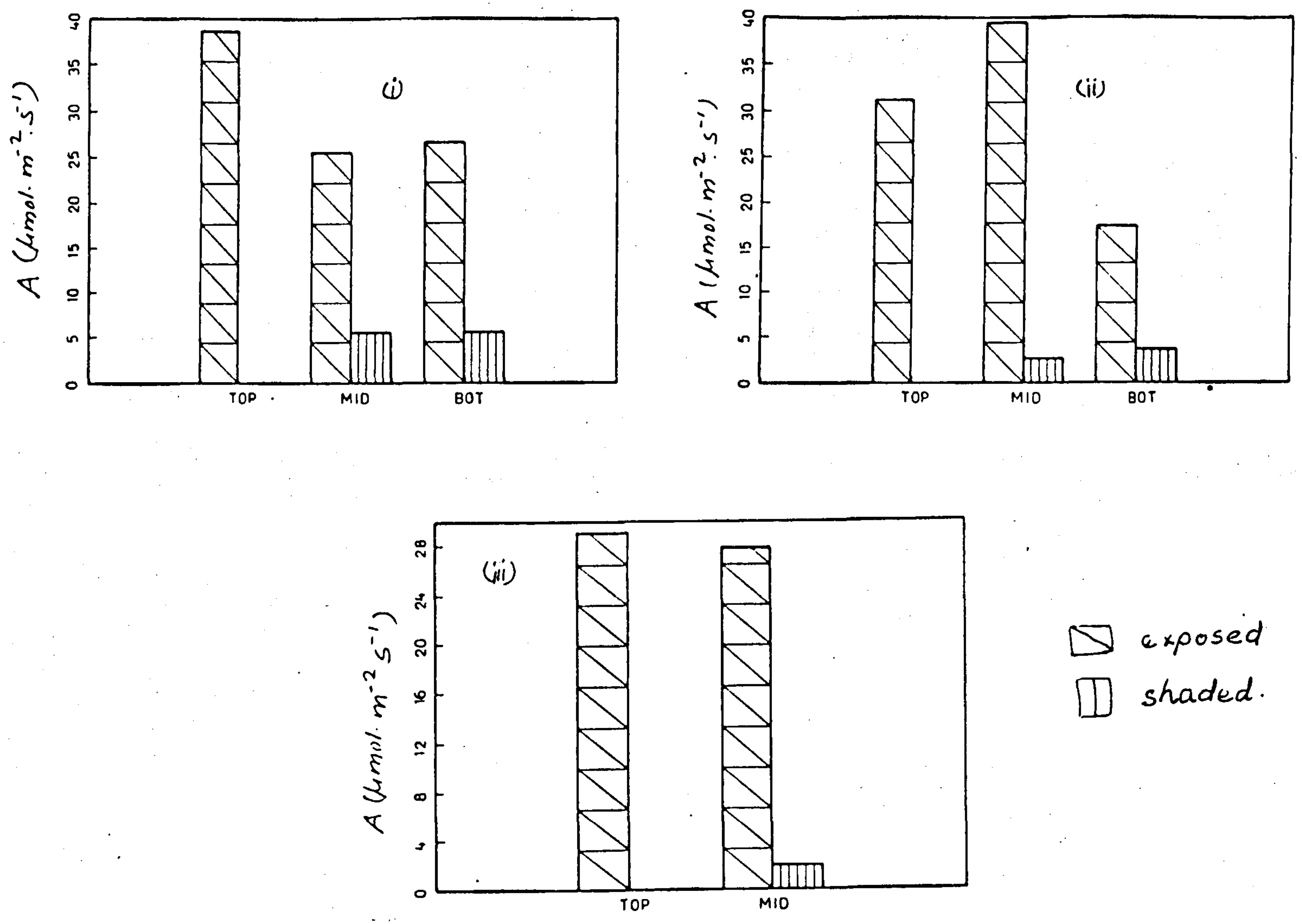


FIG IX Effect of continuous mutual shading on A in sunflower (i) amaranthus (ii) and sorghum (iii)

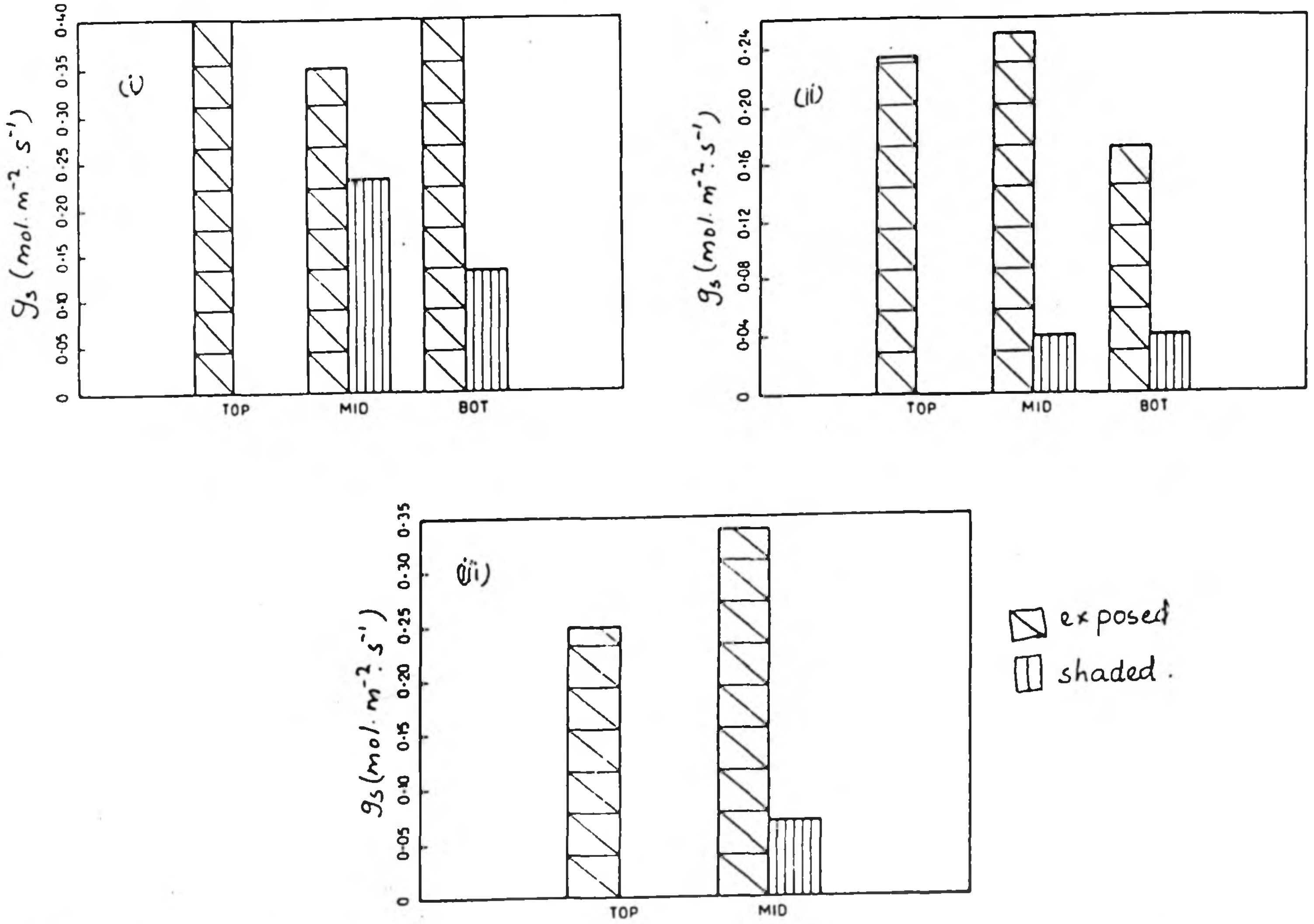
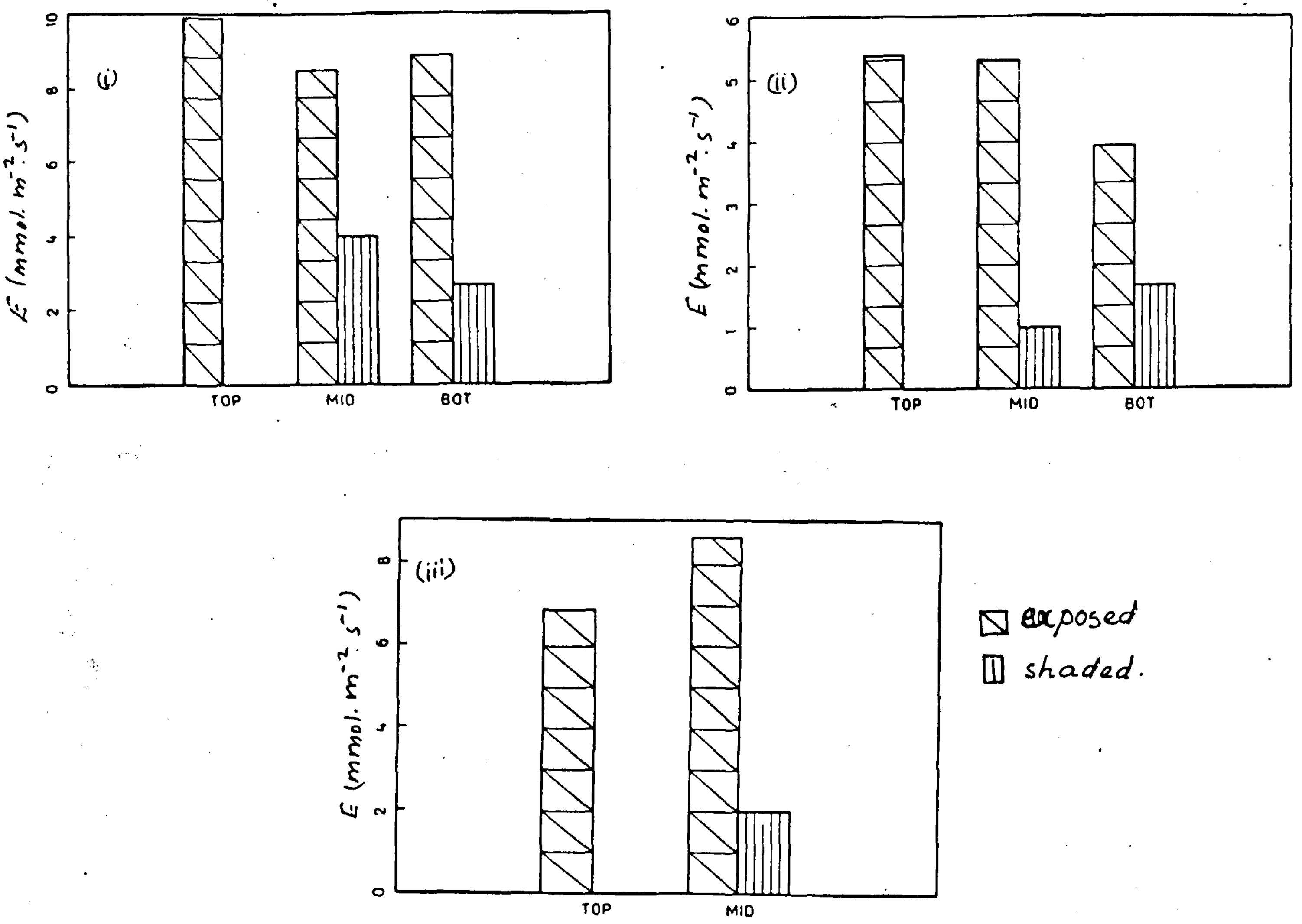


FIG X Effect of continuous mutual shading on g_s in sunflower (i), amaranthus (ii) and sorghum (iii)


(i)
E (mmol. m⁻²·s⁻¹)
0
2
4
6
8
10
TOP
MID
BOT
(ii)
E (mmol. m⁻²·s⁻¹)
0
1
2
3
4
5
6
TOP
MID
BOT
(iii)
E (mmol. m⁻²·s⁻¹)
0
2
4
6
8
TOP
MID
▧ exposed
▥ shaded.

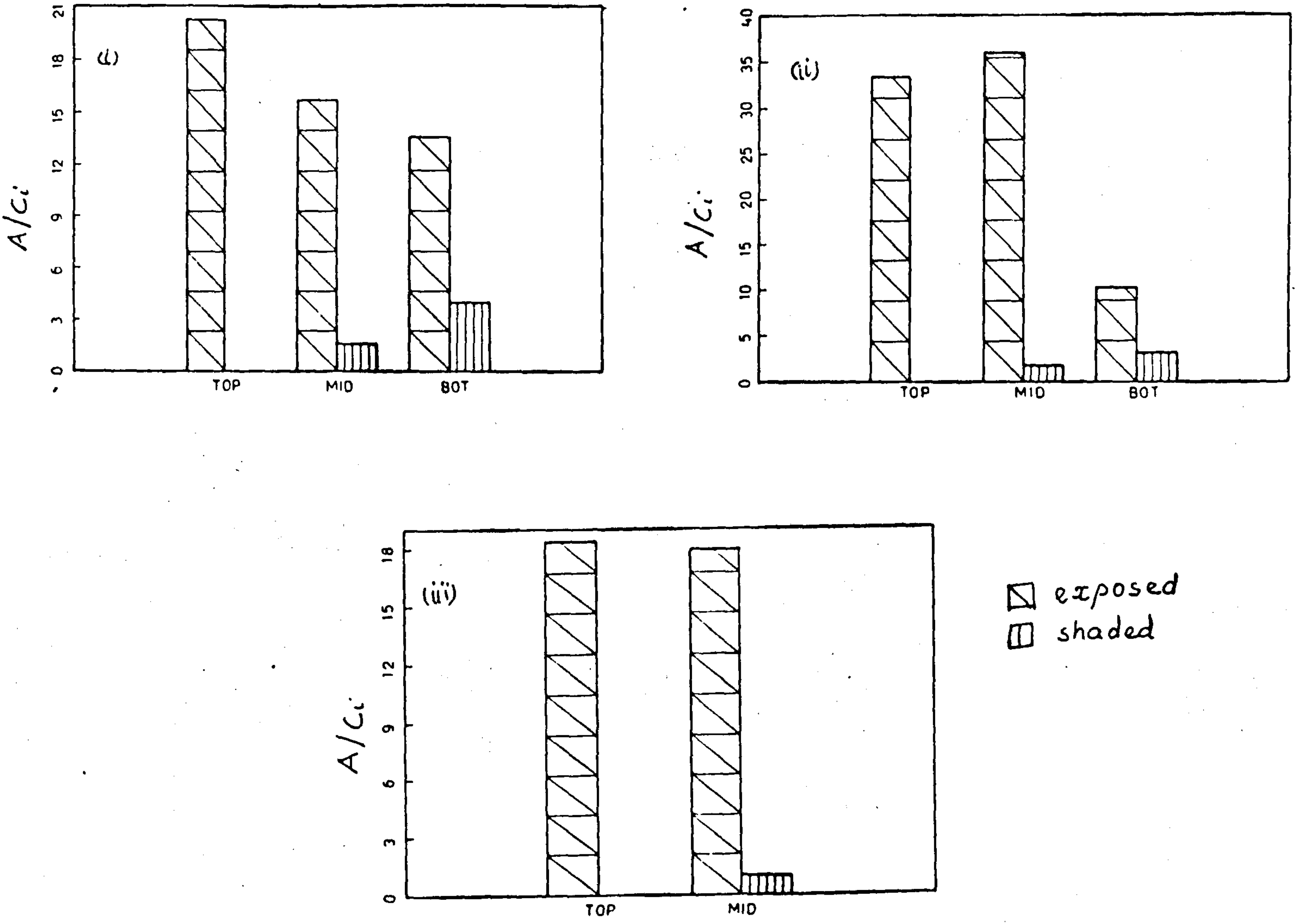


FIG XII Effect of continuous mutual shading on A/Ci ratio in sunflower (i), amaranthus (ii) and sorghum (iii)

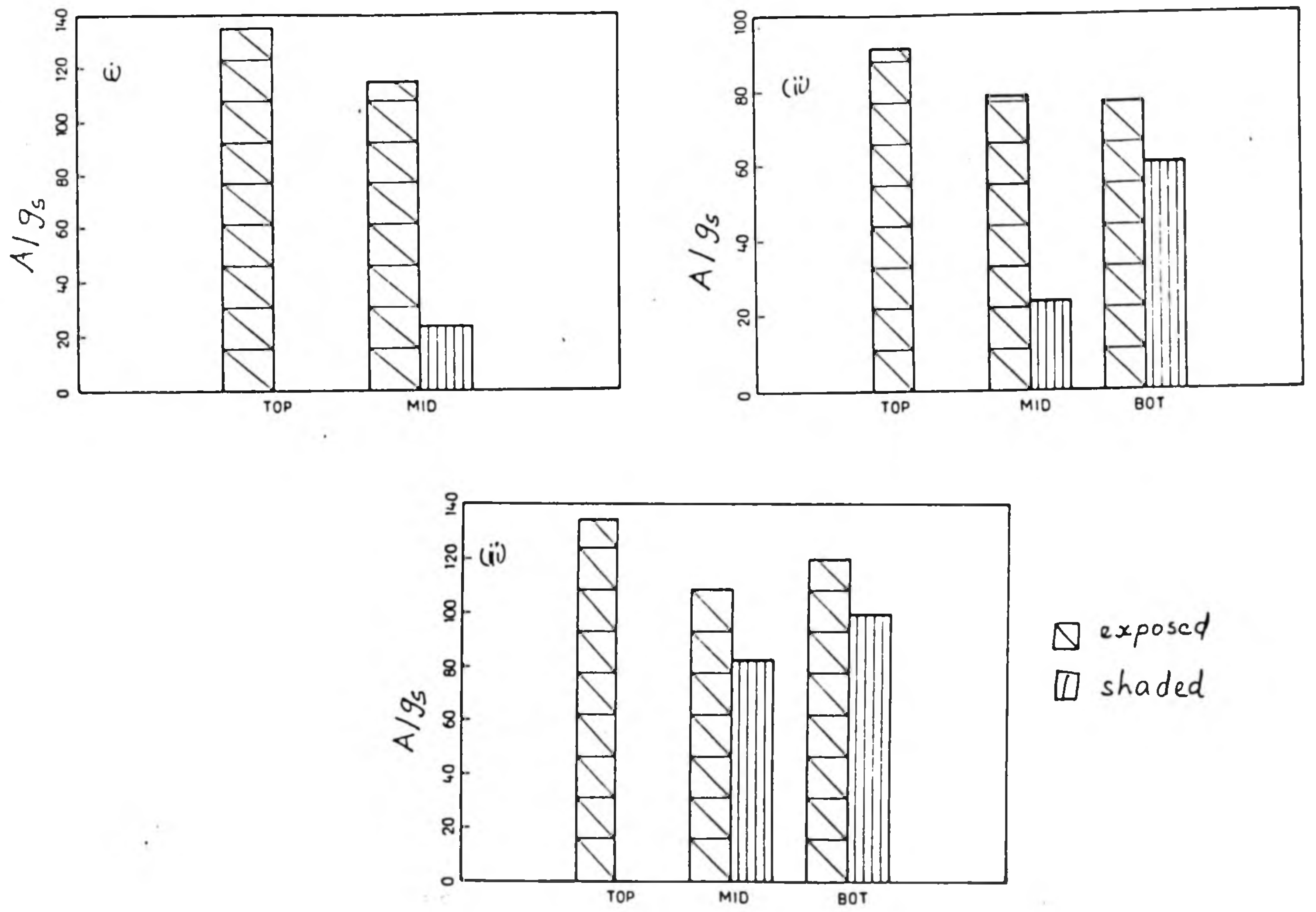


FIG XIII Effect of continuous mutual shading on A/g_s ratio in sorghum (i), sunflower (ii) and amaranthus (iii)

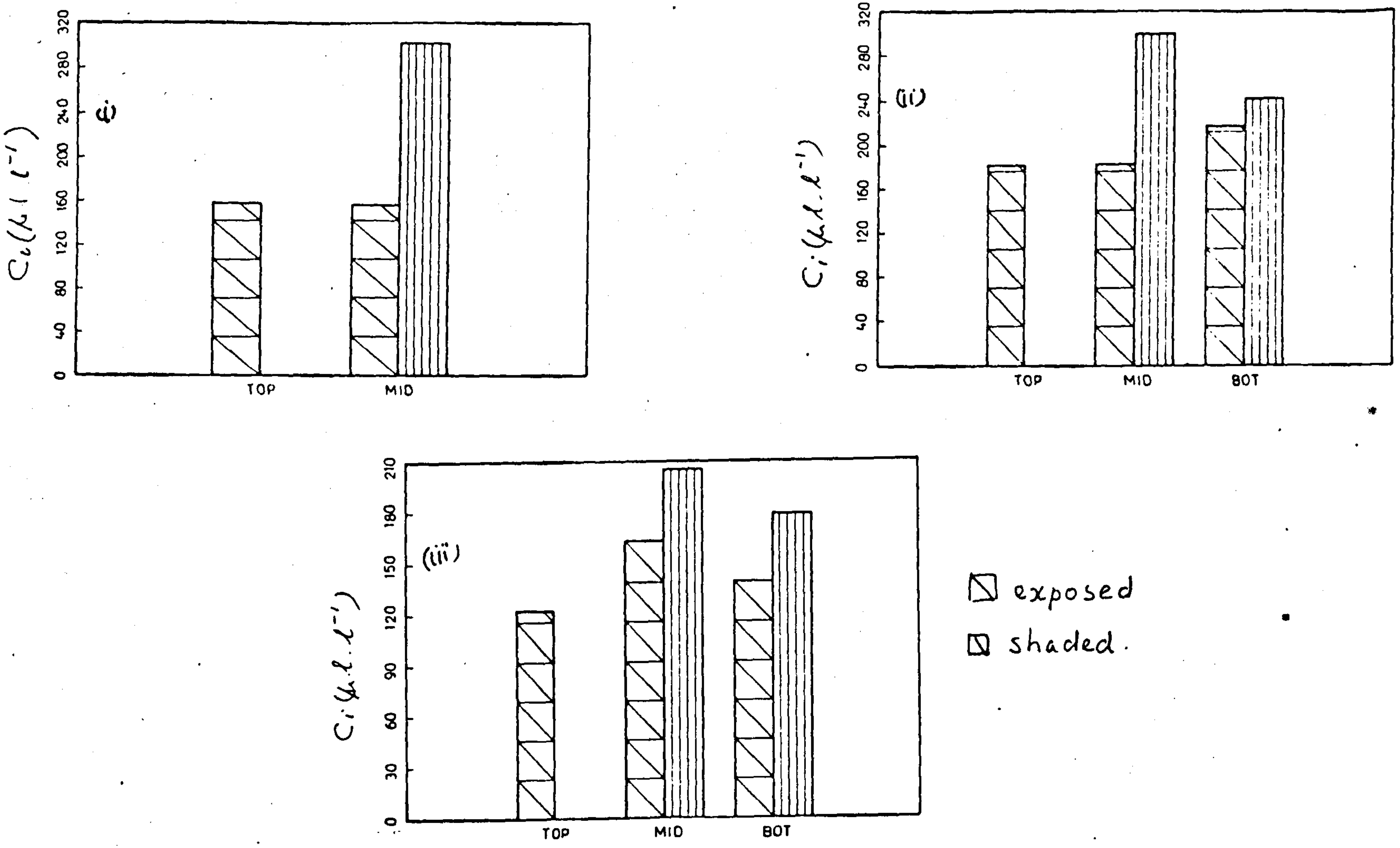


FIG XIV Effect of continuous mutual shading on Ci in sorghum (i), sunflower (ii) and amaranthus (iii)

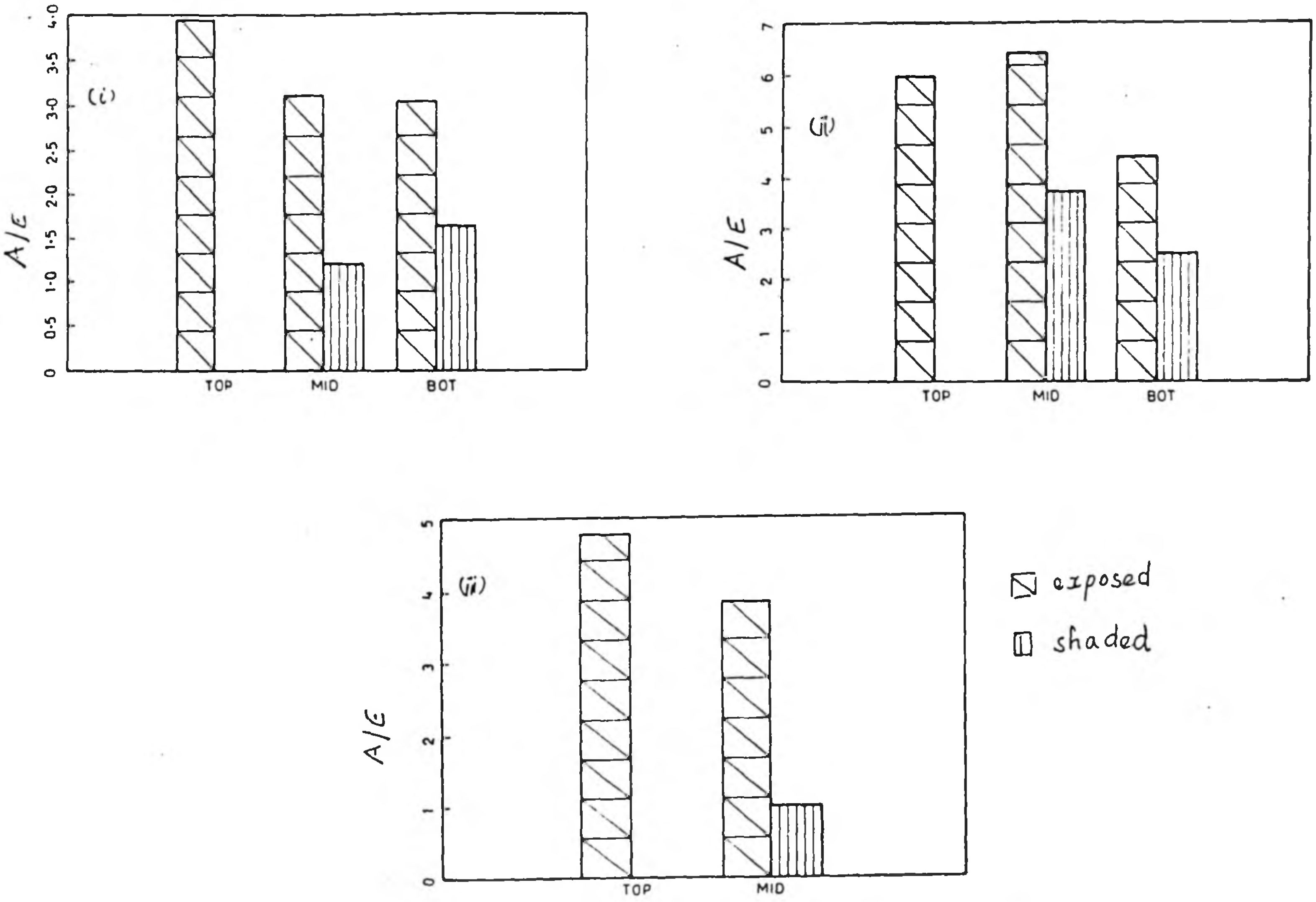


FIG XV Effect of continuous mutual shading on A/E ratio in sunflower (i), amaranthus (ii) and sorghum (iii)

TABLE 19: Regression models for A, E, g_s, and Ci in plants exposed for different periods to low light intensity

SPECIES	Y	X	Y = f (x)	dY/dx
SUNFLOWER	A	g_s	EXP (0.9197 + 4.563 g_s)	EXP (0.9197 + 4.563 g_s) (4.563)
	A	Ci	620.7 - 107.7 ln Ci	- 107.7/Ci
	A	E	-51.2083 + 21.6462 E	21.6462
	g_s	Ci	1.3518 - 0.0035 Ci	-0.0035
AMARANTHUS	A	g_s	1.9207 + 147.898 g_s	147.898
	A	Ci	228.855 - 42.581 ln Ci	-42.581/Ci
	A	E	-25.4308 + 19.2372 E	19.2372
	g_s	Ci	0.3896 - 0.0017 Ci	-0.0017
SORGHUM	A	g_s	EXP (4.965 + 1.1023 ln g_s)	EXP (4.965 + 1.1023 ln g_s) (1.1023/g_s)
	A	Ci	EXP (3.9126 - 0.0067 Ci)	EXP (3.9126 - 0.0067 CI) (-0.0067)
	A	E	-1.7148 + 11.3892 E	11.3892
	g_s	Ci	EXP (-1.4613 - 0.0027 Ci)	EXP (-1.4613 - 0.0027 Ci) (-0.0027)

increased Ci levels under shade conditions. At any given light level sunflower exhibited higher values of 'g_s' 'E' and Ci. Transferring the plants from shade back to high light intensity resulted in the recovery of all the gas exchange characteristics. Continued shading for 10 days resulted in a slight increase in 'g_s' in sunflower. All other characters remained unaltered during prolonged shading.

4.2.5 Regression models of gas exchange parameters as observed under low light conditions in sunflower, amaranthus and sorghum

The regression models fitted among 'A', 'E', 'g_s, and Ci taking any two variables at a time are presented in table 19. There was a positive relationship between 'A' and 'g_s' in these species kept under shade for different intervals. The relationship was simple and linear in Amaranthus, but was exponential in sunflower and sorghum. The relationship between 'A' and Ci was a negative one in this case since increase in Ci during shading was a result of reduced 'A'. The relationship between 'A' and Ci was a semi-logarithmic one in sunflower and Amaranthus and an exponential one in sorghum. There existed a steady and constant linear relation ship between 'A' and 'E' in all the three species studied. It may be noted that the slope of the linear curve between 'A' and 'E' was the lowest in sorghum followed by Amaranthus and sunflower suggesting that per every unit reduction in 'E' (during the period of shading) the reduction in 'A' was least in sorghum followed by Amaranthus. There was a negative relationship between 'g_s and Ci which was a simple linear one in sunflower and Amaranthus and an exponential one in sorghum.

Regression models of 'A', 'g_s', Ci, 'E' and A/E with the duration (t minutes) of shading (to a maximum of 21 min) are presented in table 20.

'A' and 'g_s showed a negative and logarithmic decrease as time of shading advanced. In the three species, Ci exhibited an exponential increase with time. The relationship between 'E' and t was negative, but simple linear in sunflower semi-logarithmic in sorghum and exponential in Amaranthus. A/E ratio showed a semi-logarithmic decrease with t in sunflower and Amaranthus and an exponential decrease with t in sorghum.

The results on gas exchange characteristics under different shade treatments reveal a time (of shading) and intensity (of shading) dependant reduction in gas exchange rates and associated parameters like A/g_s, A/Ci and A/E. An increase in Ci was also evident. Stomatal conductance and 'E' were more "resistant" to decrease in sunflower than in Amaranthus and sorghum. In the latter two species a sharp and fast reduction in 'E' and 'g_s' was very evident even under moderate shading. The results also prove the positive relationship between 'A' and 'g_s' and the negative relationship between 'g_s' and Ci. The latter need not be exclusively due to the feed back control of guard cell movements by Ci

TABLE 20: Regression models for A, g_s, Ci, and A/E with time (min.) of exposure to low light intensity

SPECIES	Y	X	Y = f (x)	dY/dx
SUNFLOWER	A	t	17.124 - 2.373 ln t	-2.373/t
	g_s	t	0.4434 - 0.0178 ln t	-0.0178/t
	Ci	t	EXP (5.6349 + 0.019 ln t)	EXP (5.6349 ln t) (0.019/t)
	E	t	3.2495 - 0.0403 t	-0.0403
	A/E	t	5.4765 - 0.6717 ln t	0.6717/t
AMARANTHUS	A	t	12.619 - 3.6607 ln t	-3.6607/t
	g_s	t	0.0961 - 0.0236 ln t	-0.0236/t
	Ci	t	EXP (4.6706 + 0.0579 t)	EXP (4.6706 + 0.0579 t) (0.0579)
	E	t	EXP (0.5995 - 0.797 ln t	EXP (0.5995 - 0.0797 in t) (-0.0797/t)
	A/E	t	5.0563 - 0.8873 ln t	-0.8873/t
SORGHUM	A	t	16.934 - 2.734 ln t	-2.734/t
	g_s	t	0.1305 - 0.0267 ln t	-0.0267/t
	Ci	t	EXP (5.1274 + 0.0717 ln t)	EXP (5.1274 + 0.0717 ln t) (0.0717/t)
	E	t	1.5674 - 0.231 ln t	-0.231/t
	A/E	t	EXP (2.4473 - 0.0457 ln t)	EXP (2.4473 - 0.0457 ln t) (-0.0457/t)

since the direct role of light in stomatal mechanism cannot be ruled out. The constant linear relationship between 'A' and 'E' agrees with the concept of optimal stomatal functioning which postulates that under conditions of an abiotic stress stomatal aperture size will be adjusted in such a way that the marginal gain of CO_2 per marginal loss of water will be held constant.

4.3 Effect of moisture stress on gas exchange rates and associated characters in sunflower, amaranthus and sorghum

Different experiments were carried out to study the effect of leaf water potential on the gas exchange rates and associated characteristics in sunflower, amaranthus and sorghum, Stressing the plants by stopping irrigation in pot culture and field culture, livewilting technique and ABA were used to alter the leaf ψ levels. The various experiments conducted in series are mentioned below.

I. Pot culture experiment

One experiment was taken up in summer 1987 to study the effect of fast wilting on gas exchange rates and associated characteristics in sunflower, amaranthus and sorghum.

II. Field experiment

Two field experiments, one in summer 1986 with sunflower and amaranthus and another in summer 1987 with sunflower, amaranthus and sorghum plants were conducted to study the effect slowly developed moisture stress on gas exchange rates and associated characteristics.

III. Cut leaf experiment

Fully expanded mature leaves were excised from field grown sunflower and amaranthus plants (summer, 1988) and allowed to live wilt. During the course of wilting gas exchange rates were measured to study how rapid depletions in leaf ψ will alter the gas exchange characteristics.

IV. Experiment with ABA

Three experiments were conducted using ABA and thus altering the gas exchange characteristics. In the first experiment, different concentrations of ABA were fed to excised leaves of sunflower and amaranthus plants through the transpiration stream to study the effects of ABA on stomatal conductance, gas exchange rates and associated characteristics with the assumption that effect of ABA would be predominantly on 'g_s and not on 'g_m'. In the second experiment EGTA and ruthenium red (RR) were used along with ABA to study whether the ABA induced reductions in 'g_s' and 'A' could be recovered by these compounds. ABA is known to affect guard cell movements through Ca^{2+} ions and these compounds are known to minimise the ABA induced stomatal closure through calmodulins. With a similar objective a third experiment was conducted in the same way in summer 1988.

4.3.1 Pot culture experiment (Summer 1987)

This experiment was conducted with an objective to study the alterations in gas exchange rates and related characteristics when plants were subjected to a fast wilting and thus lowering the leaf ψ levels faster.

Sunflower, amaranthus and sorghim plants were raised in pots under optimum conditions. When the plants were about 45 days old irrigation was withheld to stress the plants. Gas exchange characters of the leaves were monitored for 2 days in these stressed plants. Then the plants were watered and gas exchange characters measured 24 hours after stress alleviation. The results are given in Table 21. All the 3 species showed a significant reduction ψ. 'A', 'g_s' and A/Ci as the stress progressed. The reductions in 'A' were comparable in the three species. However, amaranthus and sorghum showed very drastic reduction 'g_s compared to sunflower. For instance one day after withholding water, the 'g_s' values were 0.267, 0.127 and 0.147 $mol.m^{-2}s^{-1}$ in sunflower, amaranthus and sorghum respectively. Stomatal conductance and 'E' values were always higher in sunflower than in the other two species. In all the species A/g_s increased and Ci decreased. The ratio A/Ci showed a steady decrease in sunflower. In amaranthus and sorghim it first increased on 1st day of stress and then decreased on the second day of the stress. Changes in A/E ratios were not very appreciable.

TABLE 21: Effect of soil moisture stress on gas exchange rates and associated characteristics in Sunflower, Amaranthus and Sorghum (Pot Culture Expt.1987)

Species	Days of stress	PAR	g_s	A	A/g_s	Ci	A/Ci	E	A/E	ψ
SUNFLOWER										
	0	2000	0.330	30.00	90.91	195	15.3	10.03	3.01	- 5
	1	2000	0.267	18.28	68.33	231	0.080	6.80	2.70	- 8
	2	2000	0.073	8.43	113.00	183	0.043	2.40	3.64	- 22
One day after recovery		2000	0.300	28.02	93.33	190	0.147	10.70	2.60	- 6
	F test	NS	*	*	*	*	*	*	*	*
	C D 5%		0.11	6.3	12.5	28	0.18	4.1	1.3	3
AMARANTHUS										
	0	2000	0.293	32.86	112.33	160	0.205	5.97	5.50	- 6
	1	2000	0.127	20.23	160.00	84	0.250	3.27	6.17	- 10
	2	1900	0.037	5.67	151.67	97	0.073	2.10	3.19	- 21
One day after recovery		2000	0.147	24.78	171.00	66	0.633	6.37	4.23	- 5
	F test	NS	*	*	*	*	*	*	*	*
	C D 5%		0.13	4.8	17.3	16	0.13	1.2	1.8	- 4
SORGHUM										
	0	2000	0.257	31.00	122.33	144	0.214	5.50	5.65	- 6
	1	2000	0.147	22.05	156.33	90	0.390	3.98	5.78	- 9
	2	2000	0.063	8.89	142.00	113	0.077	1.57	5.73	- 18
One day after recovery		2000	0.200	26.07	130.00	132	0.197	5.13	5.09	- 7
	F test	NS	*	*	*	*	*	*	*	
	C D 5%		0.11	5.6	18.4	30	0.18	2.2	0.9	3

One day after the stress plants were rewatered all the three species showed appreciable recovery in 'A' 'g_s' 'E' and A/Ci. The recovery rate of 'g_s' in the case of sunflower was very significant from 0.073 mols.$m^{-2}s^{-1}$ in the 2nd day of stress to 0.300 mol.$m^{-2}s^{-1}$ after recovery. The corresponding values were 0.037 and 0.147 mols.$m^{-2}s^{-1}$ in the case of amaranthus and 0.063 and 0.200 mols. $m^{-2}s^{-1}$ in case of sorghum.

4.3.1.1 Relationship between gas exchange parameters

Relationship between gas exchange parameters and the multiple linear regression models for 'A' and A/E obtained from the above experiment for sunflower and amaranthus are given in Table 22 and 23 respectively. Photosynthetic rate showed a very strong positive correlation with 'g_s' ψ and 'E' in both the species. Leaf water potential had a very strong and positive relationship with 'E' 'g_s' and A/Ci in sunflower A/E was negatively related to 'g_s' and positively to A/g_s. Similarly A/Ci was also positively related to 'E' and 'g_s'. 'E' and 'g_s were strongly and positively correlated.

In amaranthus also a strong positive correlation excised among ψ, 'E' and 'g_s'.

The multiple linear regression (MLR) model gives the contribution of the independent variables towards the observed changes in the dependent variable. The co-efficient associated with each independent variable can be considered

TABLE 22: Inter Correlation Matrix and Multiple Linear Regression Analysis of gas exchange Parameters of plants subjected to moisture stress (Sunflower Pot Culture 1987)

	E	g_s	A/g_s	Ci	A/Ci	A/E	ψ	A
E	1							
g_s	0.871	1						
A/g_s	-0.286	-0.581	1					
Ci	0.067	0.367	-0.952	1				
A/Ci	0.900	0.758	0.043	- 0.241	1			
A/E	0.750	0.601	0.513	- 0.318	- 0.473	1		
ψ	0.970	0.724	-0.447	0.161	0.844	- 0.729	1	
A	0.930	0.893	-0.161	0.008	0.960	- 0.520	0.898	1

$$A = -20,52 - 0.2184E + 1.041g_s + 0.3841\ A/g_s + 0.03493Ci + 0.2526A/Ci - 0.08876A/E + 0.0280\ \psi\ (R^2 = 88\%)$$

$$A/E = 4.49 - 2.309\ E + 0.7757\ A + 0.6024\ A/Ci + 0.3046\ \psi\ (R^2 = 85\%)$$

TABLE 23: Inter Correlation Matrix and Multiple Linear Regression Analysis of gas exchange Parameters of plants subjected to moisture stress (Amaranthus Pot Culture 1987)

	E	g_s	A/g_s	Ci	A/Ci	A/E	ψ	A
E	1							
g_s	0.708	1						
A/g_s	-0.367	-0.595	1					
Ci	0.371	0.594	-0.999	1				
A/Ci	0.101	0.052	0.589	- 0.591	1			
A/E	0.082	0.437	0.191	0.195	0.401	1		
ψ	0.790	0.762	-0.153	0.155	0.387	0.434	1	
A	0.758	0.940	-0.320	0.320	0.316	0.548	0.904	1

$$A = 6.2 + 0.3119E + 0.6706g_s - 0.00296\, A/g_s - 0.0857Ci + 0.1355A/Ci + 0.1564A/E + 0.06709\, \psi \quad (R^2 = 86\%)$$

$$A/E = 7.06 - 1{,}259\, E + 1.159\, A + 0.01645\, A/Ci + 0.375\, \psi \quad (R^2 = 88\%)$$

as the partial differential co-efficient. Among the eight variables used in the MLR, model for 'A', the most important one which could explain most of the observed variations in 'A' were 'g_s' 'E', A/E, and A/Ci in amaranthus and 'g_s', A/g_s, 'E' and A/Ci in sunflower (in the decreasing order of their contributions). Thus, in both the species, out of the variables studied, 'g_s' contribution to 'A' was the greatest with a partial derivative (dA/dg_s) of 0.6706 in the case of amaranthus and 1.041 in the case of sunflower.

In the MLR model for A/E, the important variables were 'E' followed by 'A' and ψ in amaranthus and 'E', 'A', A/Ci, and ψ in sunflower. It is also clear that, in both the species, it was 'E' and not 'A' which was more important in deciding A/E under the conditions of this study. A unit reduction in 'E' caused A/E to increase by 2.309 units where as a unit reduction in 'A' could cause only 0.7757 units reduction in A/E in sunflower similarly in amaranthus also 'E' was slightly more strong than 'A' in terms of its contribution towards A/E.

The above results suggests that when subjected to a quick water stress. 'A', 'g_s' and 'E' were reduced in sun flower amaranthus and sorghum. Reductions in 'A' were almost comparable in the three species. But sunflower maintained higher values of 'g_s' and 'E' compared to the other two species. In all the three species there was an increase in A/g_s and concommitant reduction in Ci. An increase in A/g_s ratio suggests that 'g_s' was more affected than 'g_m' under moisture stress conditions. The extent of increase in A/g_s was more in sunflower.

A very strong positive correlations existed between 'A' and other variables like 'g_s', ψ and 'E' in both sunflower and amaranthus. The individual contribution of 'g_s towards 'A' was higher than by any other single variable. This does not necessarily mean that when 'g_s was reduced under stress it imparted more limitations on 'A'. Because depending upon the total variations in 'g_s under stress its overall contribution to 'A' will be different from that of 'g_m'. Since, we do not know neither the co-efficient associated with 'g_m nor the absolute value of 'g_m', it is not possible from MLR models to say which factor was limiting 'A' to a greater extent.

4.3.2 Field experiments

A set of field experiments were carried out using sunflower, amaranthus and sorghum to study the effect of moisture stress on their gas exchange characteristics unlike, very fast, in field experiments moisture stress was developed very slowly allowing the plants to adopt to the changes in leaf water potential. This would enable the plants to acclimatise to the changing environment. The experiments were carried out during the summer seasons of 1986 and 1987.

4.3.2.1 Experiment -I (Field experiment Summer, 1986)

Sunflower and amaranthus crops were raised in the field following the standard recommendations when the plants were

Table 24 : Effect of soil moisture stress on gas exchange rates and associated characteristics in field grown Sunflower plants (Field Expt. 1986)

Days after with holding water	PAR	A	g_s	A/g_s	Ci	A/Ci	E	A/E
1	1850	45.98	1.482	31.03	290	15.78	17.03	2.59
3	1900	36.90	1.150	34.43	285	13.05	11.43	3.33
4	1800	36.00	1.482	23.73	302	11.95	14.78	2.31
5	2000	38.60	1.230	31.40	289	14.75	10.75	3.68
6	2100	37.73	1.215	30.83	290	13.10	11.00	3.48
7	1950	36.25	1.135	30.75	284	12.58	10.75	3.20
9	2000	33.63	0.715	50.53	259	13.05	11.50	2.79
12	2000	34.65	0.672	62.50	240	15.75	11.60	3.35
13	2000	26.10	0.518	52.50	256	16.18	11.25	2.21
15	1950	15.63	0.375	73.50	222	7.93	9.00	4.11
17	1950	22.55	0.360	65.25	235	9.80	8.25	2.56
18	2000	18.45	0.285	64.73	233	7.92	8.18	2.50
20	2000	11.58	0.165	111.75	114	9.55	4.00	3.61
F test	*	*	*	*	*	*	*	*
C D 5%		12.6	0.35	8.8	17	3.4	3.3	1.28

Table 25 : Effect of soil moisture stress on gas exchange rates and associated characteristics in field grown Amaranthus (Field Expt. 1986)

Days after with holding water	PAR	A	g_s	A/g_s	Ci	A/Ci	E	A/E
1	1800	41.80	0.390	106.25	169	25.25	12.25	3.38
3	2000	42.25	0.392	118.50	149	32.00	10.25	4.65
4	2000	46.00	0.400	127.00	137	34.75	10.25	4.34
5	1900	39,25	0.350	120.75	146	34.25	8.40	4.70
6	2100	35.00	0.392	95.25	187	19.25	9.40	3.92
7	2000	34.72	0.265	131.00	130	26.70	7.63	4.15
9	2000	27.16	0.220	123.25	142	20.52	6.40	4.21
12	1950	21.21	0.300	105.25	172	14.50	6.56	3.23
13	1750	29.13	0.197	150.00	100	25.75	8.50	3.38
15	1800	21.25	0.130	164.50	77	28.50	4.75	7.40
17	2000	8.60	0.168	107.75	168	12.25	4.00	5.25
18	2000	13.30	0.125	107.00	169	8.03	5.00	2.70
20	2000	10.03	0.088	114.50	157	6.53	5.25	2.10
F test	*	*	*	*	*	*	*	*
C D 5%		5.36	0.17	17.3	15	6.82	4.62	1.56

about 45 days old irrigation was stopped and gas exchange characteristic were monitored for 20 days. The data are presented in Tables 24 and 25.

There was a gradual but progressive reduction in 'A' as the stress progressed in both the species. During the early days of stress amaranthus exhibited higher values of 'A' compared to sunflower. However, in the last three observations 'A' values were slightly higher in sunflower (Tables 24 and 25) stomatal conductance showed a decline as stress progressed. Initially the reductions were less apparent, but later the reductions were more which can be considered as control - the 'g_s values were 1.482 and 0.390 mol.m^{-2}.s^{-1} in sunflower and amaranthus respectively. The corresponding values of 'g_s on the 7th day of stress were 1.135 and 0.392 mol.m^{-2}.s^{-1} and on the 20th day were 0.165 and 0.088 mol.m^{-2}.s^{-1} in sunflower and amaranthus, respectively. Reductions in 'E' were however more steady with initial marked reductions in both the species.

In general, A/g_s ratios increased as stress progressed in both the cases and the extent of increase was more in sunflower. However, in amaranthus, the values started to decline from the 17th day of stress onwards. Variations in Ci were also observed accordingly; a constant reduction in sunflower and a more or less gradual reduction upto 15 days of stress and then a gradual increase in amaranthus.

TABLE 26: Inter Correlation Matrix and Multiple Linear Regression Analysis of Gas Exchange Parameters of plants subjected to moisture stress (Sunflower Field Expt. 1987)

Inter Correlation Matrix

	E	g_s	Ci	A/Ci	A
E	1				
g_s	0.781	1			
Ci	0.743	0.815	1		
A/Ci	0.148	0.239	- 0.117	1	
A	0.652	0.756	- 0.573	0.690	1

Relationship between A/E and other parameters

	E	A	A/Ci
A/E	- 0.515	0.133	0.563 - 0.186

$A = -36.95 + 0.02236\ E + 0.08381\ g_s + 0.5746\ Ci + 0.6739\ A/Ci\ (R^2 = 93\%)$

$A/E = 3.18 - 0.8849\ E + 0.4390\ A + 0.4153\ A/Ci\ (R^2 = 75\%)$

TABLE 27: Inter Correlation Matrix and Multiple Linear Regression Analysis of gas exchange Parameters of plants subjected to (Amaranthus Field Expt. 1987)

<u>Inter Correlation Matrix</u>

	E	g_s	Ci	A/Ci	A
E	1				
g_s	0.769	1			
Ci	0.152	0.284	1		
A/Ci	0.410	0.573	- 0.587	1	
A	0.704	0.898	- 0.110	0.805	1

<u>Relationship between A/E and other parameters</u>

	E	A	A/Ci	
A/E	- 0.268	0.233	0.495	- 0.186

$$A = 0.923 - 0.01424\ E + 0.7866\ g_s - 0.2070\ Ci + 0.2167\ A/Ci\ (R^2 = 96\%)$$

$$A/E = 4.76 - 0.6811\ E + 0.2537\ A + 0.5704\ A/Ci\ (R^2 = 65\%)$$

By and large A/Ci ratios were found to decrease as stress progressed in both the species. A/E ratios showed a slow and steady increase in sunflower. However, in amaranthus, A/E ratios initially increase and during the later periods of stress showed a gradual reduction.

4.3.2.1.1 Relationship among gas exchange parameters

Inter-correlation matrix and MLR models for 'A' and A/E were developed for sunflower and amaranthus and the results are given in Table 26 and 27.

In sunflower, 'A' showed a strong positive correlation with 'g_s', 'E', Ci, and A/Ci (Table 23) strong positive correlations existed among 'E', 'g_s' and Ci. There existed a positive correlation between Ci and 'g_s' since both the parameters showed a concommitant reduction as stress progressed.

In amaranthus strong positive correlations existed among 'A', 'g_s', A/Ci, and 'E'. The MLR model fitted for 'A' by considering the variables 'E' 'g_s' Ci and A/Ci for sunflower is given in Table 26 and for amaranthus Table 27. In the case at sunflower A/Ci followed by Ci contributed to most of the variations in 'A'. In sunflower the major contributions for 'A' were from 'g_s' A/Ci and Ci. Comparing the contributions of 'E' and 'A' towards A/E was found to be more important than 'A' in both the species.

TABLE 28: Regression models involving gas exchange parameters of plant subjected to moisture stress (Sunflower Field Expt.1986)

Y	X	Y = f (x)	dy/dx
A	Time	47.2334 - 8.3862 log x	- 8.3862/x
g_s	Time	EXP (0.7188 - 0.1103 x)	EXP (0.7188 - 0.1103 x) x (-0.1103)
E	Time	15.0632 - 0.4556 x	- 0.4556
Ci	Time	317.5684 - 6.703 x	- 6.703
A/E	Time	2.8136 + 0.1125 log x	+ 0.1125/x
A/g_s	Time	EXP (3.1581 + 0.0727 x)	EXP (3.1581 + 0.0727 x) x (0.0727)
A/Ci	Time	15.0079 - 0.3205 x	- 0.3205
A	g_s	36.8769 + 13.1221 log x)	+13.1221/x
A	Ci	EXP (-4.9058 + 1.5039 log x)	EXP (-4.9058 + 1.5039 log x) x (1.5039/x)
g_s	Ci	-1.4339 + 0.0089 x	+ 0.0089
A	E	EXP (1.2029 + 0.9488 log x)	EXP (1.2029 + 0.9488 log x) x (0.9488/x)

TABLE 29: Regression models involving gas exchange parameters of plants subjected to moisture stress (Amaranthus Field Expt.1986)

Y	X	Y = f (x)	dy/dx
A	Time	49.2175 - 1.8079 x	- 1.8079
g_s	Time	0.4651 - 0.0179 x	- 0.0179
E	Time	11.4218 - 0.3622 x	- 0.3622
Ci	Time	165.8312 - 4.2442 x	- 4.2442
A/E	Time	3.6093 + 0.5308 log x	+ 0.5308/x
A/g_s	Time	111.3803 + 6.095 log x	+ 6.095/x
A/Ci	Time	42.9952 - 1.6338 x	- 1.6338
A	g_s	EXP (4.6526 + 0.9227 log x)	EXP (4.6526 + 0.9227 log x) x (0.9227/x)
A	Ci	7.9384 + 5.1533 log x	+ 5.1533/x
g_s	Ci	-0.5303 + 0.1667 log x	+ 0.1667/x
A	E	-34.3238 +33.6146 log x	+33.6146/x

Regression models describing the best function for various gas exchange parameters (taken any two at a time) are presented in Table 28-29. In sunflower, 'A' showed a logarithmic decrease with the duration of stress, whereas 'g_s' showed an exponential decay. 'E', Ci, A/Ci showed a lenier decrease with time of stress. A/E showed a logarithmic increase and A/g_s showed an exponential increase with time. Thus it is clear from these results that except 'E', Ci and A/Ci other parameters like 'A' 'g_s', A/Ci, A/g_s showed a non-linear relationship with duration of stress.

The relationship between 'A' and 'g_s, 'A' and Ci and 'A' and 'E' were non linear and positive indicating differences in the rate of change of 'A' with respect to these parameters. There was a weak linear relationship between 'g_s and Ci.

In the case of amaranthus A/E and A/g_s showed a non-linear increase with duration of stress (Table 29). However, 'A' 'g_s' 'E', Ci and A/Ci showed a linear decrease with the duration of stress.

The relationship between 'A' and 'g_s has been a positive exponential one in amaranthus. There was a strong positive logarithmic relationship between 'A' and 'E'. The best curves explaining the relationship between 'A' and Ci positive and 'g_s and Ci were positive logarithmic curves.

The functions describing the kinetics of gas exchange

TABLE 30: Effect of soil moisture stress on gas exchange rates and associated characteristics in Sunflower, Amaranthus and Sorghum (field expt.1987)

Species	Days after with holding water	PAR	g_s	A	A/g_s	Ci	A/Ci	E	A/E
SUNFLOWER									
	0	2027	0.418	30.01	95.40	186	16.1	8.68	3.51
	5	1980	0.386	32.66	92.60	191	0.192	9.22	3.89
	7	2000	0.270	24.05	96.14	186	0.134	5.32	4.96
	9	2000	0.296	27.75	85.60	203	0.196	5.92	4.26
	11	1950	0.222	26.28	118.80	150	0.176	4.54	5.83
	F test	NS	*	*	*	*	*	*	*
	CD 5%		0.11	2.8	16.5	15	0.13	2.5	1.5
AMARANTHUS									
	0	1900	0.310	38.10	123.0	144	26.6	5.96	6.47
	5	1800	0.304	37.68	124.0	142	0.266	4.86	7.79
	7	1800	0.208	30.18	146.2	106	0.296	2.44	12.74
	9	2000	0.106	15.16	149.6	100	0.154	1.48	11.24
	11	1800	0.086	12.80	149.0	102	0.132	1.04	11.87
	F test	NS	*	*	*	*	*	*	*
	C D 5%		0.12	3.4	17.5	17	0.28	1.8	2.3
SORGHUM									
	0	2100	0.290	33.98	117.0	152	22.4	5.58	5.94
	5	2000	0.178	25.28	114.0	108	0.262	6.78	4.85
	7	1850	0.116	17.08	146.2	106	0.172	2.44	7.12
	9	2050	0.126	17.88	144.2	109	0.164	2.26	7.14
	11	1900	0.080	12.50	165.6	75	0.202	1.32	9.64
	F test	NS	*	*	*	*	*	*	*
	C D 5%		0.09	3.7	12.8	14	0.17	1.3	1.8

parameters with duration of stress reveal that many parameters showed a non-linear relationship as stress progressed. It is also evident that the relationship between 'A' and 'g_s' 'A' and Ci and 'A' and 'E' during the course of water stress were non-linear. These non-linear relationship 'A' and 'g_s clearly suggests the mesophyll adoptation for better CO_2 reduction during stress. It may also be noted that A/E showed a logarithmic increase with the duration of stress in both the species suggesting that under conditions of a slowly developed moisutre stress in the field the leaves tries to maximise photosynthesis per unit amount of water transpired.

4.3.2.2 Experiment-II Field experiment (summery, 1987)

A second experiment was conducted during summer 1987 in similar lines as that of the previous one conducted in summer 1986, sunflower, amaranthus and sorghum plants raised in the field following standard practices and the crops were 45 days old irrigation was cut off and plants were stressed for 11 days. Gas exchange parameters monitored during this period and results were presented in Table 30.

In sunflower there was only a very marginal reduction in 'A' but were quite marked in amaranthus and sorghum (Table 30). Reduction in 'g_s has been slower phase in sunflower compared to the other two species. There were reduction in 'E' in all the 3 species there was a remarkable increase in A/g_s. Concomitently Ci also reduced. Sunflower exhibited

TABLE 31: Inter Correlation Matrix and Multiple Linear Regression Analysis of gas exchange Parameters of plants subjected to moisture stress (Sunflower Field Expt. 1987)

Inter Correlation Matrix

	E	g_s	Ci	A/Ci	A
E	1				
g_s	0.652	1			
Ci	0.638	0.706	1		
A/Ci	0.014	0.033	- 0.351	1	
A	0.370	0.634	0.025	0.497	1

Relationship between A/E and other parameters

	E	A	A/Ci
A/E	- 0.666	- 0.375	- 0.355

$A = 41.36 - 0.219\ E + 1.178\ g_s - 0.9306\ Ci + 0.1596\ A/Ci\ (R^2 = 90\%)$

$A/E = 4.93 - 0.929\ E + 0.7115\ A + 0.01413\ A/Ci\ (R^2 = 89\%)$

TABLE 32: Inter Correlation Matrix and Multiple Linear Regression Analysis of gas exchange Parameters of plants subjected to moisture stress (Amaranthus Field Expt. 1987)

Inter Correlation Matrix

	E	g_s	Ci	A/Ci	A
E	1				
g_s	0.952	1			
Ci	0.839	0.834	1		
A/Ci	0.629	0.730	- 0.265	1	
A	0.913	0.986	0.746	0.827	1

Relationship between A/E and other parameters

	E	A	A/Ci
A/E	- 0.799	- 0.605	- 0.238

$$A = -1.96 - 0.1135\ E + 0.8767\ g_s + 0.04579\ Ci + 0.2465\ A/Ci \quad (R^2 = 98\%)$$

$$A/E = 11.19 - 1.225\ E + 0.2299\ A + 0.3425\ A/Ci \quad (R^2 = 76\%)$$

TABLE 33: Inter Correlation Matrix and Multiple Linear Regression Analysis of gas exchange Parameters of plants subjected to moisture stress (Sorghum Field Expt. 1987)

Inter Correlation Matrix

	E	g_s	Ci	A/Ci	A
E	1				
g_s	0.735	1			
Ci	0.470	0.764	1		
A/Ci	0.363	0.214	- 0.427	1	
A	0.779	0.974	0.652	0.351	1

Relationship between A/E and other parameters

	E	A	A/Ci
A/E	- 0.773	- 0.472	- 0.186

$A = 13.11 + 0.08109\ E + 1.082 g_s - 0.2021\ Ci + 0.008214\ A/Ci\ (R^2 = 98\%)$

$A/E = 9.4 - 1.05\ E + 0.3158\ A + 0.0832\ A/Ci\ (R^2 = 65\%)$

slightly increasing trend in A/Ci ratios as the stress progressed. In amaranthus A/Ci values showed an increase upto 7th days of stress and in sorghum upto 5th day of stress and later on A/Ci ratio was reduced, short time water use efficiency (A/E ratio) showed an increase in all the three species, with amaranthus exhibiting very remarkable increase as the duration of the stress increased. For instance the A/E values for control and stressed (11 days) sunflower plants were 3.51 and 5.83 respectively. The corresponding values for amaranthus were 6.47 and 11.87 respectively.

4.3.2.2.1 Relationship among the gas exchange parameters

Inter-correlation matrics and MLR models for 'A' and A/E have been developed for the above experiment and presented in Table 31 to 33. 'A' and 'g_s' and 'g_s'and 'E' showed a very positive strong correlation in all the three species. The relationship between 'A' and 'E' and 'A' and Ci were also very strong in amaranthus and sorghum. There was a positive relation between Ci and E and Ci and 'g_s' in all the three species.

The MLR model for 'A' reveals a strong influence of 'g_s followed by Ci and 'E' in case of sunflower. In amaranthus and sorghum also contribution of 'g_s towards 'A' was significant. The contribution of 'E' towards A/E was greater than the contribution of 'A' in all the three species studied. This clearly suggests that in these species during the course. Of a slowly developed moisture stress in the field, reduction in 'E' was more responsible for the observed changes in A/E than reduction in 'A'. Thus under such conditions plants try to maximise water use efficiency by effecting more reductions in 'E' than 'A' though 'g_s is also reduced considerably.

The above results reveal that as water stress progressed there were reductions in 'A' and 'g_s' in sunflower, amaranthus and sorghum. Values of A/g_s and A/E ratios showed an increase. The increase in A/g_s ratio was more pronounced in sunflower and A/E in amaranthus. From the MLR models for 'A' it is clear that the derivative with respect to 'g_s was more than that with respect to other variables. This does not mean that 'g_s will be limiting 'A' more than 'gm' when 'A' is reduced under stress since the change in 'g_m is not be accounted in the model. The MLR model for A/E reveals that more than 'A' it was 'E' which contributed more towards the increase in A/E under stress in all the three species studied. This means that reductions in 'E' were more than the reductions in 'A' as stress progressed.

4.3.3. Cut leaf experiments

Excised leaves from the field grown sunflower and amaranthus plants were used for this experiment. After detaching the leaves from these well watered plants, they were subjected to fast live wilting under the field conditions. Such a fast wilting will not give sufficient time to the leaf to adjust to reductions in leaf ψ. The leaf water potential was

TABLE 34: Changes in Gas exchange rates and associated characteristics in detached leaves of Sunflower and Amaranthus subjected to live wilting

Species	Duration of live wilting (min)	PAR	g_s	A	A/g_s	Ci	A/Ci	E	A/E	ψ
SUNFLOWER										
	0	1985	1.10	37.6	34.18	285.31	131.78	10.3	3.65	- 5
	2	1912	1.10	41.7	37.90	279.34	149.27	11.8	3.53	- 6
	4	2132	0.88	38.5	43.75	270.00	142.59	12.0	3.21	- 10
	6	1960	0.52	30.8	59.20	245.20	125.59	6.4	4.81	- 12
	9	2071	0.44	14.7	33.40	286.54	51.30	8.3	1.77	- 18
	12	2067	0.35	-6.8				6.9		- 22
	16	2056	0.28	-4.3				7.6		- 26
	F test	NS	*	*	*	*	*	*	*	*
	C D 5%	-	0.18	3.5	3.1	12	19	3.7	1.11	3
AMARANTHUS										
	0	2117	0.42	41.7	99.28	181.14	230.20	10.8	3.86	- 6
	4	2034	0.29	42.1	145.17	107.72	390.80	7.6	5.54	- 9
	7	2119	0.18	20.01	111	162	123.51	3.6	5.51	- 16
	10	2080	0.05	3.82	76.4	218	17.52	1.7	2.25	- 22
	12	1970	0.03	-2.9				1.5	-	- 28
	F test	NS	*	*	*	*	*	*	*	*
	C D 5%		0.07	2.8	9.4	10	13	2.8	1.8	4

reduced to -26 bars by 16 minutes and to -28 bars by 12 minutes in the case of sunflower and amaranthus respectively. The leaf gas exchange rates were monitored during this period and the data are presented in Table 34.

The leaf water potential (ψ) showed statistically significant reduction as wilting progressed in both the species The reduction in ψ was faster rate in amaranthus. Both the species showed significant reductions in 'A' 'g_s'and 'E'.In sunflower the reductions in 'g_s' and 'E' were not as pronounced as in the case of amaranthus. At any given leaf water potential amaranthus maintained higher levels of 'A' and lower levels of 'g_s' and 'E' compared to sunflower. Both the species stared showing negative photosynthesis (resp iratory rate of CO_2) 12 minutes after detachment. A/g_s and A/Ci ratios showed an increase at the initial stages of wilting and later on decreased drastically. In amaranthus there was an initial increase in A/E ratio and later it decreased. In sunflower, A/E ratios were maintained constant for about 4 minutes and increased slightly about 6 minutes and then drastically reduced.

4.3.3.1 Relationship between gas exchange parameters

Regression model involving gas exchange parameters and ψ for the above experiments are given in Table 35. In both the species 'A' showed a logarithmic increase with 'g_s. The relationship between 'A' and 'E' was positive and linear in sunflower whereas it was positive and semi-logarthmic in

TABLE 35: Regression Models Involving A, E, g_s, A/E and ψ of leaves subjected to live wilting

Species	Y	X	Y = f (X)	dy/dx
SUNFLOWER	E	g_s	$5.3781 + 5.5361\ g_s$	5.5361
	A	g_s	$39.5828 + 30.5971\ in\ g_s$	$30.5971/g_s$
	A	E	$-29.011 + 5.7917\ E$	5.7917
	E	ψ	$15.5351 + 2.6076\ in\ \psi$	$2.6075/\psi$
	A	ψ	$54.3164 + 2.1909\ \psi$	2.1909
	g_s	ψ	$1.3758 + 0.0563\ \psi$	0.0563
	A/E	ψ	$EXP\ (2.0072 + 0.0877\ \psi)$	$EXP\ (2.0072 + 0.0877)\ (0.0877)$
AMARANTHUS	E	g_s	$1.3076 + 22.5531\ g_s$	22.5531
	A	g_s	$56.0228 + 12.8554\ \ln g_s$	$12.8554/g_s$
	A	E	$-0.3959 + 20.2512\ \ln E$	$20.2512/E$
	E	ψ	$EXP\ (3.0154 + 0.1104\ \psi)$	$EXP\ (3.0154 + 0.1104\psi)\ (0.1104)$
	A	ψ	$57.9668 + 1.1944\ \psi$	1.1944
	g_s	ψ	$EXP\ (2.9009 + 1.9763\ \ln \psi$	$EXP\ (2.9009 + 1.9763\ \ln\psi)\ (1.9763/\psi)$
	A/E	ψ	$EXP\ (1.8986 + 0.0192\ \psi)$	$EXP\ (1.8986 + 0.0192\psi)\ (0.0192)$

amaranthus. The relationship between 'E' and 'g_s' was linear in both the species.

Both the species 'A' showed a linear decline with ψ and 'E' showed a non-linear decrease with reduction in water potential. When sunflower exhibited a linear decrease in 'g_s'. amaranthus showed exponential decrease in 'g_s when ψ was reduced. The overall effect of reduction in ψ was to cause a week experiential decrease in A/E ratio in both the species. The function describing relationship between gas exchange parameters and ψ reveal that 'A' and 'g_s' 'E' and ψ and A/E and ψ exhibited non-linear relationship in the two species studied. In both species 'A' showed a linear reduction with decrease in ψ in sunflower the relationship was an experiential decrease in 'g_s as ψ decreased in amarathus.

4.3.4 Experiments with ABA

ABA is known to close stomata and thus reduce 'g_s'. An experiment was carried out by feeding ABA to excised leaves of sunflower and amaranthus through transpiration stream.

Though not very definitely established, the mechanism of ABA induced stomatal closure is understood to be mediated through Ca^{2+} ions. EGTA and ruthenium red (RR) are known to inhibit the formation calcium calmodulin in the cytosoil. These compounds were fed to the leaves along with ABA to minimise the ABA induced stomatal closure. This approach would be useful to understand whether Ca^{2+} ions are involved in the ABA induced stomatal movements and whether 'A' can be recovered if these compounds could inhibit the ABA action.

4.3.4.1 Experiment 1

Fully expanded mature leaves were excised from sunflower and amaranthus plants and different concentrations of ABA were fed to the leaf through the transpiration stream for about 15-20 minutes and gas exchange rates were monitored. The data are presented in Table 36.

Both sunflower and amaranthus showed an ABA concentration dependent reduction in 'A', 'g_s' and 'E'. The reductions in 'A' were comparable in both the species. But 'E' and 'g_s' were relatively more affected in amaranthus. At any given level of ABA concentration, sunflower exhibited higher values of 'g_s' and 'E'. These parameters showed significant changes in amaranthus. Amaranthus exhibited a significant increase in A/E ratios when the leaves were fed with ABA. Whereas sunflower showed slight decrease in 'A/E', ratios.

The above results suggest that ABA induced reduction in 'g_s' and 'A' in both sunflower and amaranthus. There was a very marked increase in 'A/g_s' ratios in amaranthus compared to sunflower. Concommitently in amaranthus there was also significant reduction in Ci.

TABLE 36: Effect of ABA on gas exchange rates and associated characteristics in excised leaves of Sunflower and Amaranthus

	PAR	g_s	A	A/g_s	Ci	A/Ci	E	A/E
SUNFLOWER								
Water (control)	1000	0.22	17.5	84.5	204	8.6	2.8	6.30
ABA 10^{-6}M	1000	0.13	10.5	85.0	206	5.4	2.0	5.30
ABA 10^{-4}M	1000	0.04	3.5	93.0	190	1.75	0.85	4.12
F test	NS	*	*	NS	NS	*	*	*
C D 5%	-	0.02	3.81	-	-	1.36	0.42	1.02
AMARANTHUS								
Water	1000	0.193	18.00	93	190	9.4	2.0	9.00
ABA 10^{-6}M	1000	0.07	8.85	126	138	6.4	0.65	13.62
ABA 10^{-4}M	1000	0.02	3.10	155	92	3.4	0.26	11.92
F test	NS	*	*	*	*	*	*	*
C D 5%	-	0.03	6.1	38	41	2.1	0.31	3.7

4.3.4.1.1 Relationship among gas exchange parameters

Intercorrelation matrices and MLR models for 'A' were developed for sunflower and amaranthus using the data of the above experiment and the results are presented in Table 37.

Both the species showed positive correlation between 'A' and 'E', 'A' and 'g_s', 'A' and A/Ci and 'E' and 'g_s' sunflower exhibited a strong negative correlation between 'A' and Ci. The relationship between A/Ci and Ci were strongly negative in both the species.

The MLR model for 'A' suggests a strong contribution of A/Ci followed by 'g_s' in the case of sunflower. In amaranthus 'g_s' was more important than A/Ci in terms of the relative contribution to the observed 'A'.

4.3.4.2 Experiment 2

In this experiment ruthenium red (RR) and EGTA were used to minimise the ABA induced stomatal closure. Detached leaves of sunflower and amaranthus were either fed with ABA or in combination with RR or EGTA. About 20 minutes after the treatment gas exchange parameters were determined. The data are presented in Table 38.

ABA caused significant reductions in 'A', 'g_s' and 'E' in both the species. Treatment of the leaves either with RR along or with EGTA alone also caused some reductions in these parameters compared to the control, but these reductions were not as low as with ABA treatment. The observed redcution in

'A' and 'g_s' with RR and EGTA treatments could be due to the possible effect of these compounds on chloroplast metabolism. However, the decrease in 'A' is not substantial to implicate that these compounds are potential inhibitors of 'A'.

EGTA and RR were found to cause remarkable recovery of 'A' and 'g_s' in the ABA treated leaves. For instance, the 'g_s'value showed by the ABA treated sunflower leaf was 0.04 $mol.m^{-2}s^{-1}$. When EGTA was present in the transpiration stream along with ABA, 'g_s' value was 0.19 $mol.m^{-2}s^{-1}$ with RR and ABA together, the 'g_s' value was 0.17 $mol.m^{-2}s^{-1}$. Similar trends were observed in amaranthus also.

A very remarkable recovery was also observed in the case of 'A' in both the species when EGTA or RR was present along with ABA. For instance when ABA treatment of sunflower leaves resulted in a very low value of A (2.2 $\mu mol.m^{-2}s^{-1}$), presence of EGTA along with ABA resulted in increasing this to a high level (11.5 $\mu\ mol.m^{-2}s^{-1}$). Similarly RR along with ABA also caused a significant increase in 'A' (11.1 $\mu\ mol.m^{-2}s^{-1}$). However, it may be noted that these values were slighlty lesser than the control value of 'A' (16.3 $\mu\ mol.m^{-2}s^{-1}$). The results were very similar in amaranthus also.

4.3.4.3 Experiment 3

A second experiment was also conducted in the above lines to study the reversal of ABA effects on 'g_s' by EGTA. ABA along with EGTA was fed to the excised leaves through the

TABLE 37: Inter Correlation Matrix and Multiple Linear Regression Analysis of Gas Exchange parameters in excised leaves enriched with ABA (Sunflower)

<u>Inter Correlation Matrix</u>

	E	g_s	Ci	A/Ci	A
E	1				
g_s	0.824	1			
Ci	- 0.497	- 0.455	1		
A/Ci	0.775	0.910	- 0.771	1	
A	0.817	0.957	- 0.688	0.991	1

$A = 1.995 + 0.0285\ E + 0.3403\ g_s - 0.0269\ Ci + 0.6383\ A/Ci\ (R^2 = 91\%)$

Inter Correlation Matrix and Multiple Linear Regression Analysis of Gas Exchange Parameters (Amaranthus Experiment with ABA)

	E	g_s	Ci	A/Ci	A
E	1				
g_s	0.880	1			
Ci	0.201	0.176	1		
A/Ci	0.260	0.346	-0.588	1	
A	0.802	0.914	-0.131	0.650	1

$A = 1.913 + 0.0684E + 0.7629g_s - 0.0964Ci + 0.3114A/Ci\ (R^2 = 87\%)$

TABLE 38: Effect of EGTA and RR on ABA induced changes in gas exchange rates and associated characteristics in excised Sunflower and Amaranthus leaves

	PAR	g_s	A	A/g_s	Ci	A/Ci	E	A/E
SUNFLOWER								
Water (control)	500	0.21	16.3	77.6	215	7.58	3.0	5.43
EGTA	500	0.12	12.1	100.8	178	6.80	2.9	4.17
RR	500	0.19	15.1	79.5	212	7.12	2.9	5.21
ABA	500	0.04	4.41	110	164	2.68	1.2	3.72
EGTA + ABA	500	0.19	11.5	60.5	243	4.73	2.5	4.60
RR + ABA	500	0.17	11.1	65.3	235	4.72	2.5	4.44
F test	NS	*	*	*	*	*	*	*
C D 5%	-	0.02	3.15	15.21	17.15	1.52	0.97	1.03
AMARANTHUS								
Water (control)	500	0.17	16.0	94.11	189	8.5	2.7	5.7
EGTA	500	0.14	12.5	89.3	197	6.4	2.6	4.9
RR	500	0.14	11.1	79.2	213	5.2	2.4	5.1
ABA	500	0.05	5.1	102	177	2.88	1.0	5.1
EGTA + ABA	500	0.12	11.5	95.8	187	6.1	2.0	5.75
RR + ABA	500	0.13	12.36	95.08	188	6.57	2.5	4.95
F test	NS	*	*	*	*	*	*	*
C D 5%	-	0.03	2.78	30.01	12.58	1.88	1.01	0.98

transpiration stream. Gas exchange rates were then determined 20 minutes after feeding started. The results are presented in Table 39.

ABA treatment resulted in very drastic reductions in 'A', 'g_s' and 'E' in both the species. In sunflower ABA treated leaves exhibited an 'A' of 2.0 μ mol.m^{-2}s^{-1} amd 'g_s' of 0.08 mol.m^{-2}s^{-1}. EGTA along with ABA caused an increase in 'A' to 7.5 μ mol. m^{-2}s^{-1} and 'g_s' to 0.25 mol.m^{-2}s^{-1}. However, these values were lesser than the control values. Amaranthus alos showed very similar results with ABA and EGTA.

The results of the above experiments reveal that ABA inhibited stomatal opening and so 'g_s' was reduced. There was a concommitant reduction in 'A' also. EGTA and RR were found to inhibit the ABA action. These compounds caused maintenance of 'g_s' and 'A' in presence of ABA.

4.4 Partitioning of stomatal and mesophyll limitations of photosynthesis under moisture stress

The plant factors affecting photosynthesis are broadly divided into stomatal and non-stomatal (mesophyll) factors. Stomatal factors include both stomatal frequency and aperture size. The mesophyll factors include the specific activities of the various photosynthetic and photorespiratory enzymes, chloroplast electron transport, photophosphorylation efficiency, translocation etc.

Stomatal conductance (g_s), is a reflection of the stomatal frequency and aperture size. Moisture stress is known to reduce 'g_s'. Mesophyll also offers considerable resistance to CO_2 diffusion, the reciprocal of which is termed as mesophyll conductance (g_m). Water stress reduces 'g_m' also. Unfortunately there is no method to determine 'g_m' directly unlike 'g_s'. Hence, the relative limitation of photosynthesis due to reductions in 'g_s' and 'g_m' during water stress has not been exactly quantified.

Gas exchange rates and associated parameters as affected by moisture stress studies in the various experiments described so far war subjected standard analysis of variance tests. The results of such tests would only give whether the observed variations in 'A' 'g_s' etc., were statistically significant or not. In fact water stress caused significant reductions in 'A' 'g_s' and associated parameters like Ci, A/Ci and increase in 'A/gs' and 'A/E'. This analysis, however, is not useful to estimate the extent of limitation imparted by 'g_s on 'A'. The correlation co-efficients between these parameters only give the degree of association among them. The multiple regression models developed for 'A' give the individual contribution of each variable (like 'g_s' 'A/g_s' Ci, A/Ci and E) towards the observed 'A' when each variable was changed unity keeping all others constant. In this analysis, the individual contribution of 'g_s' towards 'A' (i.e., the partial differential co-efficient of 'A' with respect to 'g_s' when other variables were kept constant) was found to be high in many cases. However, this is not sufficient to say that 'g_s was the major limitation for 'A' under stress compared to 'g_m' because of two reasons. First one is that absolute values of 'g_m' were not available to be included in the MLR models and hence the co-efficient of 'g_m' is not estimated. Secondly even if the co-efficient of 'g_m' is lesser than that of 'g_s', the total limitation imparted by 'g_m on 'A' could be more than that imparted by 'g_s'if the total change in 'g_m' is more than the total change in 'g_s'. Unfortunately absolute values of 'g_m could not be determined easily, though the per cent reductions could be calculated indirectly by knowing the per cent reduction in carboxylation efficiency (dA/dCi). This approach has been used in this study to modify the linear resistance analysis proposed by Farquhar and Sharkey (1982) to arrive at relative mesophyll limitations also. The original analysis by these authers by developing 'A'. Ci curves restricted to the determination of relative stomatal limitation only.

Experiments were conducted in potted as well as field grown sunflower and amaranthus plants with an objective to arrive at relative quantifications of stomatal and mesophyll limitations of photosynthesis during moisture stress. The results of these experiments are given below.

4.4.1. <u>Pot culture experiment (Summer, 1988)</u>

Sunflower and amaranthus plants were raised in pots under optimal conditions. When the plants were about 45 days

TABLE 39: Effect of EGTA on ABA induced changes gas exchange rates and associated characteristics in Sunflower and Amaranthus

	PAR	g_s	A	A/g_s	Ci	A/Ci	E	A/E
SUNFLOWER.								
Water (control)	400	0.40	15.0	38.0	270	5.56	3.0	5.00
EGTA	400	0.31	11.0	35.5	280	3.93	2.8	3.93
ABA	400	0.08	2.0	25.0	300	0.67	1.4	1.43
EGTA + ABA	400	0.25	7.5	30.0	290	2.59	2.0	3.75
F test	NS	*	*	*	*	*	*	*
C D 5%		0.06	2.15	3.86	12	1.05	0.56	0.97
AMARANTHUS								
Water (control)	400	0.22	21.0	95.45	187	11.22	2.1	10.00
EGTA	400	0.15	15.0	100.00	180	8.30	1.9	7.89
ABA	400	0.06	5.5	91.67	193	2.85	1.3	4.23
EGTA + ABA	400	0.16	15.1	94.38	189	7.99	2.1	7.19
F test	NS	*	*	*	*	*	*	*
C D 5%		0.04	2.13	7.35	7	2.50	0.81	1.82

old, irrigation was stopped to stress them. Three moisture regimes were imposed and the gas exchange parameters were studied when the leaf ψ was -6 bars (in control treatment), -15 bars (in moderate stress) and -24 bars (in severe stress) in both the species. After determining the gas exchange rates at normal ambient CO_2 level, all the treatments were exposed to different higher levels of CO_2 for about 10 minutes each and gas exchange paratments were again determined. Later Ci values were calculated from 'A' 'g_s' and Ca and 'A' Ci curves plotted.

4.4.1.1. Effect of moisture stress on photosynthesis under normal ambient Co_2 level

There were significant reductions in 'A' 'g_s' and A/Ci in both the species, sunflower exhibited more reductions in 'A' compared to amaranthus (table 40). At any given leaf ψ, amaranthus maintained higher values of 'A' and A/Ci. Though reduced significantly in both species, 'g_s' values were always higher in sunflower.

In both the species there was significant increase in A/g_s ratios and decrease in Ci levels. However, the changes were very marked in the case of amaranthus.

From the above results it is clear that both 'A' and 'g_s' were affected during moisture stress. Since, reductions in 'A' were concommitant with reductions in 'g_s one would expect that under conditions of a moisture stress, turgor

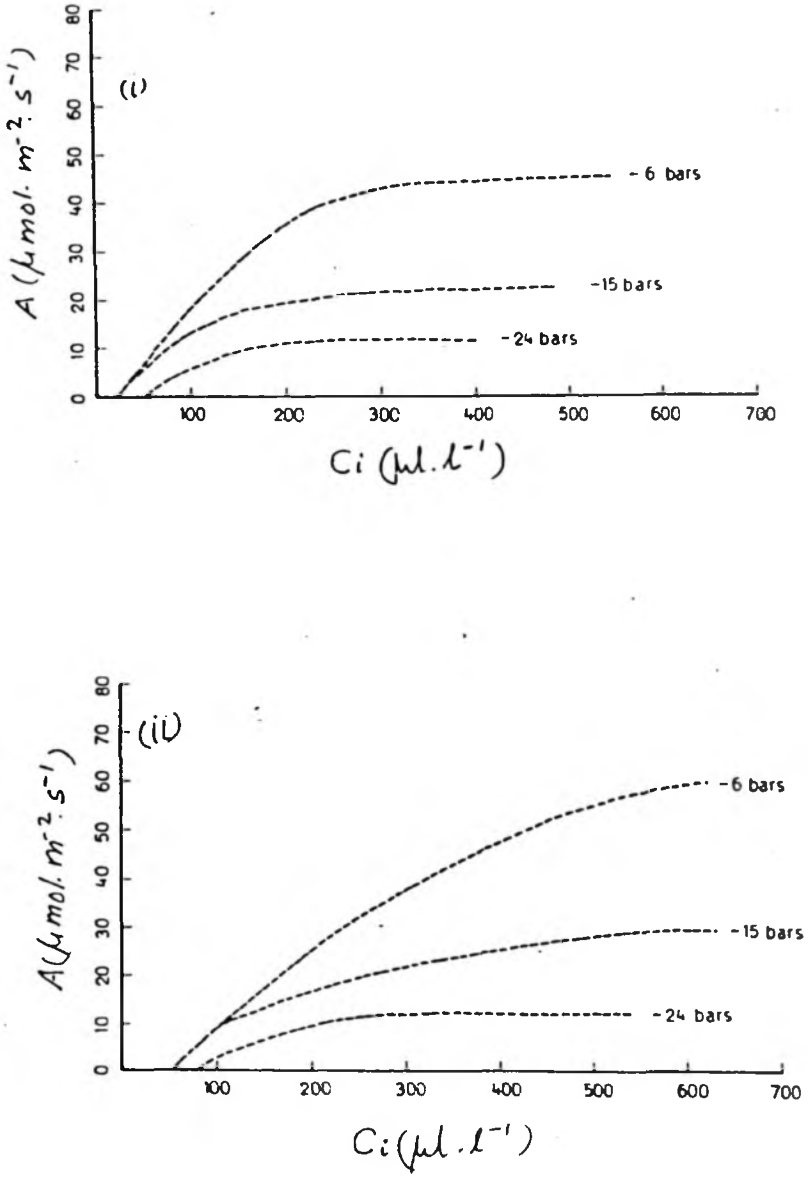


FIG XVI 'A' as a function of Ci in sunflower (i) and amaranthus (ii) plants subjected to moisture stress (Pot culture experiment) controlled stomatal aperture was a major limiting factor for photosynthesis.

However, in this study, further experiments were conducted to estimate the relative limitations of photosynthesis by 'g_s' and 'g_m' by developing 'A'. Ci curves in control and stressed plants (for a detailed methodology please refer materials and methods) and the results are given below.

4.4.1.2 Effect of increase in ambient Co_2 levels on photo synthesis

The above water stressed sunflower and amaranthus plants were exposed to different levels of Ca (upto a maximum of 900 $\mu l.\ 1^{-1}$) and photosynthetic rates were determined. Internal CO_2 concentrations for the corresponding Ca levels were then calculated and 'A'. Ci curves plotted. The curves are given in Figures 16a and b.

In both sunflower and amaranthus there was an appreciable increase in 'A' and Ci was increased. Under low leaf ψ conditions both the species showed lower values of 'A' at any given ψ suggesting that mesophyll factors were affected.

Under well watered conditions, 'A' continued to increase with Ci upto a value of 600 $\mu l.1^{-1}$ in sunflower. In amaranthus, 'A' values did not show any further increase beyond Ci values of 300 $\mu l.1^{-1}$. This is termed as break

TABLE 40: Changes in gas exchange rates and associated characteristics under moisture stress (pot culture Expt.1988)

		SUNFLOWER					AMARANTHUS				
	ψ	A	'g_s'	A/g_s	Ci	A/Ci	A	'g_s'	A/g_s	Ci	A/Ci
	- 6	29	0.88	32.95	287	10.11	33	0.73	45.21	268	12.31
	- 15	15	0.40	37.50	280	5.38	19	0.30	63.33	239	7.95
	- 24	8	0.20	40.00	276	2.90	11	0.12	91.67	193	5.70
F test	*	*	*	*	*	*	*	*	*	*	*
C D 5%	- 3	6	0.15	2.82	6	3.12	5	0.12	15	10	3.51

point by (Cowan 1977). Such a point was not seen in the case of control and moderately stressed (-15 bars) sunflower leaves. In the moderately stressed (-15 bars) amaranthus leaves, the break point appears to be below 300 ul.1^{-1}. In the severely stressed (-24 bars) leaves of both the species, the break points were at a very low Ci values, but the points were not clear since the curves showed a very gentle plateau.

The above results lead to the following conclusions. In sunflower, under control (-6 bars) and moderate (-15 bars) stress conditions 'A' showed a continued increase with Ci unlike in amaranthus. In the severely stressed (-24 bars) leaves the 'A' response to Ci was very poor in both the species.

Under control and moderate stress conditions, upto a Ci of about 300 ul1^{-1}, amaranthus exhibited higher A/Ci ratios compared to sunflower suggesting higher carboxylation efficiency in the former. Beyond this Ci level, sunflower showed higher A/Ci values. In this context, it may be recalled that higher Ci levels could have inhibited photorespiration and thus enhanced photosynthetic rate in sun flower. This could be the major reason why 'A' continued to increase over a wide range of Ci in sunflower.

4.4.1.3 Linear resistance analysis

A linear resistance analysis as proposed by Farquhar and Sharkey (1982) was done for the various 'A'. Ci curves developed in sunflower and amaranthus experiencing moisture stress. According to their original analysis they suggested a methodology to estimate the stomatal limitation (ls) of photosynthesis. In this study further calculations on mesophyll limitations for the theoretically possible maximum 'A' value (ML) and mesophyll limitation for the observed 'A' value (lm) are also made. The data are presented in Tables 41-44.

The theoretically possible maximum value of photosynthesis (AT) is defined here as that value of 'A' when Ci=Ca (i.e., when, no mesophyll and stomatal resistances are operating or rather when the stomata and mesophyll are not a limitation for photosynthesis.

In this study, a point 'Ag' has been defined on the A.Ci curve. This is that value of 'A' when normal stomatal resistances are operating and when mesophyll factors are not limiting (for a detailed description please refer materials and methods).

Another point 'Ao' has been defined on the A.Ci curve. This is that value of 'A' when stomatal resistances are zero but mesophyll limitations are existing. Calculations of ls, lm, and ML are based on these points.

The various limitations were calculated from the following formulae.

TABLE 41: Stomatal and mesophyll limitations of photosynthesis in plants subjected to moisture stress (pot culture 1988)

(Sunflower AT=55 AG = 32) (Amaranthus AT=77 AG = 37)

Stress level	SUNFLOWER							AMARANTHUS						
ψ (bars)	Ao	A	$'g_s'$	ML %	ls %	lm %	dA/dCi	Ao	A	$'g_s'$	ML %	ls %	lm %	dA/dCi
- 6 (CONTROL)	42	29	0.88	24	31	10	120	43	33	0.73	44	20	11	160
-15	23	15	0.40	58	53	35	70	23	19	0.30	70	25	48	80
-24	14	8	0.20	75	50	75	50	14	11	0.12	84	24	70	40

$$ML = \frac{AT - Ao}{Ao} \times 100$$

$$ls = \frac{Ao - A}{Ao} \times 100$$

$$lm = \frac{Ag - A}{Ag} \times 100$$

i) Relative stomatal limitation of observed photosynthesis (ls)

$$ls = \frac{Ao - A}{Ao} \times 100$$

ii) Relative mesophyll limitation of the potentially maximum photosynthesis (ML)

$$ML = \frac{AT - Ao}{AT} \times 100$$

iii) Relative mesophyll limitation of observed photosynthesis (lm)

$$lm = \frac{Ag - A}{Ag} \times 100$$

Various limitations as estimated directly from the linear resistance analysis are given in Table. 41 .

The results reveal that photosynthesis was limited by both 'g_s'and 'g_m' under all the three levels of leaf ψ. The stomatal limitation of photosynthesis (ls) was 31 per cent and the mesophyll limitation of photosynthesis (lm) was 10 per cent in sunflower in control leaves. Corresponding values of ls and lm were 20 per cent and 11 per cent respectively in amaranthus. It is clear that lm was not more than ls in these species under control conditions.

As leaf ψ decreased there was an increase in ls and lm in both the species. Increases in lm were more marked and went beyond ls as water stress progressed.

One way of indirectly estimating "g_m" is by calculating the "carboxylation efficiency". The "carboxylation efficiency" measured from dA/dCi also exhibited very significant reductions as water stress progressed suggesting a decrease in 'g_m'. Except in the extreme case of stress, amaranthus showed higher dA/dCi values compared to sunflower.

Values of AT was higher in amaranthus compared to sun flower. But Ao values were similar in both species. Values of Ao (theoretically maximum 'A' when stomatal limitations alone exist) were also found to reduce as water stress progressed in both the species indicating again a reduction the mesophull efficiency. This is also evident from the increasing values of ML observed in both the species. However, estimates of lm are more meaningful than ML since the former are estimated for the observed 'A' values and not for the theoritically projected 'A' values.

It is believed that most of the mesophyll resistance is contributed by carboxylation resistances. It is assumed that carboxylation efficiency (dA/dCi) is a measure of "g_m". More the carboxylation efficiency more the 'g_m'. Hence, it is logical to consider that the extent of reduction in dA/dCi will be equal to the extent of reduction in 'g_m' though absolute values of 'g_m' could not be arrived at Table 44 gives the per cent changes in 'g_s' "g_m", ls and lm compared to the control values (Δg_s, "Δg_m", Δls, Δlm).

TABLE: 42 Per cent changes in leaf water potential, 'g_s', 'g_m', ls and lm during water stress as compared control levels (calculated from Table 41) (pot culture 1988)

Reduction in leaf ψ	SUNFLOWER				AMARANTHUS			
	'g_s'	'g_m'	ls	lm	'g_s'	'g_m'	ls	lm
(bars)	%	%	%	%	%	%	%	%
9	- 55	- 42	71	250	- 59	- 50	+ 25	336
18	- 77	- 58	61	650	- 84	- 75	20	536

$$'g_s = \frac{'g_s'\text{ (stress)} - 'g_s'\text{ (control)} \times 100}{'g_s\text{ (control)}}$$

$$ls = \frac{ls\text{ (stress)} - ls\text{ (control)} \times 100}{ls\text{ (control)}}$$

$$lm = \frac{lm\text{ (stress)} - lm\text{ (control)} \times 100}{lm\text{ (control)}}$$

$$'g_m' = \frac{dA/dCi\text{ (stress)} - dA/dCi\text{ (control)} \times 100}{dA/dCi\text{ (control)}}$$

It is clear that the results presented in Table 42 that when leaf ψ was reduced from -6 bars to -15 bars 'g_s' was reduced by 55 per cent in sunflower and 59 in amaranthus. The corresponding reductions in "g_m" were 42 per cent and 50 per cent respectively. Thus in both the cases 'g_s' was reduced to a greater extent than 'g_m'. However, their relative limitations on photosynthesis did not follow a similar trend. In both the species lm was found to increase markedly. It may be noted that Δg_s and ls as well as "Δg_m" and Δ lm should be viewed differently. Δg_s is only a measure of the extent of reduction in g_s and it does not speak about the extent of limitation g_s is imposing on 'A'. In other words 25 per cent reduction in g_s does not necessarily mean that g_s is causing an additional 25 per cent limitation on 'A'. In fact no proportionate relationship between Δg_s and Δls was observed in this study. Similar was the case with respect to " g_m" and lm. The results show that the extent of increase in lm is more than the extent of decrease in " Δg_m". Values of Δls and Δlm are of more interest than values of g_s and "Δg_m" in the context of partitioning stomatal and mesophyll limitations of photosynthesis.

Similarly when leaf ψ was further reduced to -24 bars, it was 'g_s' that was reduced more than "g_m" in both the cases. But when the limitations of photosynthesis are considered, it was mesophyll that was more limiting than stomata since lm was much greater than ls.

TABLE 43: Stomatal and mesophyll limitations of photosynthesis under moisture stress (pot culture) expressed as per cent of total limitation (calculated from Table 41)

ψ	SUNFLOWER				AMARANTHUS			
(bars)	ls (%)	lm (%)	ls* (%)	lm* (%)	ls (%)	lm (%)	ls* (%)	lm* (%)
- 6	31	10	76	24	20	11	65	35
-15	53	35	60	40	25	48	34	66
-24	50	75	40	60	24	70	26	74

$$ls^* = \frac{ls \times 100}{ls + lm} \qquad lm^* = \frac{lm \times 100}{ls + lm}$$

TABLE 44: Stomatal and mesophyll limitations of photosynthesis under moisture stress (pot culture) expressed as per cent of exchange in total limitation (calculated from Table 41)

ψ (bars)	SUNFLOWER Δls (%)	Δlm (%)	ls^* (%)	lm^* (%)	AMARANTHUS Δls (%)	Δlm (%)	ls^* (%)	lm^* (%)
- 9	22	25	47	53	5	37	12	88
-18	19	65	23	77	4	59	6	94

$$\Delta ls = ls\ (\text{stress}) - ls\ (\text{control}) \qquad ls^* = \frac{ls \times 100}{ls + lm}$$

$$\Delta lm = lm\ (\text{stress}) - lm\ (\text{control}) \qquad lm^* = \frac{lm \times 100}{ls + lm}$$

These results suggest two points. First, when leaf ψ is lowered, 'g_s' is reduced more than 'g_m'. Secondly, though "g_m" reduction was lesser than 'g_s' reduction "g_m" offered very strong limitations on photosynthesis. Though the extent of "g_m" reduction was lesser, the limitation of 'A' by "g_m" was higher. This also suggests that "g_m" was more important in deciding 'A' than 'g_s' since there were reductions in 'g_s' without concommitant marked increases in ls. Because of the same reason it can be concluded that the stomata were under the feed back control of the mesophyll. Or rather, the turgor, operated stomatal aperture, though reduced in size under water stress, did not cause any enhanced limitation on 'A'. Certainly 'g_s' reductions caused appreciable reduction in E. It may also be noted that A/E ratios showed an increase under moisture stress.

Considering the sum of ls and lm as the total limitations, the extent of 'g_s' limitation (ls*) and 'g_m' limitation (lm*) has been calculated. The results are presented in Table 43. In this analysis also "g_m" caused increasing limitations on photosynthesis compared to 'g_s' as stress progressed. The relative contribution of lm to the total limitations was lesser than that of 'g_s' under control conditions, with lm* showing a value of 24 per cent in sunflower and 35 per cent in amaranthus. The corresponding values of ls* were 76 per cent and 65 per cent in the respective species as water stress progressed lm component of the total limitations increased steadily in both the species.

Under severe stress conditions lm* was increased to 60 per cent in sunflower and 74 per cent in amaranthus whereas ls* showed a marked reduction (40 per cent in sunflower and 26 per cent in amaranthus). This also suggests that mesophyll limitations were more in amaranthus and stomatal limitations were more in sunflower. During severe stress (-24 bars) in the case of sunflower and moderate (-15 bars) and severe stress (-24 bars) in the case of amaranthus lm* was greater than the ls*. These results suggest that as stress progressed mesophyll factors became increasingly limiting ultimately leaving stomatal limitations comparatively insignificant.

The same data was analysed in a slightly modified was as follows. The sum of the increase in ls and lm (as stress progressed) was accounted as 100 per cent and the relative concentrations of increased ls (Δ ls*) and increased lm (Δ lm*) towards this total increase was estimated. The results are presented in Table 44. The results suggest that the contribution of limitation due to mesophyll factors to the enhanced total limitation of photosynthesis (Δ lm*) under moisture stress was more than the limitation due to stomatal factors (Δ ls*) lm* was greater than ls* when the plants were under moderate or severe stresses. Thus it may also be noted that mesophyll factors became more and more limiting as water stress progressed i.e., the observed reductions in 'A' due to stress was more due to mesophyll limitations.

Linear resistance analysis (by developing A.Ci curves) has been done by (Farquhar and Sharkey 1982) to quantify the relative stomatal limitations of photosynthesis. In this study further analysis were conducted to arrive relative mesophyll limitation of photosynthesis. The A.Ci curve drawn for control treatment was extended linearly to predict the potential 'A' when neither stomata nor mesophyll were limiting. This potential value of photosynthesis (AT) was found to be higher in amaranthus.

The decreases in 'g_s and "g_m" (based on the dA/dCi values) are not true reflections of their relative limitations imposed on 'A'. Though 'g_s' was reduced more than "g_m" it was the mesophyll limitation that was greater than stomatal limitation as stress progressed.

Under control conditions, though mesophyll also imparted limitation on 'A' it was lesser than the stomatal limitation. But as stress progressed, mesophyll limitations contributed more than stomatal limitations towards the reductions in 'A'. Thus, reduction in 'g_s' appears to have been due to the feed back control of "g_m" - reduction in 'g_s' not causing any marked increase in the limitation of 'A'. Reduction in 'gs', however, resulted in more reduction in E and A/E was found to increase as stress progressed.

It was also observed that stomatal limitation of photosynthesis was more in sunflower compared to amaranthus at all the levels of leaf ψ studied.

TABLE 45: Changes in gas exchange rates and associated characteristics under different leaf water potentials (Field Expt. 1988)

Species	ψ (bar)	A	g_s	A/g_s	Ci	A/Ci
SUNFLOWER						
	- 6	23	0.90	25.56	299	7.69
	- 18	15	0.35	42.85	271	5.54
	- 24	8	0.20	40.00	276	2.90
F test	*	*	*	*	*	*
C D 5%	- 5	4	0.13	20.15	17	1.52
AMARANTHUS						
	- 6	42	0.70	60.10	244	17.21
	- 18	28	0.30	93.30	191	14.73
	- 24	17	0.13	130.77	131	12.98
F test	*	*	*	*	*	*
C D 5%	5	3	0.12	17.88	19	1.88

4.4.2. Field experiment

Sunflower and amaranthus plants were raised in the field under optimal conditions. When the plants were about 45 days old irrigation was regulated in such a way that a slow moisture stress developed by 15 days time expecting the plants to develop some adaptive mechanism. Three moisture regimes were resulted namely -6 bars (control), -18 bars (moderate stress) and -24 bars (severe stress) in both the species. The gas exchange rates were determined first under normal ambient CO_2 level. Then all the treatments were exposed to higher levels of CO_2 for about 10 minutes and gas exchange parameters were again determined. For each ambient CO_2 level, Ci was calculated from 'A' 'g_s' and Ca. Then 'A'. Ci curves were plotted and relative stomatal (ls) and mesophyll (lm) limitations estimated as described in the previous experiment.

4.4.2.1 Effect of moisture stress on photosynthesis under normal ambient CO_2 level

There were significant reductions in 'A' 'g_s' and Ci as the water stress intensity increased in both sunflower and amaranthus (Table 45). The extent of reductions in 'A' remained more or less the same in both the species. However, at any given leaf ψ, 'A' values were higher in amaranthus. Though reduced significantly in both species, sunflower maintained higher 'g_s' values compared to amaranthus. Values of Ci showed more remarkable reduction in amaranthus.

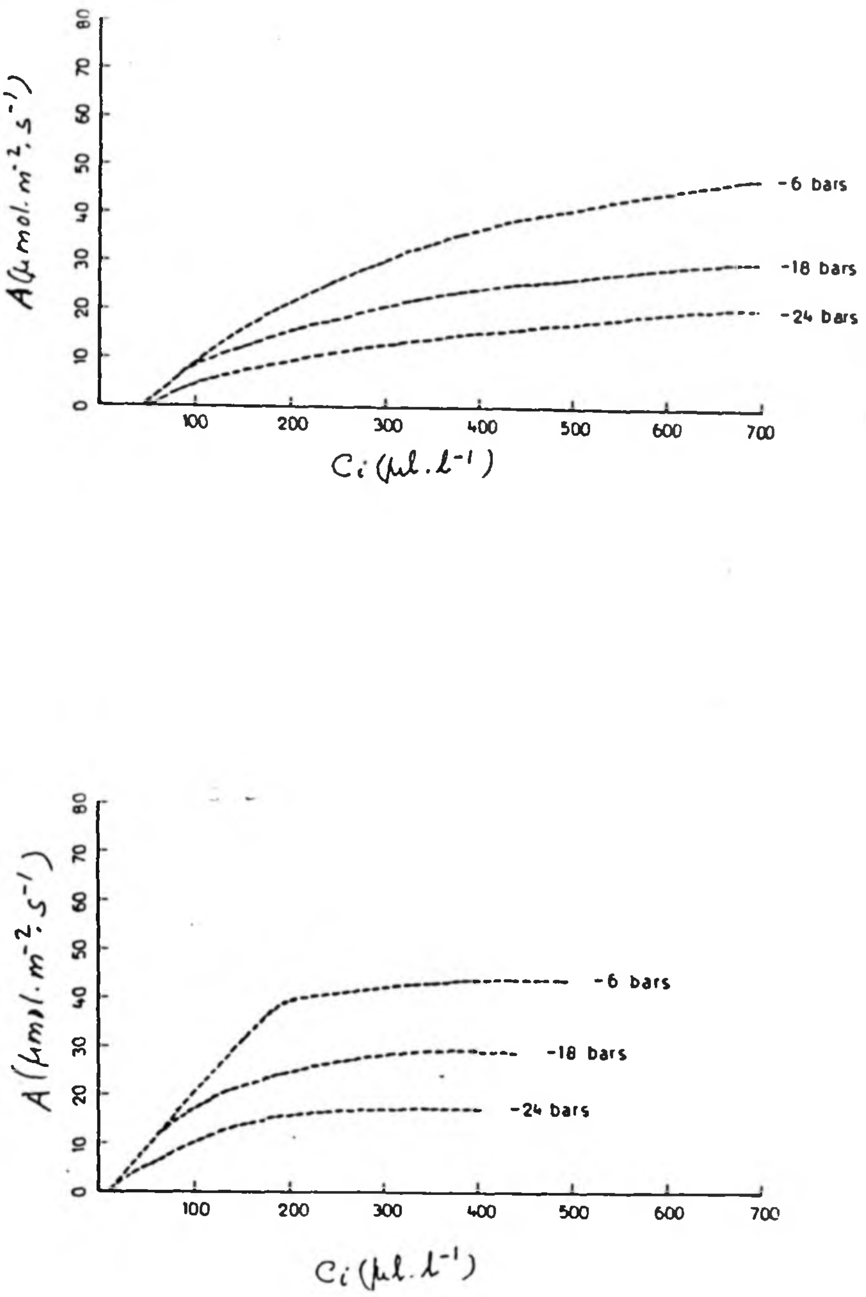


FIG XVII 'A' as a function of Ci in sunflower (i) and amaranthus (ii) plants subjected to moisture stress (Field experiment)

Both the species showed significant increase in A/g_s ratios. But the extent of increase was slightly more in amaranthus. The A/Ci ratios showed appreciable reductions in both the species, but the extent of reduction was more in sun flower.

These results show that there were concommitant reductions in 'A' and 'g_s' as the intensity of the moisture stress progressed. But this analysis will not speak about the extent of limitations imparted by the reduced 'g_s' on 'A'. This was studied by developing 'A'. Ci curves in control and stressed plants. The results are presented below.

4.4.2.2. Effect of increase in ambient CO_2 levels on photosynthesis

The above plants experiencing different levels of leaf ψ were exposed to higher levels of Ca (upto 9000 $ul.1^{-1}$) and 'A' and 'g_s' determined. Values of Ci were calculated and 'A'. Ci curves developed. The curves are given in Figures 17a and b.

Under well watered conditions 'A' continued to increase with Ci upto a value of 700 $\mu l.1^{-1}$ in sunflower. In amaranthus the break point was observed slightly beyond 200 $\mu l.1^{-1}$. No break points were observed in the case of sun flower as 'A' always showed an increase with Ci. But the rate of increase in 'A' with respect to Ci was very much reduced as the intensity of stress was high in both the species. In both moderately and severelly stressed Amaranthus leaves the break points were not clear since the slope of the A.Ci curves were very gradual.

It is evident from the above results that 'A' showed a more continued and gentle increase with Ci compared to amaranthus. In severely stressed leaves, the response of 'A' to Ci was weak compared to the control leaves. The more steady increase in 'A' with respect to Ci observed in sunflower could be because of inhibition of photorespiration at higher Ci levels.

4.4.2.3 Linear resistance analysis

Linear resistance analysis was performed for the A.Ci curves developed in the above experiment with an objective to quantify the stomatal and mesophyll limitations of photosynthesis. The data are presented in Tables 46-49.

Relative stomatal limitation of observed photosynthesis (ls), relative mesophyll limitation of observed photosynthesis (lm) and relative mesophyll limitation of the potentially maximum photosynthesis (ML) are given in Table 46.

The results reveal that 'A' was limited by both 'g_s' and 'g_m' under all levels of leaf water potential. But the mesophyll limitations (lm) were lesser than the stomatal limitations (ls) under control conditions. For instance lm was 21 per cent as against an ls of 32 per cent in sunflower in control leaves. The corresponding values of lm and ls in

TABLE 46: Stomatal and mesophyll limitations of photosynthesis under stress (Field Expt.1988).

Leaf ψ (bars)	Ao	A	gs	ML %	ls %	lm %	dA/dCi
SUNFLOWER	(AT = 55 μmol.m^{-2}s^{-1}		Ag = 32μmol.m^{-2}s^{-1})				
- 6	34	23	0.90	31	32	21	120
-18	21	15	0.35	57	29	48	60
-24	11	8	0.20	78	28	72	40
AMARANTHUS	(AT = 76 μmol.m^{-2}s^{-1}		Ag = 37μmol.m^{-2}s^{-1})				
- 6	42	35	0.70	45	17	5	180
-18	28	22	0.30	63	21	41	80
-24	17	14	0.13	78	18	62	40

$$ML = \frac{AT - Ao}{Ao} \times 100$$

$$ls = \frac{Ao - A}{Ao} \times 100$$

$$lm = \frac{Ag - A}{Ao} \times 100$$

TABLE 47: Changes in leaf ψ ,'g_s' 'g_m', ls and lm during water stress as compared to control levels (Field Expt. 1988) (Calculated from Table 46).

ψ (bars)	'g_s' (%)	'g_m' (%)	ls (%)	lm (%)	ψ (bars)	'g_s' (%)	'g_m' (%)	ls (%)	lm (%)
SUNFLOWER									
-12	-61	-50	9	129	AMARANTHUS	-57	-56	24	720
-18	-78	-67	12	242		-81	-78	6	1140

$$'g_s' = \frac{'g_s'\ (\text{stress}) - 'g_s'\ (\text{control}) \times 100}{'g_s'\ (\text{control})}$$

$$ls = \frac{ls\ (\text{stress}) - ls\ (\text{control}) \times 100}{ls\ (\text{control})}$$

$$lm = \frac{lm\ (\text{stress}) - lm\ (\text{control}) \times 100}{lm\ (\text{control})}$$

$$'g_m' = \frac{dA/dCi\ (\text{stress} - dA/dCi\ (\text{control}) \times 100}{dA/dCi\ (\text{control})}$$

TABLE 48: Stomatal and mesophyll limitations of photosynthesis under moisture stress expressed as per cent of total limitation (calculated from Table 46 (Field Expt. 1988)

	SUNFLOWER				AMARANTHUS			
ψ (bars)	ls (%)	lm (%)	ls* (%)	lm* (%)	ls (%)	lm (%)	ls* (%)	lm* (%)
- 6	32	21	60	40	17	5	77	23
-18	29	48	38	62	21	41	34	66
-24	28	72	28	72	18	62	22	78

$$ls^* = \frac{ls \times 100}{ls + lm} \qquad lm^* = \frac{lm \times 100}{ls + lm}$$

TABLE 49: Stomatal and mesophyll limitations of photosynthesis under moisture stress expressed as per cent of exchange in total limitation (calculated from Table 46 (Field Expt. 1988)

	SUNFLOWER				AMARANTHUS			
ψ (bars)	ls (%)	lm (%)	ls* (%)	lm* (%)	ls (%)	lm (%)	ls* (%)	lm* (%)
-12	3	24	10	90	4	36	10	90
-18	4	51	7	93	1	57	2	98

$$ls = ls\ (\text{stress}) - ls\ (\text{control}) \qquad ls^* = \frac{ls \times 100}{ls + lm}$$

$$lm = lm\ (\text{stress}) - lm\ (\text{control}) \qquad lm^* = \frac{lm \times 100}{ls + lm}$$

control amaranthus leaves were 5 per cent and 17 per cent respectively.

As water stress progressed both ls and lm increased and the value of lm was more than that of ls when the leaf ψ values were -18 and -24 bars in both the species. As the intensity of water stress was more the "carboxylation efficiency" (dA/dCi) also showed a remarkable reduction suggesting that 'g_m was affected.

Values of AT (theoretically maximum potential photosynthesis when stomatal and mesophyll resistances are zero) were found to be higher in amaranthus than in sunflower. Values of Ao (theoretical value of photosynthesis when 'g_m' is not limiting and 'g_s' is limiting) were higher in amaranthus compared to sunflower. But a stress intensity was increased. Ao showed marked reductions indicating again a reduction in mesophyll capacity. It may be noted that values of ML also exhibited a concommitant increase. These results are similar to the results obtained from the pot culture experiment.

Per cent decrease in 'g_s' "g_m" and per cent in ls and lm compared to the control values are given in -44 bars, 'g_s' was reduced by 61 per cent and "g_m" by 50 per cent in sunflower and 57 and 56 per cent respectively in amaranthus. Similarly the reduction in 'g_s' were by 78 and 81 per cent in sunflower and amaranthus in the severe stress treatment. The corresponding reductions in 'g_m' were 67 and 78 per cent in the respective species. It may be noted that reductions in

'g_s' were more than reduction in 'g_m'. But increase in mesophyll limitations were higher than increases in stomatal limitations like in the pot culture experiment. Similar was the result from the pot culture experiments. These results suggest that when leaf ψ is reduced, 'g_s' is reduced more than 'g_m'. This is of advantage in terms of water use efficiency as observed from the higher A/E ratios. But the limita tions imposed on 'A' by 'g_m was more than the limitations imposed on 'A' by 'g_m' was more than the limitations imposed by 'g_s' indicating that 'g_m is more important in deciding 'A' than 'g_s'. Stomata appears to be under the feed back control of 'g_m'.

The extent of 'g_s' limitation (ls*) and 'g_m'limitation (lm*) to the total limitation (ls+lm) was calculated and the results are given in Table 48. It is seen that lm* was lesser than ls* in both the species under control. But as the severity of the stress advanced lm* assumed higher values than ls* suggesting that mesophyll factors became progressively limiting photosynthesis making the stomata limiatations relatively insignificant.

The increase in total limitations (Δls+ Δlm) and the per cent contribution of ls and lm towards this increase in total limitations (Δls* and Δ lm*) are given in Table 46. These results show that Δlm* values were higher than Δ ls* values proving that the mesophyll limitations to the observed reductions in 'A' was more than the stomatal limitations.

The above results suggest that the mesophyll limitations of 'A' was more predominant than stomatal limitations under water stress conditions either in pot culture or in field experiments, though reductions in 'g_s' were greater than reductions in 'g_m' which resulted in higher A/E ratios under stress. It also appears that 'g_s' is under the feed back control of 'gm' particularly when the leaf ψ levels are low. Field experiments failed to show any reductions in the mesophyll limitations compared to the pot culture experiments though it was expected to show lesser mesophyll limitations since the stress was developed slowly. High temperatures prevailed in the field and other microclimatic factors could have been the reasons why such a result was not observed.

4.5 Gain of the feed back loops involving CO_2 and stomata

From linear resistance analysis it is evident that mesophyll factors limit 'A' more than stomatal factors under conditions of a water stress. Reduction in 'g_m' imparts a feed back control on 'g_s' in such a way that the reduction in 'g_s' under stress will not cause any significant limitation on 'A'.

In order to balance the opposing priorities of photosynthesis and transpiration plants make use of two imporatant feed back loops. They are the feed back loops involving CO_2

and transpiration (Gg). The former is also called as assimilation loop and the latter conductance loop (Farquhar et al., 1978 and Dubbe et al., 1978). If the extent of reductions in 'g_s' in relation with the extent of reductions in 'g_m' is different, the efficiency of water conservation is also different in differential feed back relationship between 'g_s' and 'g_m' will lead to differences in A/E ratios for a unit reduction in "g_m" if the reduction in 'g_s' is more it is advantageous for a better water husbandary. In this context loop gains involving stomata and 'A' were estimated for pot and field grown plants subjected to different moisture stress levels.

Dubbe et al (1978) used ABA to simulate moisture stress in excised leaves of Zea mays and Xanthium strumarium and observed an increase in stomatal and assimilation loop gains with ABA treatment. Farquhar et al., (1978) have pointed out that higher values of absolute stomatal loop gains help in husbanding more water.

Analysis of the loop gains were conducted by using the values of 'A" 'g_s' and Ci in sunflower and amaranthus plants experiencing different levels of water stress. The data from pot culture (summer 1988) and field culture (summer 1988) were used for this analysis.

Stomatal loop gain (Gg) is the product of the physical ($\partial Ci/\partial g_s$) and physiological (dgs/dCi) gains involving 'g_s'.

The physical gain component $\partial Ci/\partial g_s$ is derived by partial differentiation of the following formula.

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

$$\frac{\partial Ci}{\partial g_s} = \frac{1.6A}{g^2}$$

The physiological gain dgs/dCi is estimated from the 'g_s' Ci curve. This is nothing but the slope of the curve at the Ci level corresponding to the operating Ca. If Ci increases 'g_s' also decreases. Hence ags/dCi is negative.

The physical gain $\partial Ci/\partial g_s$ gives the rate of change of Ci when 'g_s' is altered by one unit keeping if 'g_s' is increased Ci also increases if other factors are constant, so $\frac{\partial Ci}{\partial g_s}$ is positive. The physiological gain dg_s\dCi explains the rate of change of 'g_s' with respect to Ci when 'A' and Ca were actually changing. An increase in Ci results in reducing 'g_s'. Hence dg_s/dCi is negative.

Assimilation loop gain (GA) is the product of physical ($\partial Ci/\partial A$) and physiological (dA/dCi) gains involving 'A'. The physical gain $\partial Ci/\partial A$ is calculated by partial differentiation of the following formula.

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

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

This gives the rate of change in Ci when 'A' is changed by unity keeping Ca and 'gs' constant. If 'A' is increasing Ci decreases (if other variables are constant). Hence, $\partial Ci/\partial A$ is negative. The physiological gain (dA/dCi) is estimated as the slope of the 'A'. Ci curve at the Ci level corresponding to the operating Ca. This gives the rate of change of 'A' with respect to Ci when Ca and gs were actually changing since 'A' increase with increase in Ci, dA/dCi is positive.

Open loop gain analysis were done for quick stressed (pot culture, summer, 1988) and slow stressed (field experiment, summer, 1988) plants to see whether the absolute values of the gains will be different in these two conditions. The results are discussed below.

4.5.1 Pot culture experiment (Summer, 1988)

The data of the experiment conducted to study the stomatal and non-stomatal limitations of photosynthesis described earlier are used in this analysis. The various loop gains calculated from these data are given in Table 50.

Reduction in leaf ψ resulted in appreciable reductions in 'A' and 'g_s' in both sunflower and amaranthus. The physiological gains for assimilation (dA/dCi) also showed drastic reductions in both the species suggesting that for

TABLE 50: Open loop gains involving stomata (Gg) and CO_2 assimilation (GA) at different leaf water potentials (Pot Culture 1988)

ψ (bar)	A	g_s	Physiological gain		Physical gains		Loop gains		$\frac{1}{1-G_g-G_A}$
			dA/dCi	dg/dCi	$\partial Ci/\partial A$	$\partial Ci/\partial g$	G_g	G_A	
SUNFLOWER									
- 6	29	0.88	120	-800	-1.82	0.060	-0.048	-0.218	0.79
-15	15	0.50	70	-1200	-3.20	0.102	-0.123	-0.224	0.74
-24	8	0.31	50	-1300	-5.16	0.150	-0.195	-0.258	0.65
AMARANTHUS									
- 6	33	0.73	160	-1400	-2.19	0.096	-0.135	-0.350	0.67
-15	19	0.30	80	-1600	-5.33	0.320	-0.480	-0.512	0.50
-24	11	0.12	40	-1700	-13.33	1.222	-2.077	-0.533	0.28
SORGHUM									
- 6	25	0.65	160	-1500	-2.46	0.087	-0.131	-0.394	0.66
-24	10	0.25	80	-1700	-6.40	0.256	-0.435	-0.512	0.5

unit increases in Ci, increase in 'A' was less as stress progressed. The absolute values of physiological gains for stomata (dg_s/dCi) showed more increase as stress advanced, suggesting that 'g_s' became more sensitive to Ci under stress i.e., for unit increase in Ci, there was more reduction in 'g_s'.

The physiological gains involving 'A' (dA/dCi) exhibited remarkable reductions in both the species as water stress progressed (Table 50). There was an increase in the absolute values physical gains involving 'g_s' ($\partial g_s/\partial Ci$) in both cases as stress progressed. The values of dg_s/dCi were -800 and -1300 in sunflower and -1400 and -1700 in amaranthus under control (-6 bars) and severe (-24 bars) stress conditions. This suggests that the stomatal sensitivity to Ci increased under water stress.

Physical gains involving both 'A', ($\partial Ci/\partial A$) and 'g_s' ($\partial Ci/\partial gs$) showed an increase in both the species as water stress progressed (Table 50). In sunflower the values of $\partial Ci/\partial A$ were -1.82 and -5.16 and in amaranthus they were -2.19 and -13.33 under control (-6 bars) and severe stress (-24 bars) conditions. The increase in $\partial Ci/\partial gs$ was from 0.060 (Control -6 bars) to 0.150 (severe stress -24 bars) in the case of sunflower and from 0.096 (control -6 bars to 1.222) (Severe stress -24 bars) in the case of amaranthus. Thus in both the physical gains, the absolute values as well as the extent of increase were more in the case of amaranthus.

Water stress resulted in higher loop gains in both the species. The absolute value of stomatal loop gain, 'Gg' increased from 0.048 (control - bars to 0.123, under moderate stress (-15 bars) and to 0.195 under severe stress (-24 bars in the case of sunflower. In the case of amaranthus values of 'Gg' were -0.135 (control -5 bars), 0.080 (moderate stress -15 bars) and 2.077 (severe stress -24 bars). It may be noted that at any given level of leaf ψ, stomatal loop gains were higher in amaranthus. Also the extent of increase in 'Gg' under stress was higher in amaranthus compared to sunflower.

During water stress 'GA' increased in both the species and the increase was more prominant in amaranthus. The values of 'GA' under control (-6 bars), moderate stress (-15 bars) and severe stress (-24 bars) conditions were 0.218, 0.224 and 0.258 in sunflower and 0.350, 0.512 and 0.533 respectively in amaranthus. However, the extent of increase in 'GA' was much lower than the extent of increase in 'Gg' in both the species.

The ratio (1/1-Gg-GA) is the extent to which Ci will increase when Ca is increased by one unit. It is evident that if 'Gg' = GA = 0 (i.e., if no feedback loops operate), Ci will increase at the same rate at which Ca is increased. In fact, it is not occuring like that for any increase in Ca, Ci will increase only by a small fraction of Ca due to the operation of these feed back loops. Thus this ratio can be considered as a measure of stomatal sensitivity to CO_2.

Because more the ratio, more is the entry of CO_2 into the mesophyll or rather lessed the 'g_s' sensitivity to CO_2. Sensitivity of stomata to changes in CO_2 levels in different in different species. This is also evident from the loop gain analysis where the values of dg_s/dCi are different in sunflower and amaranthus. This ratio was found to decrease in both species as stress increased in both the species indicating that the extent of increase in Ci with increase in CA progressively reduced as stress advanced. This is due to combined effects of reduced 'g_s' and increased stomatal sensitivity to CO_2. At any given leaf ψ levels, this ratio was lesser in amaranthus. This could be due to the higher degree of 'g_s' sensitivity to CO_2 (again evident from the higher 'Gg' values in amaranthus). The values of this ratio more or less agree with the slope (dCi/dCa) of the Ci, Ca curves. It may be noted that sunflower has a higher slope compared to amaranthus.

The above results reveal that both 'Gg' and 'Ga' increased under stress. A high gain of conductance loop helps to husband water. The increase in 'Gg' was more pronounced in amaranthus indicating its intrinsic capability to husband more water. A low 'Gg' observed in sunflower would be of advantage only under conditions of abundant water supply. A higher value of 'Gg' in amaranthus under stress also suggests that stomata were more sensitive in this species. Both the species exhibited an increase in 'GA' as the intensity of water stressed was increased. In amaranthus this increase was more apparant than in sunflower. In increase in 'GA' is not an advantage, obviously. But it is consequence of the increase in the absolute value of physical gain of 'A', ($\partial Ci/\partial A$) as well as a distant effect of the feed forward control of 'Gg' over 'GA'. However, in both the species the extent of increase in 'GA' was much lesser than that of 'Gg' indirectly indicating the higher WUE under stress.

4.5.2 Field experiment (summer, 1988)

The 'A'. Ci curves developed for the study of stomatal and mesophyll limitation of photosynthesis was affected by field water stress has been used here to estimate the various loop gains. The results are presented in Table 51.

Reduction in leaf ψ resulted in appreciable reduction in 'A' and Ci in sunflower and amaranthus. The physiological gains for assimilation (dA/dCi) also showed a drastic reductions in both the species. This suggests that the increase in 'A' for unit increase in Ci progressively reduced as the stress advanced indicating a reduction in carboxylation efficiency. The physiological gains for conductance loop (dgs/dCi) showed an increasing trend in both the species suggesting that 'g_s' became more sensitive to CO_2 under stress.

The physical gains involving 'A', ($\partial Ci/\partial A$) and involving 'g_s' ($\partial Ci/\partial g_s$) showed and increase in both the species as

TABLE 51: Open loop gains involving stomata (Gg) and CO_2 assimilation (GA) at different leaf area water potentials (Field Expt. 1988)

ψ (bar)	A	Physiological gain			Physical gains		Loop gains		$\frac{1}{1-G - GA}$
		g_s	dA/dCi	dg/dCi	$\partial Ci/\partial A$	$\partial Ci/\partial g$	Gg	GA	
SUNFLOWER									
- 6	23	0.90	120	-1050	-1.78	0.045	-0.048	-0.214	0.79
-18	15	0.35	60	-1400	-4.57	0.196	-0.274	-0.274	0.65
-24	8	0.20	40	-1700	-8.00	0.320	-0.544	-0.540	0.54
AMARANTHUS									
- 6	35	0.70	180	-1500	-2.286	0.124	-0.186	-0.412	0.63
-18	22	0.20	80	-1800	-8.000	0.920	-1.660	-0.640	0.30
-24	14	0.10	40	-1900	-16.000	2.240	-4.260	-0.640	0.17

stress progressed (Table 53.) For instance absolute value of $\partial Ci/\partial A$ was 1.78 in control and 8.00 in severe stress in the case of sunflower. The corresponding values of $\partial Ci/\partial A$ in amaranthus were 2.286 and 16.00 respectively. This shows that the extent of increase in $\partial Ci/\partial A$ was more in amaranthus.

The physical gains of conductance loop ($\partial Ci/\partial g_s$) also showed an increasing trend as stress advanced. The absolute values of $\partial Ci/\partial g_s$ and extent of increase were more in the case of amaranthus at any given leaf ψ.

Both the species showed an increase in stomatal loop gain (Gg) and assimilation loop gain (Ga) as stress progressed. The absolute value of 'Gg' was 0.048 in sunflower and 0.186 in amaranthus under control conditions when the leaf ψ was further decreased to -24 bars the absolute value of 'Gg' increased to 0.544 in sunflower and 4.26 in the case of amaranthus. Similarly the absolute values of assimilation loop gain 'GA' showed an increasing trend in both the species as the stress progressed. In sunflower 'GA' was 0.214 in control and 0.54 in the severely stressed leaves. The corresponding values in amaranthus 0.412 (control) and 0.640 (severe stress). The above result suggests that the absolute values and the extent of increase of 'Gg' were more in amaranthus. The absolute values of 'GA' were always more amranthus. These results are in agreement with the results obtained from pot culture experiment. The absolute values as well as the extent of increase in both 'Gg' and 'GA' were more in amaranthus as evident from the pot culture studies. However, the results of the field experiment described above indicate that the extent of increase of 'GA' is slightly more in sunflower though the absolute values of 'GA' were more in amaranthus.

The ratio (1/1-Gg-GA) is a measure of the extent of increase in Ci per unit increase in CA. This ratio is also very close to the slope (Ci/ Ca) of the linear function fitted between Ci and Ca. The slope is slightly higher in most of the C-3 plants and lower in most of C-4 plants. The results obtained in these study also revealed similar trend (Table 51). At any given ψ sunflower exhibited a high value compared to amaranthus. The values showed a reducing trend as leaf ψ decreased. This means that for unit increase in Ca the increase in Ci was less in the stressed leaves. This indirectly suggests that stomatal sensitivity to CO_2 increased. Under stress the extent of decrease in this ratio was more in amaranthus suggesting that amaranthus showed a higher degree of stomatal sensitivity to CO_2.

As in the pot culture experiment in the field experiment also the extent of increase in 'Gg' was more than the extent of increase in 'GA'. An increase in 'Gg' under stress is certainly of advantage in terms of high WUE. However, an increase in 'GA' is not of any advantage, it may be noted that the increase in 'GA' always lesser than the 'Gg'.

4.6 Studies on chlorophyll fluorescence quenching as affected by leaf water status in sunflower, amaranthus and sorghum

Water stress induces photooxidation of chlorophyll molecules and results in photoinhibition of photosynthesis electron transport. This leads to changes in the chlorophyll fluroscence emission characteristics (Govindjee *et al.*,1978).

Experiments were conducted in this study, to vary the leaf water potential by floating leaf discs in PEG solutions of known osmotic potential or by live wilting excised leaves to known levels of leaf water content. The leaf discs were then incubated in dark for about 10 minutes and the choloro-phyll flurorescence spectrum was recorded for 50 seconds after illumination. The per cent quenching of relative flurorescence intensity (25 and 50 seconds after illuminating) as compared to the initial peak value was calculated.

4.6.1 Experiment-1: Effect of floating leaf discs in PEG solution on chlorophyll fluorescence

Leaf discs were cut from fully expanded mature leaves of well watered potted plants of sunflower, amaranthus and sorghum and floated in PEG solutions with osmotic potentials -6 bars, 12 bars and -15 bars for two different durations namely 1.5 hours and three hours water was used as control chlorophyll fluorescence was recorded after the treatment and

TABLE 52: Percent Fluroscence Quencing (at 25th and 50 second) of leaf discs after treating with PEG solutions of different osmotic potentials.

Duration of treatment (hrs)	Species	Water		-6 Bars		-12 Bars		-15 Bars	
		Per cent Fluorescence Quencing							
1.5 hours		25s	50s	25s	50s	25s	50s	25s	50s
	AMARANTHUS	10.9	12.3	7.9	9.2	0.0	0.67	-0.70	-1.40
	SORGHUM	11.5	12.7	2.7	3.7	9.3	10.90	0.64	0.64
	SUNFLOWER	5.8	6.0	1.3	0.63	-2.8	-5.6	-1.80	-3.04
3 hours	AMARANTHUS	9.5	10.3	2.8	3.5	1.7	1.7	-3.1	-5.5
	SORGHUM	10.7	11.3	6.0	7.3	4.0	5.7	4.6	5.9
	SUNFLOWER	5.4	6.0	-1.3	-2.1	-0.63	-1.9	-1.5	-2.3

per cent quenching calculated. The data are presented in Table 52.

There was a consistant reduction in the per cent quenching of fluorescence in all the three species studied as the osmotic potential of the PEG solution was reduced (Table 57). Fluorescence quenching was more 50 seconds after illuminating compared to 25 seconds after illuminating. For instance, in the water treatment (1.5 hours) there was 10.9 per cent quenching 25 seconds after illuminating and 12.3 per cent quenching 50 seconds after illuminating. The corresponding values were 11.5 and 12.7 per cent in the case of sorghum and 5.8 and 6.0 per cent in the case of sunflower. When floated in PEG (-6 bars) for 1.5 hours, per cent quenching of fluorescence 50 seconds after illumination was 9.2 per cent in amaranthus 3.7 per cent sorghum and 0.63 per cent in sunflower. Sunflower showed an increase in fluorescence emission (negative quenching) when floated in PEG solutions of -12 and -15 bars osmotic potentials. In the case of amaranthus negative quenching was observed when leaf discs were floated in PEG solution of -15 bars osmotic potential.

Similar were the results when leaf discs were floated in PEG solutions for 3 hours. In this case sunflower started showing negative quenching for -6 bars onwards.

The above results suggest that the present fluorescence quenching decreased as the osmotic potential of PEG solution in which the leaf discs were floated reduced. This means that when the severity of osmotic stress was increased the intensity chlorophyll fluorescence emission failed to reduce suggesting that the integrity of pigment systems was affected as severity of the stress was more. Since sunflowr exhibited lesser quenching at any given level of osmotic stress it can be concluded that the pigment systems were more affected compared to amaranthus and sorghum.

4.6.2 Experiment-2: Effect of live wilting of excised sunflower, amaranthus and sorghum leaves on chlorophyll fluorescence:

Fully expanded mature leaves were excised from well watered potted plants of sunflower. Amaranthus and sorghum and allowed to live wilt under laboratory conditions At different stages of wilting a disc of leaf was removed and used to study the fluorescence emission. Then the water loss was also calculated by gravimetric method. The results are presented in Table 53.

As the leaf water content was reduced, there was a reduction in the per cent quenching of fluorescence in all the three species studied. For instance, when there was 60 per cent water loss in amaranthus, the fluorescence quenching was only 3.0 per cent compared to the 6.7 per cent quenching in the control (0% water loss) leaves. In the case of sorghum, negative quenching was observed at 45 and 60 per cent water loss and in sunflower at 80 per cent water loss. In general fluorescence quenching was less in sunflower

TABLE 53: Percent Fluroscence Quenching (at 25 and 50 second) of leaf discs after live wilting

Species	Time after leaf excision (min.)	Per cent water loss	Per cent Quenching	
			25	50
AMARANTHUS	0'	0%	9.7	6.7
	5'	40%	7.8	7.8
	10'	50%	4.1	4.9
	15'	55%	6.0	6.0
	20'	60%	3.0	3.0
SORGHUM	0'	0%	1.9	5.0
	5'	20%	1.2	4.0
	10'	35%	0.6	2.3
	15'	45%	- 0.6	- 0.6
	20'	60%	- 3.7	- 5.2
SUNFLOWER	0'	0%	5.4	5.8
	5'	30%	1.5	2.3
	10'	65%	0.9	2.8
	15'	70%	0.0	0.9
	20'	80%	- 1.0	- 3.0

compared to amaranthus and sorghum at any given leaf water level indicating that pigment systems were more affected in sunflower. It may also be noted that at any given time per cent water loss was more in sunflower suggesting a high rate of water loss resulted in quicker wilting of sunflower leaves compared to the other two species.

DISCUSSION

V. DISCUSSION

Leaf area and photosynthetic CO_2 assimilation rate per unit leaf area are two important components determining dry matter production under any given conditions. Photosyn thetic rate (A) is a complex metabolic, process involving a number of biochemical reactions. At the same time it is a highly regulated physical process also. CO_2 gas diffuses into the site of reduction, namely chloroplast stroma through boundary-air-layer, stomatal pore, substomatal cavity, mesophyll cell wall, cytoplasm and cell membrances. At each level of this diffusion CO_2 experiences either a physical or a biochemical resistances. The most prominent physical resistances in the CO_2 diffusion pathway are boundary air layer resistance and stomatal resistance. The resistance offered by mesophyll is mostly biochemical involving many enzymes like carbonic anhydrase, enzymes of Calvin cycle and photorespiration etc. It is believed that most of the mesophyll resistance is at the carboxylation step.

There are numerous external factors which influence A. They are light intensity, atmospheric temperature, humidity, wind velocity, plant water and nutrient status etc. These environmental parameters will either have a direct or indirect effect on photosynthesis. For instance light intensity has direct effect on photosynthesis since photo-machinery directly derives energy from light. At the same time the stomatal aperture size which decides the stomatal conductance for CO_2 varies depending on light intensity. Variations in 'g_s' cause variations in 'A'. Thus light has a direct effect as well as indirect effect on 'A'. Similarly other environmental variables like humidity, temperature, water status, nutrition level also have direct and indirect effect on the physics and physiology of photosynthesis.

Among the various abiotic stresses, water stress is probably the most important one. A rapid reduction in leaf water status causes rapid reduction in 'g_m' (Mooney _et al._, 1977). Many enzymatic reactions are also known to be affected because of water stress. Those enzymes include RUBISCO, carbonic anhydrase (Jones, 1973), Ribulose 5-P Kinase and PEP case (Huffaker _et al._, 1970) and ATPase (Yoomis _et al._, 1979). Regeneration of RUBP is also affected by water stress (Farquhar and Sharkey, 1982). A slow developed stress may lead to maintenance of turgor due to osmotic adjustments (Jones and Rawson, 1979) water stress is known to affect the activity of photosynthetic pigment systems (Boyer and Bowen, 1970), Mohanty and Boyer, 1976; Govindjee _et al._, (1981). This could lead to a reduction in Hill activity (Fry, 1970) and inhibition of photophosphorylation (Keck and Boyer, 1974).

Water stress is known to reduce stomatal conductance (Jacob, 1982 ; Kramer, 1983). There are differences in sensitivity of stomata to reduction in leaf water content because the threshold ψ for the stomata may be different in different species (Boyer, 1976). Similarly species vary in their stomatal sensitivity to light intensity depending on age and environment. The light intensity required for 35 per cent of maximum 'g_s' varies from 100-200 u moles $m^{-2}s^{-1}$ (Turner, 1974). Stomatal response to light varies in the morning for opening and in the evening for closing (Ramos, 1981). Changes in atmospheric humidity also alters 'g_s' irrespective of leaf water status (Hall et al., 1976), Extremes of temperature resulted in irreversible reductions in 'g_s' (Bauer, 1978).

Thus the various abiotic stresses are known to influence 'g_s' and 'g_m' and thus reduce 'A'. Different abiotic stresses may affect 'g_s' and 'g_m' differently in different species also. The stress induced reduction in 'A' is hence due to the effect of abiotic stress on 'g_s' and 'g_m'. It is important to know which of these factors limits 'A' more under a given set of environmental conditions and the interactions between 'g_s' and 'g_m'.

Understanding the control processes of photosynthesis will reveal how plant adopts to a particular environment. Identifying the limiting factors is therefore important to attempt further manipulations to maintain more stability of 'A' under stress.

It has been the general contention that stomatal conductance imparted more limitations on 'A', particularly under condition of as abiotic stress (Farquhar and Sharkey, 1982). This conclusion was obviously based on the concommitant reductions in 'A' and 'g_s'. A very strong positive correlation existing between 'A' and 'g_s' also led to this important misconception that 'g_s' is limiting 'A' more than 'g_m'. Unfortunately all these conclusions were based on little experimental support because quantification of either absolute value of 'g_m' or the limitations imparted by reduced 'g_m' on 'A' were never done precisely.

In this context experiments were conducted in this study with the following objectives, selecting sunflower, amaranthus and sorghum which have very distinct gas exchange characters. The 1st objective was to stduy the effect of abiotic stresses like nitrogen stress, low light stress and water stress on 'A', 'g_s' and associated characteristics.

The second objective was to study the inter-relations among different gas exchange characters as affected by above mentioned stresses.

The third objective was to estimate the relative limitations imparted by stomatal and mesophyll factors on 'A' under conditions of moisture stress in sunflower and amaranthus.

The fourth objective was to determine the stomatal and assimilation loop gains to understand the adaptations of these species to water stress.

Different experiments conducted to meet these objectives include the following. The 1st set of experiments included raising sunflower, amaranthus and sorghum plants at different N levels. In the second category of experiments the experimental plants were subjected to low light stress. In the third set of experiments water stress was imposed in poted plants, field grown plants and in excised leaves by live wilting. In another set of experiments ABA was used to reduce 'g_s'. In all the above experiments gas exchange characteristics were measured. Lastly chlorophyll fluorescence emission spectrum was studied in the leaves which experienced different intensities of moisture stress. A detailed discussion of these experiments is given below.

Effect of different levels of fertilizer N on gas exchange characteristics in sunflower, amaranthus and sorghum

Sunflower, amaranthus and sorghum plants were grown in pots under different N levels. The purpose of altering N level was to alter predominently the mesophyll factors because any reduction in the N nutrietion level will result in reduced enzyme contents in the leaf. It is evident from this fact that reductions in the values of 'A' were greater than the reductions in 'g_s' resulting in reduced A/g_s ratio. Under low N conditions. This suggests that mesophyll factors were affected to a greater extent than stomatal factors. In other words 'g_m' was reduced more than 'g_s'. It may be noted that the reductions in 'A' in the case of amaranthus and sorghum is to a lesser extent compared to reductions in 'A' of sunflower. This indicates that 'g_m' was affected greatly in sunflower compared to amaranthus and sorghum. However, sunflower failed to exhibit any significant reduction in 'g_s' compared to the other two species. Thus indirectly indicates that the feed back control of 'g_s' by 'g_m' was weak in the case of sunflower. This is also evident from the fact that the decrease in A/g_s and the resulting increase in Ci were very prominent in sunflower.

The ratio A/E which is a measure of the instantaneous WUE was reduced in all the 3 species with reduction in N level. The decrease in A/E ratio was very prominent in sunflower. Similarly the A/Ci ratios also showed significant reductions in all the three species and they were very marked in sunflower, indicating a greater reduction in the carboxylation efficiency in sunflower. It appears that the lack of a strong feed back control of 'g_m' and the appreciable reductions in A/Ci values are responsible for the remarkable reductions in A/E ratios in sunflower.

The relationship between 'A' and 'g_s' was a non-linera one in all the 3 species. This observation is in agreement with the results of Goudriaan and Ven Kevlen (1979). They failed to get any strong correlation between 'A' and 'g_s' in sunflower subjected to N stress.

Medina (1972) obtained curvilinear relationship between 'A' and 'g_s' for many C-3 species.

The relationship between 'A' and E was also a non-linear one in all the three species studied. This means that the rate of change in 'A' with respect to E ($\frac{dA}{dE}$) was not a constant. This contradicts the hypothesis of optimal stomatal behaviour proposed by Cowan and Farquhar (1977). The non-linear relationship between 'A' and E suggests that rather than optimising 'A' these plants subjected to N stress were trying to maximise 'A' per unit reduction in E.

Effects of low light intensities on-gas exchange characteristics

Light is known to have direct influence on photosynthesis since the energy for the process is derived directly from light through the photochemical reactions. Light also effects photosynthesis indirectly by modulating 'g_s'. Thus, light has a two fold effect on 'A'. In this context sunflower, amaranthus and sorghum plants were exposed to moderate or very low light intensities and gas exchange chracteristics studied. In the first set of experiments in this section, plants from high light intensities (2000 μE $m.^{-2}$ $s.^{-1}$) were shifted to a moderate light intensity of 400 to 600 $\mu Em.^{-2}s^{-1}$. In a second experiment they were shifted to a low light intensity of 100 $\mu E.m.^{-2}s^{-1}$. In a 3rd experiment effect of sequential transfer to moderate and low light intensities on gas exchnge paramters were studied. The fourth experiment was to study the effect of continuous mutual shading on gas exchange paramters in field grown sunflower and amaranthus plants.

All the 3 species tested exhibited a light dependent reduction in 'A', 'g_s', A/g_s. A/Ci and A/E (Tables 16-18). The reductions in 'A' was to a greater extent compared to reductions in 'g_s' indicating that 'g_m' was affected to a greater extent than 'g_s' under conditions of low light intensity. This means that light has a more regulatory role on 'g_m' compared to 'g_s'. It is generally known that the light saturation point for 'g_s' is lower than light saturation point for 'A'.

Though the values of 'g_s' and 'E' exhibited a statistically significant reduction with light intensity as well as duration of shading the values were always higher in sunflower compared to amaranthus and sorghum. The reductions in 'g_s', and 'E' were very remarkable in the case of amaranthus (Table 4), and sorghum (Table 5). It is also evident that the extent of reduction in A/'g_s' ratio was more in case of sunflower (Table 3). The reduction observed in the values of 'A' were comparable in all the 3 species. However, since 'E' values were fairly high and the extent of reduction in 'E' was less, A/E values showed more reductions in sunflower compared to other two species.

Transferring the plant back to high light after a time exposure to moderate or very low light intensity resulted in the recovery of all the gas exchange characteristics to almost the control levels. It is interesting to note that in amaranthus and sorghum A/E values were more than that observed before shading, the reason being that though 'A' recovered fairly well, 'E' did not recover to the same extent even though recovery of 'g_s' was almost complete. This implies that the microhabitat around the leaf has some key role in regulating 'E', particularly in amaranthus and sorghum. Such a regulatory mechanism which could be a function of boundary air layer resistance or the leaf morphology also has many important ecological significances, especially under conditions of flactuating light either due to clouds or due to sunflecks within the canopy.

Continuous shading due to mutual shading resulted in remarkable changes in the gas exchange characteristics of leaves similar to the changes observed in the short term shading experiments. But it was observed that the exposed leaves of the bottom canopy level was as good as the exposed middle leaves in the case of sunflower (Table 19) unlike in amaranthus (Table 20) or sorghum (Table 21).

Like in the case of N stress, low light stress also affected 'g_m' more than 'g_s' resulting in reductions in A/'g_s' values. In general sunflower exhibited a greater extent of reduction in A/'g_s' ratios. This means the reduction in 'g_s' vis-a-vis the reduction in 'g_m' was less in sunflower compared to amaranthus and sorghum. For instance 10 minutes after shading sunflower showed a 'g_s' of 0.325 μ mol.$m^{-2}s^{-1}$. At the same time the corresponding values were

0.110 and 0.077 μ mol.m^{-2}s^{-1} in amaranthus and sorghum respectively. This leads to the conclusion that the feed back control of 'g_s' by 'g_m' was weaker in sunflower compared to the other species. Again, it may be noted that this weak feedback control of 'g_s' by 'g_m' agrees with the fact that the extent of reduction in A/E ratios under low light conditions was also more in sunflower.

It may be noted that N stress and low light stress were imposed on well watered plants. Here rather than minimising the cost (dE) in transpiration by more reductions in 'g_s', the strategy of the plants would be to maximum the marginal gain (dA) in photosynthesis. In such a situtation where water is non-limiting there is more significance for higher absolute values of 'A' as against the instantaneous WUE (A/E). But if the light intensity is too low as the low light used in this study (100 μE) m^{-2}s^{-1}) it will be impossible to maintain sufficiently high values of 'A' just because light is limiting even if 'g_s' maintained high. However, in moderate light (about 500 μE.m^{-2}s^{-1}) sunflower could maintain slightly higher levels of 'A' compared to amaranthus and sorghum. It appears that in such a situation, the weak feedback control of 'g_s' by 'g_m' was of some advantage in sunflower. It may also be observed that only under moderate light sunflower maintained higher values of 'A' and A/E ratios compared to the other two species.

In both N stress and low light stress, there were significant reductions in A/'g_s' and concommitant signifcant increases in Ci in all the three species studied. It appears that Ci acted as a messenger to effect a feedback control on 'g_s by 'g_m'. However, the extent of decrease in 'g_s with increase in Ci differed markedly among the three species tested, sunflower maintained a "more resistant" 'g_s' to increase in Ci compared to the other two species. This means that 'g_s' in sunflower was more insensitive to Ci than in amaranthus and sorghum. This less sensitivity of sunflower 'g_s' to Ci can be explained only by the weak feedback control of 'g_s' by 'g_m' existing in this species. But the reasons for differential feedback controls in these two species under N stress and low light stress are not clear.

In this context it may be recalled that models based only upon stomatal response to Ci would not completely explain the stomatal responses to light of many species (Wong *et al*., 1978; Wong, 1979, Ramos, 1981). For some species, blue light has a special effect on guard cells and the action spectrum differs from that of 'A' (Raschke, 1979). The responses of 'g_s' to photon flux follows a rectangular hyperbole and sometimes different functions are more appropriate (Burrows and Milthorpe, 1976).

Relationship among gas exchange paramters

The functions fitted to explain the best relation between 'A' and 'g_s' during the course of a short term shading (low light) reveals that a non-linear relationship existed between them in sunflower and sorghum and a linear relation existed in amranthus. The best fit between 'A' and 'E' was simple linear regression model in all the three cases. This relationship is in agreement with the theory of optimal stomatal behaviour. It may be observed that dA/dE was highest in sunflower suggesting that per unit reduction in 'E', the associated reduction in 'A' was more in sunflower in comparison with the other two species. This points to the fact that the extent of reductions in A/E ratios would be more in sunflower under very low light intensities. It appears that these species developed such distinct characteristics in the course of evolution and adaptation to their natural environments.

Curvilinear relations among gas exchange parameters Their implications

It is very difficult to understand the physiological implications of many curvilinear relations obtained between various gas exchange parameters estimated under conditions of N stress and low light stress. Curvilinear relations are characteristic in biological systems. Curvilinearity in the relationship between two gas exchange parameters which is otherwise expected to be linear could be explained only on the basis of interactions from other variables which creep into the system without the knowledge and control of the experimenter. When gas exchange parameters under abiotic stress are considered, there is every likelihood that the abiotic stress in question would alter the microhabitat which would no~~t~~w modulate the orginal relationship. Th effect of such uncontrollable but inevitable variables on the physics and physiology of gas exchange process is difficult to be understood and quantified.

Culvilinear relations between 'A' and 'g_s' have been reported for two C_3 species under long term drought situations (Hall and Schulze, 1989). But linear association between 'A' and 'g_s' with short term changes in photon flux density has been reported for several species by Wong et al., 1979; Wong, 1979).

Effect of moisture stress on gas exchange characteristics of sunflower, amaranthus and sorghum leaves

The third kind of abiotic stress studied in this work was soil moisture deficit. The details of the results of experiments on soil moisture stress are discussed belwo.

Soil moisture deficit is probably the most important abiotic stress affecting crop productivity. Water stress reduces canopy photosynthesis by reducing leaf area. Low ψ levels inhibit cell divsion and cell expansion. Reduction in leaf area can be considered as an adaptation strategy since the total transpiring area is reduced. Under such conditions greater stability of 'A' assumes high significance.

Considerable work has been done on the effects of reduced leaf ψ on 'A'. Moisture stress reduces 'g_s' and 'g_m' and thus 'A' (Hsiao, 1973) Slayter, 1973; Mooney, et al., 1977; Krieg and Hutmacher, 1986; Jacob, 1988). But it is not very clear how exactly 'g_m' is reduced under moisture stress and to what extent it is reduced. Involvement of ABA in the closure of stomata during water stress is a well known phenomenon, though the exact mechanism is not very clear (Raschke, 1979). Certainly it acts as a messenger from mesophyll to guard cells in causing a feed back control on 'g_s' by 'g_m'. There are a number of subcomponents for 'g_m' and how they are affected during water stress is still an active area of research.

Since both 'A' and 'g_s' are affected during stress it is important to know their relative reductions. Attempts were made in this study to understand the effect of moisture stress on 'A' 'g_s' and associated characteristics when stress was developed in potted plants and field grown plants and by live wilting excised leaves. These different ways of water stress treatments were adopted to understand whether the photosynthetic machinery would show any kind of adjustments if the stress is developed slowly (field grown plants) as compared to a fast wilting in pot culture or cut leaf experiments. Moisture stress experiments were conducted again in sunflower, amaranthus and sorghum which have very distinct gas exchange characteristics.

As the intensity of water stress was increased there were significant reductions in 'A', 'g_s', A/Ci, Ci and 'E' in all the three species studied. Concommitantly signifcant increases were observed in A/'g_s' and A/E ratios. The increase in A/'g_s' ratios were more pronounced in sunflower. Though reduced significantly, 'g_s' and 'E' values were generally higher in sunflower at any given level of leaf ψ and hence the extent of increase in A/E ratios was generally lower in sunflower compared to amaranthus and sorghum. These results are by and large in agreement with the various published results.

Johnson et al., (1974) reported an increase in A/E ratio wtih reduction in leaf ψ in a few arid and alpine tundra species. Blum and Sullivan (1986) found an increase in A/E ratios in sorghum and pearl millet under mositure stress. This was because of more reduction in 'E' than 'A'. This is possible only if 'g_s' in reduced more than 'g_m'; which will result in increased A/'g_s' ratios. The results of the present investigations also show that both 'A'/'g_s' and A/E ratios increase when plants were subjected to slow stress and quick stress. Even in the cut leaf experiment there was an initial increase in A/'g_s' and A/E ratios in amaranthus. In sunflower, A/E ratios were initially maintained constant and later decreased in the cut leaf experiment. An increase in A/'g_s' ratio with a concommitant increase in A/E ratio has been reported by Sashidhar 1987, Udaya Kumar et al., 1988 and Jacob and Udaya Kumar, 1988).

Significance of variations in A/'g_s' ratio and Ci under stress

Under optimum conditions Ci appears to be a reflection of the mesophyll conditions. A high A/'g_s' ratio results in low Ci values. If the 'A' values for a given 'g_s' is high it suggests that the mesophyll is more efficient in reducing CO_2. In the present investigation the Ci values were lower in amaranthus and sorghum compared to sunflower suggesting better mesophyll conditions in the former two species. But under any stress situation like in the case of a moisture stress if 'g_s' is reduced more than 'A', a reduction in Ci results. This Ci value is not a direct reflection of mesophyll factors. It only suggest that reduction in 'g_m' or the mesophyll factors were affected to a greater extent than the stomatal factors. Similarly if 'A' is reduced faster than 'g_s' as in the case of N stress and low light stress Ci levels increase. This means that mesophyll factors are affected to a greater extent than the stomatal factors. Thus absolute values of Ci under a given stress condition is not a direct reflection of mesophyll factors. But it has to be compared with the absolute Ci values of control treatment (optimal conditions) to understand the extent to which 'g_m' is reduced. Hence, A.Ci correlations across a stress treatment is of little relevance.

Similarly A/'g_s' at any given time also has to be compared with the control values. Because under conditions of a stress, A/'g_s' ratio can increase if 'g_s is reduced faster than 'A'. This only means that 'g_m' is affected to a lesser extent than 'g_s', when comparing two species or two situations, if the extent of increase in A/g_s is more in one case, it can be concluded that 'g_m' was better maintained in that case. Hence more than the absolute values of A/g_s, the extent of increase has to be considered.

Ecological significance of increase in A/g_s ratio during moisture stress

Reductions in absolute values of 'A' during stress is a result of reductions in absolute values of 'g_s' and 'g_m'. However, if 'g_s' is reduced more than 'g_m' it is if advantage to the plant in terms of water husbandary. The responses for the above mentioned behaviour can be understood by considering the gas exchange as a pure physical process of diffusion of CO_2 ~~into~~ and vapour in the opposite direction both sharing a common pathway. It can be summarised as follows.

$$A = f\ (g_s,\ g_m)$$

$$E = f\ (g_s)$$

Hence, if 'g_s' is reduced more than 'g_m' 'E' is reduced more than 'A' resulting in high A/E ratios.

The correlations between 'A' and 'g_s' as well as 'E' and 'g_s' were high and positive in the different water stress experiments conducted in this study. Similarly 'A' and 'E' also showed a significant positive correlation. These strong positive correlations do not mean that a linear regression between 'A' and 'g_s' or between 'A' and 'E' was a best fit. In all the moisture stress experiments the function describing the relationship between 'A' and 'g_s' was a non-linera one so also was the relationship between 'A' and 'E' except in sunflower in the cut leaf experiment.

The way in which 'A' and 'g_s' are related has great ecological significance (Schulze and Hall, 1982). A linear relation between 'A' and 'g_s' has been reported for a large number of species varying environmental conditions (Wong, 1979; Wong <u>et</u> <u>al</u>., 1979). The slope of the AVs 'g_s' regression lines vary significantly between C_3 and C_4 species (Wong, 1979). It is interesting to note that a linear association between 'A' and 'g_s' (Farquhar 1978) is consistant with concept of optimal stomatal functioning (Cowan and Farquhar, 1977). In other words constant rate of change in 'A' with respect to 'g_s' will reflect in constant rate of change in 'A' with respect to E. That is to say, that,

$$\text{if} \qquad \frac{dA}{dg_s} = K, \qquad \frac{dE}{dg_s} = K^1$$

$$\text{then} \qquad \frac{dA}{dE} = K^*$$

Where K^1 and K^* are constants.

However in the present investigations on moisture stress affected on gas exchange parameters of sunflower, amaranthus and sorghum plants neither dA/dg_s nor dA/dE remained constant. On the countrary, both the rates showed an increasing trend. This suggests that under conditions of a moisture stress stomata behave in a such a way as to maximise the marginal gain dA for every unit of marginal loss dE rather than to optimise dA with respect to dE.

It may also be noted that 'E' and 'g_s'exhibited a linear relationship with constant slope (dE/dg_s) in both sunflower and amaranthus used in the cut leaf experiment (Table 44). However, dA/dg_s was never a constant in any of the species. Hence,

$$\frac{dA/dg_s}{dE/dg_s} = \frac{dA}{dE} \text{ was also not a constant.}$$

It may again be mentioned that $\frac{dA}{dE}$ was a function of 'E' in such a way that for any additional loss of water, the additional reduction in 'A' progressively reduced. This is the basis of the argument that rather than optimisation of 'A', the stomatal behaviour was suggestive of maximisation of A/E by minimising the reduction in 'A' for every unit of 'E'. Similar were the trends in the case of N stress. However, the picture was entirely different in the case of the ~~species~~ light studied. It is probably because light for any of the species studied has independent and direct roles on photosynthesis as well as stomata.

The multiple linear regression models developed for 'A' and A/E gives the strength of the contribution of individual variables when changed by one unit keeping variations in the other variables constant. The co-efficients attached to each variable in an MLR model is thus a partial derivative. In general the co-efficient associated with 'g_s' was higher in the MLR models for 'A' in all the three species studied. This does not necessarily mean that reductions in 'g_s' imparted more limitations on 'A' compared to 'g_m' since the latter is not accounted in the MLR model. The MLR model for A/E reveals that more than 'A' it was 'E' which contributed more towards the observed A/E values at any given time. This indirectly means that increase in A/E ratios observed during water stress was due to the more reduction in 'E' compared to 'A'. This leads to the conclusion that 'E' is more sensitive to moisture stress than 'A'. This is because of greater reductions in 'g_s' compared to 'g_m'

It is of importance to quantify the stomatal and mesophyll limitations of photosynthesis. Hence further studies were conducted to quantify the limitations of 'A' by 'g_s' and 'g_m' under different levels of moisture stress on potted and field grown plants of sunflower and amaranthus. Discussion of these studies follow.

Calculation of relative stomatal and mesophyll limitations of photosynthesis from A.Ci curves

Reduction in 'g_s' has been believed to exert a large amount of limitation on 'A' under conditions of a water stress. This important misconception was based on the paired observations of 'A' and 'g_s' and the extent of a very strong positive correlation existing between them. Misinterpertation of the linear resistance analysis also lead to similar conclusions.

Farquhar and Sharkey (1982) proposed a simple method to estimate relative stomatal limitations of 'A'. They observed that the 'g_s' induced limitations of 'A' did not increase with stress though there was reduction in the absolute values of 'g_s'. Hence, they concluded that the mesophyll limitations of 'A' were more under stress. Kreig and Hutmacher (1986) also adopted the same methodology and arrived at similar conclusions. But mesophyll limitations were not quantified.

A slightly modified method was adopted in this study to estimate relative mesophyll limitations also apart from stomatal limitations. The basic approach remained the same as that of the above authors. The first step was to make A.Ci curves for sunflower and amaranthus plants experiencing different levels of moisture stress in pot and in field.

The various limitations calculated from these curves are (a) relative stomatal limitation of the observed photosynthesis (ls), (b) relative mesophyll limitations of the observed photosynthesis (lm) and (c) mesophyll limitations of potential photosynthesis (ML).

It may be observed that at any given level of leaf ψ, both 'g_s' and 'g_m' are imparting certain amount of limitations on 'A' (Tables 41 and 46). It agrees with the theoretical conclusion that 'A' is a function of both 'g_s' and 'g_m' at any given time.

$$A = f\ (g_s,\ g_m)$$

This means that both 'g_s' and 'g_m' could be imparting certain limitation on 'A'. It is interesting to note that the relative stomatal limitation (ls) was more than relative mesophyll limitations (lm) under well watered conditions in both the species. This suggests that the major constraint for photosynthesis lies in the stomatal factors under control conditions. In other words stomata was not permitting sufficient amount of CO_2 into the mesophyll to meet the demand of the intrinsic carboxylation. It is difficult to explain the evolutionary implication of this fact. Because when water is not limiting it is not necessary for the plant to regulate 'g_s' in such a way that it is imparting a limitation of 'A'. However, as the intensity of water stress increased the mesophyll limitations were more than the stomatal limitations.

The extent of reduction in 'g_s' was calculated from the absolute value at different stress levels. However, absolute values of 'g_m' were not available. An indirect approach was followed to calculate the per cent reduction in 'g_m under mositure stress conditions. (Tables 41 and 47).

Most of the mesophyll resistance is believed to be at the carboxylation site. More the carboxylation efficiency more the 'g_m'. The slope of the A.Ci curve (dA/dCi) is considered as the carboxylation efficiency. This slope was found to reduce due to water stress indicating a reduction in 'g_m'. The extent of reduction in dA/dCi can be argued to be equal to the extent of reduction in 'g_m' believing that these two parameters are linearly related. Thus per cent reductions in 'g_m' were calculated though absolute values were not estimated. The results reveal that as stress advanced there was reduction in 'g_m'. But the extent of reduction in 'g_m' was lesser than 'g_s' (Tables 42 and 45). This is the reason why A/g_s ratios were high as stress progressed. Because A/g_s will increase only if 'g_s' is reduced more than 'g_m'.

To quote an instance, the per cent reduction in 'g_s' was 77 and 84 per cent respectively in sunflower and amaranthus when the plants were under severe stress (Table 42). The corresponding reductions in 'g_m' were 58 and 75 per cent in sunflower and amaranthus respectively. However, it is interesting to note that the per cent increase in mesophyll limitation was much more than stomatal limitation. For instance when stomatal limitation increased by 61 per cent, mesophyll limitation increased by 650 per cent in sunflower under severe stress. Similarly in amaranthus the corresponding increase in stomatal limitation was only 20 per cent and mesophyll limitation was 536 per cent. The significance of such a paradox in the extent of reductions in 'g_s' and 'g_m' and the extent of increase in their relative limitation on 'A' is discussed at a later stage.

The contribution of mesophyll limitation to the total limitation was less under well watered conditions (Table 43 and 48). But this increased as water stress progressed. For instance the per cent contribution of mesophyll limitation to the total limitation was 24 per cent in control in the case of sunflower. This increased to 60 per cent under severe stress (Table 43). Similarly in amaranthus also it increased from 35 per cent (control) to 74 per cent (severe stress). This suggests that as the water stress progressed mesophyll limitations were more prominant than stomatal limitations though the extent of reduction in 'g_m' was lesser than 'g_s'.

These results reveal that though 'g_s' were reduced more than 'g_m' it was the mesophyll limitations of 'A' that was more prominent than the stomatal limitation. It suggests that the reduction in 'g_s' did not come in the way of CO_2 diffusion as much as the reduction in'g_m' limited CO_2 diffusion into the chloroplast stroma. It seems, then, that the 'g_s' was under the feedback control of 'g_m' because 'g_s'

was tuned so perfectly that its limitations on 'A' was insignificant, but at the same time drastically reducing 'E'. This has a lot of ecological significances. This fine control system operating under conditions of moisture stress would result in enhancing A/E ratios though absolute values of 'A' may reduce. The ABA which is synthesised in response of moisture stress certainly acts as a messenger between mesophyll and guard cell and hence the extent of feedback control of 'g_s' by 'g_m' would be noted. Sunflower exhibited only very weak feedback controls in the experiments with low light stress where Ci appeared to be the best candidate for the feedback control. In moisture stress conditions, it appears that the strength of the feedback regulation of 'g_s' was enhanced even in sunflower. ABA could be, probably, a more potent compound than CO_2 in causing stomatal closure. It is also shown that ABA makes the stomata more sensitive to CO_2 (Dubbe et al., 1978).

Experiments with ABA

ABA is known to cause stomatal closure (Cummins et al., 1971). It is known to be synthesised during moisture stress (Hsiao, 1973). It has been shown that ABA can affect mesophyll factors also. But it might have a more pronounced effect on 'g_s'. In this context a set of experiments were conducted using excised leaves of sunflower and amaranthus.

Different concentrations of ABA were fed to excised leaves through the transpiration stream and gas exchange rates measured. In another experiment EGTA and ruthenium red were also fed along with ABA. Though it is not very clearly understood, there are indirect evidences to suggeststhat ABA causes stomatal closure by increasing the permeability of the guard cell plasma membrane to calcium, calcium might then act as second messenger to regulate the tonic fluxes that determine the guard cell turgor (De Silva _et al_., 1985 and Schwartz, 1985). Similar results were demonstrated in our laboratory also (Anonymous, unpublished 1988). EGTA which is a calcium sequesting agent and RR which is a calcium channel blocker were used in the light of the existing knowledge that they could prevent Ca^{2+} mediated processes.

A concentration dependent reduction 'A', 'g_s' Ci, A/Ci and E were observed when sunflower and amaranthus leaves were fed with different concentrations of ABA (Table 36). There was a significant increase in A/g_s with increase in the ABA concentration. This suggests that the reduction in 'g_s' was more than 'g_m'. Hence, the effect of ABA was predominantly on 'g_s'. ABA induced reductions in 'g_s' and 'A' were recovered markedly when EGTA or RR was also supplied along with ABA (Tables 38 and 39). This suggests that the ABA action was mediated through Ca^{2+}.

Under conditions of moisture stress, the ABA synthesised could affect 'g_s' and 'g_m'. But it appears that the effect is predominantly on 'g_s'. ABA can, in addition sensitize guard cells to CO_2. Hence, one can expect a very strong feedback control of 'g_m' under moisture stress conditions. It may also be noted that the extent of feed back control was less in the case of N stress and low light stress. In the case of moisture stress also, sunflower appears to have a weaker feed back control on 'g_s' compared to amaranthus.

Even at lower levels of leaf ψ, amaranthus maintained higher values of 'A' irrespective of very low 'g_s' compared to sunflower (Table 3). The fact that 'A' started showing negative values when 'g_s' was fairly high in sunflower suggests that the feed back control of 'g_s' by 'g_m' was very weak. It may be noted in this context only in sunflower compared to the other two species in the pot culture and field experiments. It appears that a strong feedback control of 'g_s' by 'g_m' is necessary to keep the A/E ratios high even when the absolute values of 'A' are reduced as observed in the case of amaranthus and sorghum in the different water stress experiments.

Stomatal and assimilation loop gains

If there is a strong feedback control of 'g_s' by 'g_m' one would expect an increase in the loop gains, particularly the stomatal loop gain (Gg). Loop gain analysis helps to under stand the differential behaviour of 'A' and 'g_s' in a given situation. In this study 'g_s' and assimilation loop gain (GA) were estimated for sunflower and amaranthus plants raised in pots and field and experiencing different intensities of moisture stress.

Physiological gain involving assimilation (dA/dCi) showed drastic reductions as stress intensity was more (Tables 50 and 51). This suggests a reduction in carboxylation efficiency. The physiological gain of conductance loop (dg_s/dCi) also increased as water stress intensity was more. This suggests that 'g_s' became more sensitive to Ci as stress intensity was increased. Stomata are known to become more sensitive to Ci during moisture stress and in presence of ABA (Dubbe et al., 1978).

The physical gains involving assimilation ($\partial Ci/\partial A$) was found to increase with stress. The increase was more pronounced in amaranthus similar was the trend in the physical gain of conductance loop ($\partial Ci/\partial g_s$). These leads to increase in the absolute values of 'Gg' and 'GA' under stress. The results are in agreement with the values of various gains obtained by Farquhar _et al_ (1978) and Dubbe _et al_., (1978).

An increase in 'Gg' under stress is an advantage in terms of better water husbandary. More increase in 'Gg' leads to the conclusion that the extent of feedback control of 'g_s' by 'g_m' is also more. Since the extent of increase in 'Gg' is more in amaranthus it can be concluded that the feed back control was also stronger in this species. This may be the reason for the more enhanced increases in A/E ratios in amaranthus compared to sunflower under moisture stress conditions.

An increase in 'GA' is not of advantage as it is going to reduce the WUE. But this is an inevitable consequence under water stress because of the increase in the physical gain involving assimilation. But it may be noted that the extent of increase in 'GA' was much lesser than 'Gg' in both the species.

The ratio 1/(1-Gg-GA) is a measure of the stomatal sensitivity to CO_2. If this (Gg=GA=0) this ratio becomes 1, this means Ci will increase to the same as that of Ca due to the lack of any feedback loops operating. In thrue situations this ratio is always lesser than 1. This ratio agrees closely with the slope of the linear curve fitted between Ci and Ca (Farquhar et al., 1978).

In the present investigations this ratio was found to be lesser in Amaranthus compared to sunflower at any given leaf ψ. It may be recalled that the slope dCi/dCa is lower in most of the C_4 species (about 0.45) compared to many C_3 species (about 0.7). The values obtained in this study were slightly higher than these but the trends were maintained. The ratio 1/(1-Gg-GA) was found to decrease as the intensity of stress was increased. This means the Ci became more "resistant" to change with respect to changes in Ca. In other words 'g_s became more sensitive to CO_2 under moisture stress conditions. A similar observation has been reported in the literature.

Experiments conducted in this investigation pertain to studies on the effect of abiotic stress like low N low light and moisture stress on gas exchange rates and associated characteristics in sunflower, amaranthus and sorghum. These abiotic stresses caused remarkable reductions was different for different species and under different types of stress. For instance, under conditions of low N or low light intensity 'A' was reduced to a greater extent than 'g_s' leading to reductions in A/g_s ratios. This suggests that these stress situations caused more reductions in 'g_m' compared to 'g_s'. However, under conditioins of moisture stress A/g_s ratio, in general showed an increasing trend suggesting that 'g_s' is more sensitive to low water potentials compared to 'g_m'. The increase in A/g_s ratio was more pronounced in sunflower compared to amaranthus and sorghum. But the absolute values of 'g_s' and 'E' were higher in sunflower at any given leaf ψ.

It appears that under optimum conditions A/g_s and hence Ci are reflections of the mesophyll capacity for carboxylation. Under conditions of an abiotic stress the absolute values of A/g_s and Ci may vary depending upon whether 'g_s' or 'g_m' is affected to a greater extent. Hence, rather than the absolute values, the extent of variations in A/g_s or Ci reveals better the relative stability of mesophyll factors.

An increase in A/g_s ratio under moisture stress conditions helps in better water utilization in relation to carbon fixation. It appears from the relationship between 'A' and 'E' under moisture stress that stomatal mechanism is so evolved in such a way that maximisation photosynthesis for every unit of water transpired is the plant strategy. It is evident from the results of the relative stomatal and mesophyll limitations of 'A', that 'g_s' was never a major constraint under moisture stress conditions. However, under well watered conditions the stomatal factors were more limiting than the mesophyll factors.

Since mesophyll limitations were very hight under drought stress an attempt was made to understand the extent of damage to the photochemical mechinary due to simulated moisture stress by studying the extent of quenching of chlorophyll fluorescence. More the quenching, better the photochemical activity. It was found that as the leaf ψ declined there was lesser and lesser quenching of chlorophyll fluorescence. It appears that photo-chemical reactions were more sensitive to moisture stress in sunflower compared to amaranthus and sorghum. However, the stability of 'g_m' was better in sunflower as evident from the extent of increase in A/g_s ratio. It is possible that factors other than photosynthetic electron transfer might have been limiting in the case of amaranthus and sorghum

Though 'g_m' was reduced relatively to a lesser extent than 'g_s', the 'g_m' limitations of 'A' was higher. This suggests that 'g_s' was under the feed back control of 'g_m'. The reductions in 'g_s' while inhibited 'E', did not come in the way of supplying enough CO_2 to meet the mesophyll requirements. Thus it appears that the role of stomata is more on regulating 'E' than 'A'.

The high degree of feed back control of 'g_s' by 'g_m' under moisture stress conditions resulted in higher stomatal loop gains in sunflower and amaranthus.

The various experiments conducted in this investigation reveal that mesophyll limitations to photosynthesis is very strong under drought stress. There exists a very strong feed back coupling between 'g_s' and 'g_m' in such a way that any reduction in 'g_s' during drought stress resulted in preventing trnaspiration to a greater extent than inhibiting photosynthesis.

SUMMARY

VI SUMMARY

The present investigation was carried out with the basic objective of understanding how gas exchange parameters were affected when plants were subjected to different types of abiotic stresses. The inter relationship between the various gas exchange parameters was also analysed in this study. The ultimate purpose of these experimentations was to quantify the relative changes in g_s and g_m and their impact on 'A'. It has been the interest of many research workers in the field of crop improvement to quantify the relative limitations imparted on 'A' by 'g_s' and'g_m' during stress. Finally determination of the stomatal and assimilation loop gains was also done to understand the adaptation strategies of plants when subjected to moisture stress. To meet these objectives a series of experiments was conducted with sunflower, amaranthus or sorghum which differ in their photosynthetic characteristics.

A series of pot culture and field experiments were conducted in this investigation to study the effect of low N, low light and drought stresses on gas exchange and associated characteristics in sunflower, sorghum and amranthus. In all the experiments in situ measurements of A, 'g_s' and 'E' were made by using a portable photsynthesis system (LI6000). Then Ci, A/Ci and A/E were calaculated.

In the first experiment sunflower, amaranthus and sorghum plants were raised in pots under different levels of

fertiliser N. Low N treatment resulted in altering the mesophyll factors predominently. This resulted in greater reductions in 'A' compared to 'g_s' leading to reduced A/'g_s' ratio. Mesophyll factors were found to be affected more in the case of sunflower compared to amaranthus and sorghum, since the extent of decrease in A/g_s ratios was more in sunflower. However, there were no marked reductions in 'g_s', in sunflower irrespective of the fact that 'g_m' was affected. This suggests that there did not exist any feedback control of 'g_s' by 'g_m' particularly in sunflower. All the three species showed a reduction in A/E ratios, with sunflower exhibiting more reduction. So also was A/Ci values which showed a very marked reduction in sunflower.

In the second set of experiments sunflower, sorghum and amaranthus plants were exposed to different low light intensities. Low light was also found to have a more profound effect on 'g_m' than on 'g_s'. As in the case of low N stress, low light stress also resulted in reducing 'A', 'g_s', 'E', A/g_s and A/Ci values in sunflower, amaranthus and sorghum. Amaranthus exhibited very marked reductions in g_s and E compared to sunflower. The extent of reductions in A/g_s and A/E were more in sunflower. However, absolute values of 'E" and 'g_s' were always more in this species. This also suggests that the feed back control of 'g_s' by 'g_m was weaker in sunflower.

In both low N stress and low light stress, Ci was found to increase. However, the extent of decrease in 'g_s' per unit increase in Ci varied drastically, sunflower exhibited a more "resistant" stomata to CO_2.

The analysis of the relationship existing between various gas exchange parameters revelaed interesting facts. There existed a non-linear relationship between 'A' and 'g_s' in all the three species tested under low N and low light stress. But 'A' and 'E' is in support of the concept of optimal stomatal behaviour.

The results of the experiments with low N and low light stress could be summerised as follows:

- Both low N and low light stress resulted in decrease in 'A' 'g_s' and associated characteristics.
- Reductions in 'A' were more than 'g_s' leading to low A/g_s ratios under low N and low light stress.
- This suggests that these abiotic stress affected 'g_m' more than 'g_s'.
- The extent of reduction in A/g_s was more in sunflower suggesting that 'g_m was affected more in this species compared to amaratnhus and sorghum.
- Decrease in A/g_s resulted in increase in Ci.
- The extent of reduction in g_s in comaprison with the extent of increase in Ci was less in sunflower

compared to the other two species.

- This lead to the conclusion that the extent of feed back control of 'g_s' by 'g_m was weaker in sunflower.

- Low N and low light stresses resulted in reducing A/Ci and A/E ratios.

- The weak feed back control of 'g_s' by 'g_m' resulted in lesser reductions in 'E'. Hence, the above behaviour observed in sunflower.

- The relationship between 'A' and 'E' was linear one in the case of low light stress supporting the concept of optimal stomatal behaviour. But in low N stress the relationship was non-linear.

A third set of experiments was conducted to study the effect of mositure stress on the gas exchange parameters of the above mentioned crops Pot grown and filed grown sunflower, sorghum and amaranthus plants were subjected to low leaf ψ. Moisture stress reduced A, 'g_s', Ci, A/Ci and E. Unlike in the case of low N or low light stress 'A' was reduced to a lesser extent than 'g_s resulting in an increase in the A/g_s ratios as the stress advanced. This suggests that 'g_s' was more sensitive than 'g_m to low leaf ψ. An increase in A/g_s ratios resulted in reducing Ci. The "carboxylation efficiency" (A/Ci) was found to decrease as the stress progressed suggesting a decrease in 'g_m also.

Moisture stress caused marked reductions in 'E'. The reductions in 'E' were more than 'A' and hence A/E ratio increased with stress intensity. The increase in A/g_s was more pronounced in sunflower compared to amaranthus and sorghum. Though reduced significantly, absolute values of 'g_s' and 'E' were always higher in sunflower at any given leaf ψ leading to low A/g_s and A/E ratios in this species.

The way in which 'A' and 'g_s are related has great ecological significances. An increase in A/g_s ratio under moisture stress conditions leads to better water utilisation. The extent of increase in A/g_s ratio appears to be a reflection of the stability of mesophyll factors. An increase in A/g_s and A/E ratios with concommitant reductions in 'A', 'E' and 'g_s' under moisture stress conditions suggests that stomata behave in such a way as to maximise rather than optimise photosynthesis for every unit of transpiration. This also suggests that 'E' is more senitive to low leaf ψ than A. This is because of greater reduction in 'g_s' compared to 'g_m'.

The salient features of the stuides on moisture stress effect on gas exchange parameters can be summarised as follows:

- Moisture stress decreased 'A', 'g_s' and associated parameters.
- Reductions in 'A' were less than 'g_s' leading to high A/g_s ratios under low leaf ψ levels.

- This suggests low leaf ψ affected 'g_s' more than 'g_m'. The extent of increase in A/g_s ratio, in general was more in sunflower comapred to amaranthus or sorghum
- Increase in A/g_s resulted in decrease in Ci.
- The absolute values of A/g_s ratio were always more in amaranthus and sorghum compared to sunflower.
- The absolute values of A/g_s ratio under optimum conditions is a reflection of the mesophyll conditions.
- The extent of increase in A/g_s ratio appears to be a measure of the extent of stability of mesophyll factors.
- A/Ci ratios decreased as moisture stress advanced indicating a reduction in 'g_m'.
- There was more reduction 'E' as compared to 'A' under mositure stress conditions. This means 'E' is more sensitive than 'A' to low leaf ψ.
- This behaviour resulted in increased A/E ratios under low moisture situations.
- The relationship between 'A' and 'g_s was generally non-linear but positive. This was because of high A/g_s ratios obtained under stress.

- Similarly the relationship between 'A' and E was also non linear and positive suggesting stomata behave in such a way as to maximise photosynthesis for every unit of transpiration.

When these plants were subjected to moisture stress there was concomitant reduction in 'A' and 'g_s'. But the extent of limitations offered by 'g_s' and 'g_m' for photosynthesis of more relevance. Hence further studies were conducted to quantify the relative stomatal and mesophyll limitations of photosynthesis in potted and field grown sunflower and amaranthus plants experiencing different intensities of moisture stress. In situ measurements of 'A' and 'g_s' were measured at different levels of Ca from sunflower and amaranthus leaves experiencing different intensites of moisture stress. The Ci was calculated and 'A'.Ci curves plotted. The method of Farquhar and Sharkey (1982) was used to estimate stomatal limitations. Mesophyll limitation was estimated by modifying their model. It was found that at any given level of leaf ψ, both 'g_s' and 'g_m' impart a certain amount of limitation on 'A'. Under well watered conditions stomatal limitations were slightly more than mesophyll limitations. Compared to amaranthus, the stomatal limitations were generally higher in sunflower at any given leaf ψ.

A decrease in leaf ψ resulted in appreciable decreases in 'g_s' and 'g_m'. But the extent of reductions in 'g_s' was more than 'g_m'. However, mesophyll limitations of 'A' increased many fold as moisture stress advanced leaving the stomatal limitations relatively significant. In other words, under low leaf ψ conditions mesophyll factors were the major constraints for 'A' and hence a decrease in 'A' under such conditions could be attributed more to mesophyll factors.

The results also suggest that reductions in 'g_s' did not cause any appreciable limitations on 'A' as compared to the mesophyll limitations. It appears that 'g_s was under the feed back control of 'g_m'. This control system is so adjusted that any reduction in 'g_s' will not come in the way of CO_2 diffusion to meet the requirement of the mesophyll. Thus, the role of the stomata appears to be more on regulating transpiration rather than photosynthesis.

The above results can be summarised as follows.

i) At any given leaf ψ both 'g_s' and 'g_m' impart a limitation on 'A'.

ii) Under well watered conditions 'g_s' limitation was more than 'g_m' limitation.

iii) Mesophyll limitations contributed to most of the total limitations under low leaf ψ levels.

iv) The decrease in 'A' under moisture stress was more due to mesophyll limitations suggesting that the major constraints for 'A' under moisture stress conditions lie in the mesophyll and not in the stomatal factors.

v) In general stomatal limitations were more in sunflower and mesophyll limitations more in amaranthus.

vi) The extent of reduction in 'g_s' was more than 'g_m' under low leaf ψ level irrespective of the fact that 'g_m' imparted more limitation on 'A'.

vii) This suggests that 'g_m exerted a feed back control on 'g_s

viii) The role of stomata appears to be more on regulating water loss than photosynthesis.

Since mesophyll was found to be limiting photosynthesis more than stomata an attempt was made to study the effect of low ψ on photochemical reactions by monitoring the chlorophyll fluorescence quenching. Low leaf ψ resulted in less per cent quenching of fluorescence. It was found that photochemical reactions were more sensitive to low ψ in sunflower compared to amaranthus or sorghum.

There appears to have a strong feed back control of 'g_m' by 'g_s' particularly during moisture stress. Drought is known to cause synthesis of ABA which is the potent messenger for this feed back control. Experiments were also conducted by using ABA to simulate moisture stress in excised leaves of sunflower and amaranthus by feeding it through the transpiration stream. ABA caused remarkable reductions in 'g_s' in both the species. It is believed that ABA induced stomatal closure is Ca^{2+} mediated. When EGTA (a calcium sequesting agent) or ruthenium red (calcium channel blocker) were used along with ABA, there was appreciable recovery in 'g_s'.

Estimations of stomatal and assimilation loop gains were also done to understand the extent of feed back control of 'g_s' by 'g_m' and the adaptation of sunflower and amaranthus plants to moisture stress. There was substantial increase in the stomatal loop gain (Gg) in both the species suggesting a strong feed back control of 'g_s' by 'g_m'. An increase in 'Gg' helps in better water husbandry. Similarly there was an increase in the assimilation loop gain (GA) also. This is not of any advantage. It may be noted that the extent of increase in GA was much lesser than 'Gg'.

The ratio 1/(1-Gg-GA) can be considered as a measure of stomatal sensitivity to CO_2. Higher the value, lesser ~~later~~ the sensitivity. This ratio was always more in sunflower compared to amaranthus suggesting that stomata were more intensitive to CO_2 in the former. As water stress progressed this ratio was found to decrease indicating that stomatal sensitivity to CO_2 increase under water stress conditions.

The conclusions of the above results are as follows.

i) Moisture stress resulted in increasing 'Gg' and GA in both sunflower and amaranthus.

ii) Increase in 'Gg' was more than the increase in GA.

iii) The absolute values of 'Gg' as well as the extent of increase in 'Gg' were higher in amaranthus compared to sunflower.

iv) The ratio 1/(1-Gg-GA) was lower in amaranthus compared to sunflower indicating more sensitivity of stomata to CO_2 in the former.

v) Stomatal sensitivity to CO_2 was found to be more under moisture stress conditions.

Under conditions of a drought stress the major constraint for stabilizing photosynthesis appears to be the mesophyll factors. The reduction in stomatal conductance helps in preventing transpiration more than limiting photosynthesis. The results also prove that stomata are under the strong feed back control of mesophyll particularly during drought stress.

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