PHOTOSYNTHETIC RATES IN A FEW C_3 AND C_4 SPECIES UNDER NON-STRESS AND ABIOTIC STRESS CONDITIONS – ASSESSMENT OF STOMATAL AND MESOPHYLL LIMITATIONS

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DEDICATION

The career of students depends on teachers.
I whole heartedly dedicate my *thesis* to *all* teachers.
Especially to *Smt. Premalatha*
and my mother *late Vasantha*

DEPARTMENT OF CROP PHYSIOLOGY
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BANGALORE

CERTIFICATE

This is to certify that the thesis entitled **Photosynthetic rates in a few C_3 and C_4 species under stress and non stress conditions - Assessment of stomatal and mesophyll limitations** submitted by **M.S.SHESHSHAYEE** in partial fulfilment of the requireents for the degree of **DOCTOR OF PHYLOSOPHY** in **CROP PHYSIOLOGY** of University of Agricultural Sciences, Bangalore is a record of research work done by him during the period of his study in this university under my guidance and supervision and the thesis has not previously formed the basis of the award of any degree, diploma, associateship, fellowship or other similar titles.

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Abbreviations used

A = Assimilation rate (μ moles.$m^{-2}.s^{-1}$)
APS = Apparent photosynthesis
ASU = Air Supply Unit
ATP = Adenosine TriPhosphate
CER = Carbon Exchange Rate
Ci = Intercellular CO_2 concentration (ppm)
g_s = Stomatal conductance (mmole.$m^{-2}.s^{-1}$)
g_m = Mesophyll conductance.
Γ^* = CO_2 compensation point.
IRGA = Infra Red Gas Analyzer
K_{cat} = Specific activity of RuBisCO.
K_m = Affinity constant of RuBisCO for CO_2
l_m = Relative mesophyll limitation of photosynthesis
l_s = Relative stomatal limitation of photosynthesis
LCA = Leaf Chamber Analyzer
NADPH= Nicotinamide Adenine Dinucleotide Phosphate (reduced)
Pi = Inorganic phosphorus
PR = Photorespiration
RuBP = Ribulose, 1-5, BisPhosphate
RuBisCO = Ribulose, 1-5, BisPhosphate Carboxylase/Oxygenase
SPS = Sucrose Phosphate Synthase
VPD = Vapour Pressure Deficit
V_{max} = Maximum velocity of an enzyme catalyzed reaction

INTRODUCTION

INTRODUCTION

There has been a continuous effort for crop improvement over several decades. Significant increase in grain yield has been attributed mainly to optimization of agronomic inputs (Gifford, 1987), maximizing light interception by optimising canopy architecture (Nelson, 1988) and by enhancing partitioning coefficients (Austin et al., 1980). Further improvements in either dry matter production or grain yield is possible only through an increase in single leaf photosynthetic rates as a plateau has been reached in other physiological parameters of canopy photosynthesis.

Although considerable genotypic variation in assimilation rates has been reported in various crop plants (Fischer et al., 1981; Mohan and Hobbs, 1981; Hobbs and Mohan, 1985; Austin et al., 1986; Shankar et al., 1990 and Najareddy, 1992), progress in exploiting this variation for improving photosynthesis to achieve higher yield has been slow. Because of the multigenic control of photosynthesis, breeding for higher rates either through conventional breeding techniques or by recent genetic engineering have not been encouraging.

However, at present much of the research effort is being diverted to evaluate and understand various limiting factors of photosynthesis. If a few important limiting factors of photosynthesis are identified, incorporation of those traits can be achieved with a higher degree of success to enhance bio productivity.

Though essentially a biochemical phenomenon, photosynthesis can be considered as a diffusive process also. Assimilation rate is controlled by the diffusive characteristics of stomata and the ability of the mesophyll factors to fix carbon. Understanding the extent of limitations imposed by stomatal or mesophyll factors would lead us a step ahead in improving photosynthesis.

Apart from these intrinsic factors, photosynthesis is modulated to a large extent by the existing environmental factors. Since crop plants experience a number of environmental stresses during their growth periods, assessing the impact of various environmental variables on assimilation rates is also essential for crop improvement via photosynthesis.

With these informations, an investigation was conducted with the following objectives.

1. To assess the potential carbon assimilation rates of chloroplasts in a few species.

2. To quantify the relative limitations of `A' in a few C_3 and C_4 plants under non stress conditions.

3. To quantify the relative limitations of `A' under different abiotic stresses

4. To evaluate a few mesophyll subcomponents responsible for the high mesophyll limitation under moisture stress.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Crop improvement for enhanced dry matter production was achieved either by increasing the harvest index or by increasing the light interception by the canopy. Further increase in dry matter production can be achieved only by improving the photosynthetic efficiency of the crop canopy.

Though photosynthesis is a biochemical process, it is often referred to as a diffusive process (Kriedman and Downton, 1981). The path of movement of CO_2 from the ambient air to the carboxylation site at chloroplasts occurs always downward along a concentration gradient. Thus the movement of CO_2 can be considered as a diffusive process. This diffusion of CO_2 across the leaf from the ambient air to the chloroplasts experience a number of resistances. These resistances can be classified under stomatal and non stomatal or mesophyll resistances (Kriedman and Downton, 1981). Photosynthesis, apart from the intrinsic stomatal and mesophyll regulation, has a tremendous degree of environmental modulation.

It has been clearly established that various environmental variables impose a certain degree of regulation to the process of photosynthesis either by their effect on stomatal or mesophyll factors. Even at controlled environmental conditions, a great extent of interspecific and intra specific variations in photosynthesis exists. These variations can be broadly attributed to the variations in stomatal or mesophyll factors.

Assessment of limitations imposed by stomatal and mesophyll factors becomes a prerequisite for crop improvement for yield through increased photosynthetic rates. It is also essential to probe into the limiting factors of photosynthesis when plants experience abiotic stresses.

If the cause of low assimilation rates in normal and stressed conditions could be quantified, manipulation of the causative component either genetically or through agricultural management techniques could be attempted with the intention of minimizing the losses in productivity. The present status of understanding the limitations of photosynthesis both under non stress and abiotic stress conditions is reviewed in this chapter.

Limitations to photosynthesis under non-stress condition

Under a specified unlimited resource condition, photosynthesis is regulated more by the intrinsic plant characteristics. The diffusion of CO_2 through the stomata, diffusion of carbon in the mesophyll cells, the efficiency of the photochemical machinery to supply the required reducing power, factors associated with RuBisCO and the subsequent utilization of triose phosphates for end product synthesis are a few of the most important plant factors that constitute the major limitation of photosynthesis.

Limitation of photosynthesis due to diffusion of CO_2

At ambient CO_2 levels (330 ppm), photosynthetic rates are predominantly limited by the diffusive characteristics for the CO_2 transfer. These include the resistance to CO_2 diffusion through stomata (Jones, 1983; Schulze, 1986 and Ball, 1981) and the aqueous phase of the leaf. Among the two diffusive processes, the stomatal factors offer the maximum limitation to photosynthesis.

Stomatal limitation of photosynthesis

It is well documented that 'A' can be enhanced by increasing the stomatal conductance (gs) under unlimited water supply conditions, especially in the C_3 plants (Jones, 1986).

Occurrence of a positive correlation between 'A' and gs was observed among various species and among different physiological treatments (Wong et al., 1979). They noted as others have since, when the gs is smaller, the capacity of assimilation also tends to be smaller.

Recently, analyzing the relationship between stomatal resistance and photosynthesis in potato leaves, Perumal et al., (1989) found that the upper leaves of a canopy had more 'A' because of a lower stomatal resistance. Similar inferences were drawn by Kuroda and Kumura (1989) in rice plants. They showed that the variation in 'A' was positively correlated with variations in gs. They further concluded that stomata limit 'A' under well watered conditions. Based on carbon isotope discrimination Ehleringer (1990) suggested that stomatal conductance is related to photosynthetic rate of crop plants.

They confirmed that the variation in assimilation rates were due to thee possible variation in the extent to which stomata limit the photosynthetic gas exchange rates.

Recent evidences clearly suggest that when the crops are grown under unlimited resource conditions, the stomatal limitation to photosynthesis predominates (James Jacob, 1988; Kirschbaum and Pearcy, 1988; Xu and Xu, 1989; Ephrat et al., 1990; Jacob and Lawlor, 1991 and Heitholt et al., 1991).

It can be concluded that the predominant limitation of photosynthesis comes from the stomatal diffusive characteristics under non stress conditions.

Limitations of photosynthesis due to mesophyll factors

Once the substrate CO_2 is made available at the site of carboxylation, the limitation of photosynthesis occurs at the metabolic level. Fixation of CO_2 into organic compounds require electron transport associated with lamellar polypeptide, carboxylation of RuBP associated with RuBisCO, the calvin cycle enzymes and the end product synthesizing machinery.

Photochemical reactions

The photochemical reactions have been shown to be limiting 'A' upto a light intensity of 400 to 500 μ moles/m2/sec. A linear increase in 'A' with light intensity exists up to this level (Austin, 1989). However, it has been often reported that photochemical reactions are not limiting and the lower 'A' is associated with other factors. But the existing information in the literature do not unequivocally substantiate this argument.

Therefore, it is necessary to examine the limitations imposed by photochemical aspects under moderate to high light intensities with the existing ambient CO_2 levels.

Experimental evidences on the following three aspects substantiate to conclude that the photochemical reactions may not pose any limitation.

a. CO_2 response curves
b. Increase in`A' with increase in temperatures
c. Increase in `A' with altered end-product synthesis.

a. CO_2 response curves:

An increase in `A' with increase in intercellular $CO_2$ concentration (Ci) is one approach to infer that photochemical reactions do not pose a limitation at moderate and high light intensities. It is assumed that an increase in `A' at a given light intensity by increasing Ci would impose a greater demand for ATP and NADPH for CO_2 reduction process. An instantaneous increase in `A' with an increase in Ci substantiate the fact that a feed back stimulation of electron transport chain occurs to generate more ATP and NADPH to meet the demand of carbon fixation.

A large body of literature support the fact that `A' increases with increase in Ci (Caemerer and Farquhar

1981,Brooks 1986, Sage et al 1988,1989, Campbell et al., 1987; Blechschmidt et al 1989). Apart from reducing photorespiration a stimulation of oxygen evolution with increase in Ci has been shown (Badger, 1985).

The exact increase in `A' with increase in Ci varies amongst species and with other environmental factors. But it is essentially the same for many $C_3$ species. A mere doubling of Ca has been shown to increase `A' at a given light intensity though the range varies any where from 20 to 300 per cent (Jarvis et al 1981).

In studies where `A' was measured at high or saturating light intensities a significant increase in `A' with increase in Ci has been shown. In soybean plants grown under natural light intensities an increase in Ci, substantially increases `A' from 15 $\mu$ moles.$m^{-2}.s^{-1}$ to as high as 32 $\mu$ moles.$m^{-2}.s^{-1}$. Similar instances of increase in `A' has been shown by Caemerer and Farquhar (1981), Sage et al. (1988) in Phaseolus vulgaris, and by Brooks (1986) in spinach. In five different C_3 species also a significant increase in `A' was shown with an increase in Ci in a recent study by Sage et al. (1989). Even a significant increase has been shown in C_4 plants like maize (Canvin et al 1980) and Amaranthus (Blechschmidt et al. 1989).

The observed increase in `A' at high light can not be attributed to decrease in photorespiration only. Such an inference can be drawn from three types of studies.

i) The instances where they have shown more than 100 to 200 per cent increase in `A' with increase in Ci in C_3 plants.

ii) Increase in `A' in C_4 plants at high Ci (Badger, 1985).

iii) Direct evidences showing the stimulation of oxygen evolution with increase in Ci (Canvin *et al* 1980).

Both in C_3 plants *Helianthus annus*, *Helianthus incarna* *Phaseolus vulgaris* and also in C_4 plants *Zea mays* stimulation of oxygen evolution with increase in Ci has been shown at high light intensities (Canvin *et al* 1980,Badger, 1985). However, such a response was often noticed when `A' was not co-limited by light intensity . When gas exchange rates were measured at low light intensities the response to increase in Ci was marginal (Canvin *et al* 1980, Badger, 1985).

From several of the studies it can be inferred that in plants acclimatized to natural light intensities, with increase in Ci maximum assimilattion (A_{max}) significantly increases, when measured at saturated light intensities, thus substantiating the fact that there is a feedback stimulation of photochemical reactions when carboxylation efficiency was enhanced by higher substrate levels.

b. Increase in `A' with increase in temperature

When temperature is enhanced from suboptimal to optimum level the kinetic properties of RuBisCO will be altered favoring higher assimilation (Badger and Collatz, 1977; Caemerer and Frquhar, 1981). A significant increase in `A' at higher temperature has been shown in Phaseolus species with increase in Ci. Similarly a stimulation of oxygen evolution with increase in temperature has been clearly demonstrated in a recent study by Stitt (1986) in spinach. This in turn has been attributed to better inorganic phosphorus (Pi) recycling at high temperatures. Irrespective of the reasons for increase in 'A' it clearly substantiates the fact that when other limitations imposing on 'A' are removed there will be a feed back stimulation of photochemistry to meet the demand for assimilatory power.

c. Increase in 'A' with altered end product synthesis

While reviewing the possible feedback inhibition of photosynthesis Neals and Incoll (1968) concluded under normal conditions the photosynthetic rates of leaves are far less than their potential. The main reason attributed was inadequate synthesis and thus lack of utilization of end products. Several studies have shown an increase in photosynthetic rate either by shading (Herold, 1980) or defoliation (Sweet and Wareing, 1966) or DCMU spray to the earhead (King et al., 1967). When earhead photosynthesis was decreased by DCMU an increase in 'A' of flag leaf was noticed. Similarly when leaf number was decreased by defoliation an increase in leaf assimilation rate was noticed (Sweet and Wareing, 1966). However, in all these studies measurements were made days after imposing the treatments. Hence, it is difficult to pin point the actual processes associated with observed increase in 'A'. More recently it has been shown that the decrease in leaf number increases assimilation rate substantially in sunflower and soybean within 2 to 4 hours after imposition of the treatment. However, no other measurements except gas exchange characteristics were recorded in this study (Annonymous, 1989-90).

Evidences supporting photochemical reactions limiting `A'

There are several studies where low `A' was attributed to photochemical reactions. The following four evidences show the possibility of photochemical reactions limiting `A'.

1. No increase in `A' with increase in Ci at a given light intensity.
2. No increase in `A' with increase in light intensity at a given Ci.
3. No increase in `A' at high light and saturating CO_2.
4. Differences in `A' at a given light intensity between species and varieties.

Several researchers have used the nature of CO_2 response curve to arrive at the limitation imposed either by RuBisCO kinetics or photochemical components. Under high light intensities less increase in `A' with increase in Ci has been attributed to inadequate supply of photochemical energy. Though at high $CO_2$ the photochemistry may limit `A', in a series of recent studies by Taylor and Terry (1984) it is argued that energy supply may co-limit `A' even at ambient CO_2 concentrations. By using iron deficient and sufficient plants with different photochemical electron transport capacities, the co-limitation by photochemistry was also examined. Based on the initial slope of CO_2 response curve which was reduced substantially in leaves with low chlorophyll content, it was suggested that photochemistry co-limits assimilation rate even at low Ci also.

The inadequate response to increase in Ci (Taylor and Terry, 1984) may be due to the fact that the plants were acclimatized at 800 μ moles.$m^{-2}.s^{-1}$ of light intensity and CO_2 response curves were taken at 3000 μ moles.$m^{-2}.s^{-1}$

The importance of the photochemical reactions to achieve high 'A' has been shown in a study by Malkin _et al_. (1981) in four desert species differing in total photosystem II (PSII) reaction centers. The high 'A' in _Camissonia_ at high irradiances was attributed to higher PSII number. There was a good correlation between photosynthetic rate and concentration of PSII reaction center. No such correlation was found with PSI reaction centers.

In a study to assess the reasons for variation in 'A' between diploid and tetraploid wheat Austin _et al_. (1987) emphasized the importance of photochemical reactions. In diploids with high photosynthetic rates, uncoupled oxygen evolution and CO_2 dependent oxygen evolution, was high. In a similar comparative study Hobbs (1986) showed that the differences in chlorophyll 'a' and 'b' content correlated with the differences in photosynthetic rate. So the photochemical reactions are responsible for differences in photosynthesis, because RuBisCO and soluble protein did not show any correlation with photosynthetic rate.

End product inhibition - Recycling of inorganic phosphate as a limitation

Studies for last five years have clearly established that maximum rates of photosynthesis can be restricted by the rate of end product synthesis (Stitt 1986, Sharkey et al 1986, Stitt and Quick, 1989).

The partitioning of carbon between starch and sucrose as the two important end products of carbon assimilation has been clearly understood. The carbon fixed in the Calvin cycle in chloroplast is exported to the cytosol as triose phosphate. This export occurs across a phosphate translocator protein with a strict counter exchange with inorganic phosphate. The triose phosphates are metabolized to form sucrose in the cytosol and regenerate Pi which is available for further import of triose phosphate from the chloroplast. Restriction of sucrose synthesis will lead to a decreased export of triose phosphate (TP) from the chloroplast resulting in greater accumulation of TP in the chloroplast which is later converted to starch. The conversion of TP into starch in the stroma also facilitates the release of Pi. From these sequences of events it can be envisaged that inadequate rate of end product synthesis mainly sucrose could impose a limitation to Pi recycling and indirectly on carbon exchange rate (CER).

The mechanisms involved in regulating the reactions involved in sucrose synthesis from TP in cytosol has been extensively reviewed (Stitt and Quick, 1989).

The two enzymes,sucrose phosphate sysnthatase (SPS) and fructose 1-6, bis phosphatase have been considered as regulatory enzymes largely responsible for altering Pi recycling (Stitt and Quick, 1989).

A decrease in the activity of sucrose phosphate synthetase results in the accumulation of fructose 6- phosphate. This in turn gets converted into fructose 2, 6- bisphosphate a potent inhibitor of Fr. 1,6 bisphosphatase. As a consequence of this triose phosphates are not metabolized and gets accumulated.

The TP accumulation in cytosol may occur due to higher rate of influx compared to its conversion into sucrose via SPS or due to intrinsic low SPS activity or due to lack of efflux of sucrose (Stitt, 1986; Sharkey et al., 1986).

Inadequate Pi levels in the stroma as a consequence of decreased Pi recycling from the cytosol is the major factor affecting the ATP generation from photophosphorylation (Stitt, 1986; Sharkey et al., 1986). Such a phenomenon will have several direct and indirect effect on down stream mechanism of photosynthesis, for instance a marked decrease in the activation state of RuBisCO. Accumulation of PGA and a resultant decrease in stromal pH has been attributed to be one of the reasons for decreased activity of RuBisCO (Sharkey 1985).The decrease in photosynthesis by low rate of end product synthesis and thus Pi recycling has been shown to occur under a few specific situations like (a) under conditions of saturated CO_2 (Stitt 1986), (b) decreased efflux of sucrose due to decreased activity of sinks, (c) in mutants and possibly in some genotypes with low intrinsic SPS activity and (d) under environmental factors which decrease the efflux of sucrose or its synthesis by SPS.

In view of these facts it is necessary to evaluate whether Pi is a limitation under ambient CO_2 and saturating light intensities. There are two types of evidences to show that Pi is not a limitation.

1. CO_2 exchange rates are highly sensitive to oxygen concentrations under ambient CO_2 levels.

Recently oxygen insensitive photosynthesis has emerged as an important criterion to assess the relative Pi limitation on 'A'. It has been shown that in oxygen insensitive zone of CO_2 concentrations a decrease in oxygen concentration decreases the 'A' suddenly and later comes back. It was attributed to much greater decrease in Pi level due to reduction in photorespiration. Several studies have indicated that a decrease in oxygen concentration will increase the 'A' indicating that at ambient CO_2 concentrations 'A' is sensitive to oxygen concentration and hence Pi recycling is not a limitation.

2. Other evidence is that a substantial increase in 'A' at higher levels of CO_2 suggesting the maintenance of adequate Pi recycling even at elevated CO_2 concentrations.

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In leaves exposed to high CO_2 concentrations an instantaneous increase in 'A' has been shown by many workers (Campbell et al., 1987; Sage et al., 1987; Sage et al., 1988) and maintenance of the rate even for a period of 20-30 minutes (Steady state) has been reported (Annonymous, 1989-90). A substantial increase in 'A' and its maintenance at high CO_2 levels *in plants acclimatized to high CO_2 has been shown* (Sage et al., 1988; Campbell et al., 1987). From such evidences it can be inferred that at ambient CO_2, high light intensities and optimum temperature recycling of inorganic phosphate may not pose a significant limitation to photosynthetic rate (Stitt, 1986).

RuBisCO characteristics

Rubisco is the most important enzyme that catalyses the primary carboxylation reaction of photosynthesis. This enzyme gets further importance because of the fact that it also catalyses the wasteful process of oxygenating RuBP, the primary reaction of the photorespiratory cycle. Thus RuBisCO plays a very prominent role in the regulation of photosynthesis. The photosynthetic influx of CO_2 depends on the rate at which CO_2 is being fixed into organic molecules by RuBisCO to maintain the CO_2 diffusion gradient. Because of these conspicuous roles of the enzyme, much of the research effort has been put to understand the possible mechanisms of limitations by RuBisCO. Though there are contradicting evidences and hypotheses answering this question, it is still relevant and rewarding to probe carefully further into this question to arrive at better or more realistic solutions.

The plants allocate over 50 per cent of its soluble proteins towards RuBisCO protein. This further reiterates the importance of this enzyme.

Many early studies prior to 1975 showed a good positive correlation between light saturated CER, and RuBisCO activity particularly when the relation was observed between leaves of ontogenic stages and environmental history. Correlation coefficients to an extent of 0.9 were obtained between a range of Rye grass genotypes and small grain cereal cultivars (Treharne,1972). A few other studies claimed correlations between flag leaf RuBisCO activity and grain yield. Whether such correlations have physiological significance is an open question.

Dornhoff and Shibles (1970) and Frey and Moss (1976) showed that much of the variation in RuBisCO activity per unit leaf area simply reflects the leaf thickness. However, Randall et.al .,(1977) discovered a dacaploid tall Fescue which has very high RuBisCO activity and low levels of other leaf enzymes per unit leaf protein. They also showed two times more assimilation rate per unit leaf area in such varieties of decaploid tall Fescue. Examining the relationship between kinetic properties of RuBisCO in wheat species and genotypes Evans and Seemann, (1984) clearly demonstrated *a relationship between carboxylase activity and* carboxylation efficiency. The two factors associated with higher carboxylation was RuBisCO protein content and specific activity.

The following are the three important RuBisCO characteristics associated with total activity per unit leaf area (a) RuBisCO content, (b) activation state, and (c) kinetic properties.

RuBisCO content and photosynthetic rates

RuBisCO content and the per cent of soluble protein allocated to RuBisCO has been shown to be one of the important factors associated with higher RuBisCO activity and hence drawn attention of several workers examining the interrelation between RuBisCO content and carboxylation efficiency.

Recently, Sung and Chen, (1989) related ontogenic changes in apparent photosynthesis to the variation in the amount of RuBisCO per unit leaf area. They further concluded that the increased `A' in lanceolate leaf types of soybean is associated with more enzyme content which in turn is due to an increased chloroplast number per cell.

In rice, Shich and Liao,(1989) showed that `A' was significantly correlated with RuBisCO activity . This activity was shown to correlate with RuBisCO protein content and total soluble proteins. The ratio of RuBisCO activity to RuBisCO content remained constant throughout the growth of the plant suggesting that `A' was regulated via the RuBisCO protein content. Keeberg et.al.,(1989) observed an increased biosynthesis of RuBisCO in blue light with a corresponding increase in assimilation rate.

Although Frey and Moss, (1976) concluded that RuBisCO activity per unit leaf weight can be used as a selection criterion to identify high 'A' types, Gifford, (1987) argued that such a selection probably cause redistribution of N away from other photosynthetic components with consequential undesirable imbalances in CO_2 fixation. But however, The fact still remains that the influence of increased RuBisCo protein content on 'A' and the effect on other protein components of photosynthesis must be looked into very carefully before drawing any conclusions.

Activation state .

Activation of the enzyme to become catalytically functional is the most important and widely studied aspect of the enzyme regulation of photosynthesis. RuBisCO is known to be in the inactive or in the "deactivated" form in the absence of CO_2 and light. Since, the overall activity of the enzyme depends on its activation state, it is worthwhile to understand the mechanisms of regulation and the physiological significance of the activation.

There are a number of theories and hypotheses to explain how the enzyme is activated <u>in vivo</u> (Salvucci, 1989). Activation state of the enzyme is defined as the number of active sites that are made available functionally to catalyze either carboxylation or oxygenation process. This number is usually expressed as a percentage of the total number of active sites actually present on the enzyme.

The activation process involves the initial binding of an activator CO_2 covalently to the epsilon amino group of a specific Lysine residue (201 position), believed to reside in the cleft of the active site (Miziorko and Lorimer, 1983). This CO_2 bound to the amino group is called 'carbamate'. Once the carbamate is formed, Mg is rapidly co-ordinated on to the site which is then capable of carboxylating RuBP. This process is referred to as CO_2-Mg dependent activation often termed as 'carbamylation'.

But this mechanism of activation requires three times as high CO_2 concentration as in the atmosphere and the process is a relatively slow one (Portis <u>et.al.</u>,1986). Recently a new stromal protein has been identified that catalyses the carbamylation process at the physiological CO_2 concentration (10 ppm). The enzyme has been identified as RuBisCO activase (Streusand and Portis, 1987). Maintenance of the activation state in high RuBP concentrations seems to be the other primary function of RuBisCO activase (Portis <u>et al.</u>, 1986). The activation catalyzed by RuBisCO activase requires ATP.

There are evidences to clearly suggest the role of light and pH of the stroma in mediating the activation of RuBisCO (Campbell <u>et al.</u>, 1988; Mott and Berry, 1986). CO_2 and Mg^{+2} are very important for the activation. However, it is not clear what is the minimum concentration of CO_2 required for the purpose. Campbell et al. (1988) working with soybean, concluded that activation in vivo is effectively CO_2 saturated at concentrations less than 110 ppm measured external to the leaf. But Caemerer and Edmondson (1986), found Raphanus sativus RuBisCO to be 50 per cent activated at a Ci approximately 10 ppm.

Nevertheless, the activation of RuBisCO is a very essential part of photosynthetic induction in leaves. But the activation in leaves is seldom 100 per cent in most of the crop plants. This fact raises a question whether the in vivo activation state of the enzyme is limiting photosynthesis. If so, would it be beneficial to further manipulate and enhance the activation state to achieve higher assimilation rates.

Though it is clearly demonstrated by Sage and his co-workers (1987) that the activation state may not pose a limitation at elevated CO_2 concentrations, it has an important role to determine the initial carboxylation efficiency at ambient CO_2 concentrations.

Kinetic properties of RuBisCO

Analysis of photosynthetic gas exchange and RuBisCO activity have revealed that the maximum rate of CO_2 assimilation in plants under ambient conditions can be predicted from the amounts and also the kinetic properties of the enzyme (Makino et al., 1985 and Evans, 1986).

The affinity of this enzyme for CO_2 has been recognized as a factor limiting photosynthetic carbon assimilation. Therefore, genetically based variations in the kinetic properties and amount of this enzyme may provide targets for breeding approaches to improve leaf photosynthesis. Keeping this in view several attempts have been made to look into the variations in some RuBisCO kinetic characteristics like the K_m and K_{cat} in the recent years (Paul and Yeoh,1987.,Seemann et al,1984.,Wessinger et al,1989.)

Kinetic studies on RuBisCO from taxonomically diverse plants have revealed that K_m (CO_2) values are associated primarily with differences in photosynthetic pathways and correlated to some extent with taxonomic groupings (Yeoh et al., 1980, 1981 and Yeoh and Hattersley, 1985).

A large variation in K_m (CO_2) [7.8 to 14 uM of CO_2] has been shown amongst 16 cassava cultivars suggesting that K_m (CO_2) values have potential application in cassava systematics (Paul and Yeoh, 1987).

In a similar study, Makino et al. (1987) attempted to assess the differences in K_m (CO_2) and V_{max} of RuBisCO in three distinct types of Oryza sativa L. (Japanica, Indica and Javanica types). The overall variations in the kinetic constants of RuBisCO and its ratio to soluble proteins were very small, especially the two main cultivar types japanica and indica.

Although the V_{max} from Javanica was slightly high, this was also associated with high K_m (CO_2).

In addition to the differences in K_m (CO_2), the differences in specific activity and K_{cat} have been examined as the possible factors associated with the carboxylation efficiency. The *in vitro* RuBisCO activity for unit of leaf nitrogen was found to be 30 per cent greater in *Triticum aestivum* than in *T. monococcum* and it was attributed mainly to the higher specific activity of the enzyme, since the amount of RuBisCO protein per unit leaf protein did not differ between the two sub-species.

The specific activity and also K_{cat} have been shown to significantly vary in species differing in photosynthetic pathways. For instance, the species though have lower K_m the specific activity and K_{cat} were less compared to the C_4 plants that had higher K_m (CO_2) [Seeman *et al.*, 1984 and Wessinger *et al.*, 1989]. The kinetic properties of RuBisCO appear to have modified to adjust to the changes in substrate availability. C_4 plants have a higher concentration of CO_2 available to RuBisCO than the plants (Keys, 1986). Therefore, the K_m (CO_2) values of the C_4 species and also a few Algae acclimatized to higher HCO_3 concentrations have much higher K_m (CO_2) values than the C_3 plants (Keys, 1986). However, species with high K_m (CO_2) also showed high K_{cat} suggesting that the K_m (CO_2) and K_{cat} are linked and the variation in K_{cat} and K_m (CO_2) ratios may remain stable. But the possibility of differences in K_{cat} at a given K_m (CO_2) can not be completely ruled out. In one of the C_4

species, Flavaria vaginata, compared to F. australasica, the K_{cat} was significantly high though the K_m (CO_2) differences were marginal (Wessinger et al, 1989).

Although great extent of understanding of various biochemical processes has been achieved, few reports are available to explain or attribute the differences in 'A' to variations in particular mesophyll reactions. Recently, however, there are efforts deviated for the quantification of a possible limitation imposed by the stomatal and mesophyll factors.

Approaches to quantify limitations of photosynthesis

Development of a technique which would enable partitioning of limitations of observed assimilation rates under both non stress and abiotic stress conditions between stomatal and non stomatal factors is of great importance from the practical and theoretical stand points.

The problem of quantifying the limitation of photosynthesis has been addressed by many workers over the past several decades. As a result, many methods to apportion the relative limitations of photosynthesis between stomatal and mesophyll factors have evolved. Among them, the following two methods are generally adopted to quantify the limitations of photosynthesis.

1. Resistance analysis originally proposed by Gaastra, (1959).

2. sensitivity or path dependent analysis (Jones, 1985b; Assman, 1988).

Resistance analysis

Based on the resistance analysis originally proposed by Gaastra (1959), Farquhar and Sharkey (1982) developed a straight forward technique to quantify thee stomatal limitation to photosynthesis. They proposed that the stomatal limitation can be calculated by knowing the 'A' when the stomatal conductances are infinite when Ci and Ca would equilibrate. The percentage limitation is then taken as $(1-A/A_o)100$.

Sensitivity analysis

The other approach generally adopted for the partitioning of limitations of photosynthesis is the path dependent method other wise termed as the sensitivity analysis (Cornic and Miginiac, 1983; Jones, 1985; Assmann, 1988). If 'A' can be described as a function of a number of environmental variables, then the sensitivity of 'A' to any one variable can be explained by a partial derivative with respect to that variable. This type of sensitivity analysis which was initially developed by Kascer and Burns (1973) and Heinrich and Rapaport (1974), have recently begun to be used for the analysis of control functions and to quantify limitations to photosynthesis (Dietz, 1986; Woodrow, 1986: Stitt and Quick, 1989).

Although several approaches have been proposed for the quantification of limitations, misconceptions still persist in the literature (Blackman,1905; Gaastra, 1959; Jones, 1973a; 1983; 1985 & 1990; Farquhar and Sharkey,1982; Priol et al., 1984 and Assmann, 1988).

Environmental factors affecting assimilation rates

The process of photosynthesis depends, to a large extent, on the environmental factors. It has been clearly demonstrated that even under situations where leaf photosynthesizing machinery is capable of fixing higher amounts of carbon, the rates of carbon fixation is limited or reduced considerably by the existing environmental conditions such as CO_2, soil and plant moisture status, light intensity during the day, RH of the air and temperature.

Effect of water stress on photosynthesis

Among many of the abiotic stresses, moisture status of soil and air and the light intensity can be considered as the most important factors determining the assimilation rates.

Diffusive resistances to CO_2 are extremely susceptible to the environmental conditions. Any change, therefore, in these resistances will ultimately affect the net carbon exchange rates. The most frequent environmental variable that adversely affects these diffusive resistances is a lack of moisture in the soil. The soil moisture stress can affect both the diffusion of CO_2 into the leaf and its fixation into organic molecules by the biochemical processes in chloroplasts.

Limitations of photosynthesis under moisture stress

A large body of literature is available to explain the possible mechanisms of inhibition of photosynthesis due to drought stress (Kaiser, 1987 and Chaves, 1991).

The effect of water deficit has been studied based on the inhibition in stomatal movements, mesophyll photosynthetic characteristics and plant growth. The effect of moisture stress on these aspects are largely inter dependent. However, at certain situations the effects could be quite independent. Any such effect, alters the carbon balance as well as the carbon partitioning and thus causing a reduction in productivity by the plants. Understanding the effect of water deficit on stomatal aspects and mesophyll capacity is relevant at this juncture because they in turn regulate the photosynthetic carbon exchange rates.

It is now well established that the rates of CO_2 assimilation in the leaves is reduced at moderate leaf water deficits or even before leaf water status is changed in response to a drop in air humidity (Bunce, 1981), or in soil water potential (Davies and Sharp, 1981; Gollan et al., 1986). In such cases the stomatal control of CO_2 diffusion plays the most important role in controlling the photosynthesis under moisture stress conditions.

Stomatal control of photosynthesis under moisture stress

During the early part of the century it was an accepted dictum that stomatal factors limit photosynthesis to the largest extent at low leaf water potentials (Verduin and Loomis, 1944; Scarth and Shaw, 1951; Brix, 1962). The reduction in photosynthesis under moisture stress was considered to be due to the reduction in the supply of CO_2 because of the closure of stomata.

The implications of the regulation of photosynthesis has been clearly studied from the time of Gastraa (1959) who for the first time derived different diffusive resistances to CO_2 and gave utmost importance to the stomatal resistances. CO_2 uptake for photosynthesis and transpirational loss of water occurs through stomata (Fischer and Turner, 1978). Thus any attempt to increase photosynthesis by stomatal opening invariably results in a higher loss of water by transpiration (Jones, 1986; Farquhar et al., 1987).

The effect of environmental factors like moisture stress, light, CO_2 and temperature on photosynthesis through stomata are clearly understood (Raschke, 1979; Zeiger, 1983; Assman and Zeiger, 1985; Mott, 1988; Shaish et al, 1989 and Chaves, 1991)

Stomatal inhibition of photosynthesis under water stress is more common in plants. Nevertheless, though not many, there are information regarding the stomatal inhibition of photosynthesis even in C_4 plants. For instance, Pier (1987) imposed drought stress and temperature stress on Sorghum plants and concluded that water stress inhibited photosynthesis mainly through stomatal closure in response to a decrease in leaf water indirectly to the evaporation of the atmospheric air or to the soil water status. Jensen and his associates (1989) working with lupins and wheat showed that there was no significant reduction in leaf water potential for small changes in soil water potential. But the leaf conductance decreased by 60 per cent

Water stress commonly results in stomatal closure which is thought to result from the accumulation of ABA since this stomatal closure can be mimicked by application of exogenous ABA to the unstressed leaves (Walton, 1980).

Recent reports mention that roots behave as the sensory organs of the plants to sense the possible reduction of water status. They send an immediate signal to the leaf to close the stomata and there by avoiding an inhibitory effects on photosynthesis by lowered soil moisture status (Jensen et al, 1989; Schulze, 1986). This signal is speculated to be transduced in the form of abscisic acid synthesized in the root in response to decreasing soil moisture status (Ztang and Devies, 1989; Downton et al 1988; Jensen et al, 1989; Ztang and Davies, 1991).

However, it is not yet clear whether, under moisture stress, ABA itself is transmitted to the leaves after having been synthesized in the roots or whether a stimulus is transferred to leaves where ABA could be synthesized.

Response of stomata to abscisic acid

CO_2 and ABA are the most important variables that induce stomatal closure. Stomatal response to these two factors have been widely examined although there is still some controversy as to whether the levels of one can influence the activity of the other and vice versa. However, Orton and Mansfield (1974) and Mansfield (1976), found that the response of stomata of Xanthium strumarium was virtually the same in CO_2 free and normal air.

Majority of the data are gathered with well watered plants, whose response to the applied ABA is not confounded by the endogenous ABA levels. It seems to be a common assumption that the plants supplied externally with ABA would respond to CO_2 much in the same way as a stressed plant would (Walton, 1980; Wilson, 1981).

Robinson and associates (1988) fed 10^{-5} M ABA through the petioles of detached leaves of Apricot, Sunflower and Spinach. They showed that in Spinach, the effect of ABA was entirely on stomatal conductance. Thus reduction in photosynthesis was entirely due to the stomatal closure. In Sunflower, though gs decreased significantly, there was no reduction in electron transport rate or RuBisCO activity. A similar conclusion was made by Downton et al (1988), who reported that the inhibition of photosynthesis in ABA fed leaves was completely explainable by the effect of the ABA on stomatal closure.

A number of reports have clearly demonstrated that the mesophyll processes are relatively resistant to ABA. A lack of sensitivity of photosynthesis to ABA has been described in isolated mesophyll cells (Mawson et al., 1981; Raschke and Hedrich, 1985), in isolated chloroplasts (Keck and Boyer, 1974). The in vitro activity of rubisco also was not affected by ABA (Kriedman, 1975; Robinson et al., 1988). These reports suggest that the inhibitory effects of ABA seems to be mediated through stomatal closure.

Response of stomata to VPD

The stomata also respond quickly to the first signs of drought in air by reducing the aperture when vapour pressure deficit increases due to a reduction in RH of the air (Hall et al, 1976; Losch and Tenhunen, 1981; Schulze, 1986). Recently Gantz (1990) reviewed the location and mechanism of sensing humidity in plants. The plant responses to humidity was also considered. The stomatal response was considered in detail with reference to data on soybeans, sugarcane and Commelina sp.

Hirasawa et al., (1989) reported a reduction in stomatal conductance in rice with an increase in the leaf to air vapour pressure difference or as leaf water potential decreased in the range of above -5 to -6 bars. They concluded that above these potentials, the reduction in `A' was predominantly due to the stomatal limiting factors.

It is sufficiently explained that the inhibition of `A' under stress occurs due to a reduction in gs which decreases the

CO_2 concentration at the carboxylation site.

However, at this juncture the question still remains whether stomatal closure completely accounts for the inhibition of 'A' under moisture stress.

Non stomatal control of photosynthesis under moisture stress condition

It is now well established that the rates of CO_2 assimilation in leaves is depressed at moderate leaf deficit or even before water status is changed in response to a drop in air humidity (Lange et al., 1971; Bunce, 1981; Schulze, 1986) or in soil water potential (Davies and Sharp, 1981; Gollan et al., 1986). As discussed earlier, in such cases, stomatal control of CO_2 diffusion plays the most important role in controlling photosynthesis.

When the drought period is lengthened, dehydration is made more severe or other environmental stresses are super imposed, changes may occur in metabolic functions (Kaiser, 1987a) and/or whole plant behavior (Schulze, 1987). Classically, the importance of stomatal closure in regulating photosynthesis under water deficit was recognized by the numerous findings of parallel reductions in photosynthesis and transpiration as drought develops (Kozlowsky, 1982).

Shimshi as early as 1963, calculated that although the stomata decreased its aperture size, there was a component of photosynthetic inhibition that could not be attributed to stomatal closure. Hutmacher and Krieg (1983) have shown the reductions in mesophyll photosynthetic capacity and reduction in stomatal conductance under water deficit may to a significant degree, be coupled.

Farquhar and Sharkey, (1982) suggested that the water stress also affects the photosynthetic capacity of the mesophyll cells. The stomatal limitations under water stress can be overcome by increasing the external CO_2 concentrations or by incubating small leaf slices in bicarbonate medium (Kaiser, 1987).

In a classical work with selected Triticum species, Johnson et al., (1987) concluded that stomata played an important role to achieve high `A' under well watered conditions. But under water deficit, the high `A' was related more to the maintenance of higher capacity for mesophyll photosynthesis. The reduction in assimilation rate of both drought tolerant and susceptible species of Triticum was mainly due to a reduction in mesophyll capacity to fix CO_2. They noticed that the maintenance of higher `A' in drought tolerant species was because of better mesophyll reactions.

Such results along with the gas exchange measurements of photosynthesis at high CO_2 concentrations indicate that a component of mesophyll mediated inhibition of photosynthesis under moisture stress usually occurs (Johnson et al., 1987; Farquhar and Sharkey, 1982; Sharkey, 1985)

The strong correlation between stomatal conductance and photosynthetic rate seems to represent an adjustment of stomatal conductance to match the intrinsic photosynthetic capacity rather than indicating a causal relationship. Therefore stomatal mechanisms are strongly controlled by the capacity of the mesophyll cells (Wong et al., 1979; Jarvis and Morison, 1981).

Stomata respond to inter-cellular CO_2

Earlier in 1977, Cowan and Farquhar proposed a theoretical model of functioning of stomata so that an optimum balance between loss of water and CO_2 uptake may be maintained. This theory fits the observations of Farquhar and Sharkey, (1982) that stomata operate to keep the CO_2 concentration in chloroplast close to the transition point between substrate CO_2 and RuBP regeneration limitations of photosynthesis.

However most of the investigators assume that the stomata respond to the CO_2 concentrations in the intercellular spaces of the mesophyll cells. Sensing of the Ci by the guard cells is an attractive hypothesis because as the mesophyll demand for CO_2 increases, Ci will decrease promoting stomatal opening thus increasing Ci (Farquhar et al., 1978).

However, Raschke (1975) suggested that guard cells might exchange CO_2 along the entire wall of stomatal pores and therefore could sense approximately the average of Ci and Ca. But Heath as early as in 1949 had reported that in darkness the tightly closed stomata do not open even if CO_2 free air was passed on the leaf. This suggests the stomata respond more to the CO_2 concentration of the inter-cellular spaces.

The fact that the guard cells indeed respond to Ci was recently demonstrated by Keith Mott (1988). In this study asymmetric CO_2 concentrations for the two surfaces of an amphistomatous leaf were used to vary intercellular and leaf surface CO_2 concentrations independently in <u>Xanthium strumarium</u>. Stomata responded to Ci when Ca at the leaf surface was held constant. In addition stomata did not respond to changes in leaf surface CO_2 concentration when the Ci was held constant. This evidence clearly suggests that the stomatal movements are strictly controlled by the mesophyll factors. Thus it is plausible at this juncture to predict that the stomatal closure under moisture stress conditions could be also due to a decreased capacity of photosynthesis in the chloroplast. This further supports the earlier findings of Wong <u>et al</u>. (1979) and Farquhar and Sharkey (1982).

Effect of moisture stress on mesophyll factors

Several data showing inhibition of CO_2 assimilation at high internal CO_2 partial pressure simultaneously with stomatal closure led Farquhar and Sharkey (1982) to conclude that the photosynthetic apparatus were inhibited by water stress and that the stomatal limitation decreased under stress. Accordingly different lines of evidence where internal partial pressure of CO_2 has remained unchanged in leaves of plants subjected to water stress in spite of a decline in both stomatal conductance and CO_2 fixation indicate that mesophyll capacity to photosynthesize was affected by dehydration (Wong et al., 1985; Raschke and Recemann, 1986; Cornic et al., 1987). In the in vitro experiments conducted earlier by Boyer (1976a) and recently by Kaiser (1987a) further confirmed that the capacity of photosynthesizing cells was indeed damaged by dehydration.

In a recent study Quick et al., (1990) comparing four species showed that soil water depletion over a period of several day (4-7 days) may result in different effects on mesophyll. Similarly Speer et al., (1988) working with Nicotiana rustica and Chaves (1991) concluded that slowly imposed dehydration may affect mesophyll photosynthesis at relative water contents higher than those that inhibit photosynthetic capacity in rapid wilting. Bunce (1988) reported a reduction in photosynthesis even at high transpiration rates without stomatal closure in stressed field grown tomato plants, indicating a possible non- stomatal limitation of photosynthesis. These data confirmed the previous findings when slow stress resulted in a reduction in the initial slope of the CO_2 response curve as well as the CO_2 saturated photosynthetic rate suggesting a damage to the carboxylating machinery as well as the regeneration of RuBP (Jones, 1973; Radin and Ackerson, 1981; Jones and Fanjul, 1983; Bjorkman and Powels, 1984).

Earlier, based on chlorophyll-a fluorescence, Sivak and Walker (1985) and recently Bukhov et al., (1989) demonstrated clearly that the CO_2 fixation potential of leaf was extremely sensitive to stress.

Many of the previous works in the early seventies based their conclusions on the calculated intercellular concentrations of CO_2 (Ci). When stomata close without considerable damage in the mesophyll capacity to photosynthesize, the Ci inevitably decrease. However, under water stress Ci often remains high or at least higher than should be expected from the decrease in photosynthesis and stomatal conductance. However,Caemmerer and Farquhar (1984) and Sharkey (1984) argued that though the Ci did not show any increase under stress the mesophyll capacity could still have been damaged and hence a non-stomatal limitation of photosynthesis could be operating.

These aspects have been reviewed by Bradford and Hsiao (1982) and Schulze (1986). This is usually interpreted to indicate a direct damage to the photosynthetic machinery by dehydration. No increase in Ci under water stress was also interpreted to indicate an inhibition to mesophyll capacity for photosynthesis.

Is patchy stomata responsible for the apparent non- stomatal limitation?

It has been found that stomata can close in patches over some sections of the leaf when the leaf water status is low. This non uniform stomatal closure can bring in a lot of error in terms of over estimating the Ci and thus the interpreted non stomatal limitation of photosynthesis. Farquhar et al., (1987) suggested that non uniform stomatal closure under water stress previously shown by Laisk et al., (1980) and Laisk (1983) might lead to an incorrect calculation of Ci.

Downton et al., (1988a) adopted the fluorescence quenching mechanism to compare the ABA fed leaves on control plants. They found that the inhibition of photosynthesis in the species studied was indeed fully due to the effect of ABA on stomatal closure. They also observed that stomatal closure in response of ABA was very heterogenous. In a subsequent study Downton et al., (1988b) showed that an increased amount of ABA was induced by withholding water from *Vitis vinifera*, *Nerium oliander* and *Eucalyptus ficifolia*. In all these species, non uniform stomatal closure occurred. This aspect was further demonstrated by Sharkey and Seeman (1989) in *Phaseolus vulgaris*.

Though the discovery of the occurrence of patchy stomata has been employed in explaining the non-stomatal inhibition of photosynthesis in water stressed leaves or those supplied by ABA, there is no evidence yet that satisfactorily explains why stomata respond in such a non uniform manner.

However, Tereshima and coworkers (1989) have made emphasis on the anatomy of leaves to explain the non uniform stomatal response. They argue that in many leaves the network of vascular bundles causes the effective isolation of sections of intercellular spaces and that there is restricted lateral diffusion of CO_2 between the sections. Leaves with such "Heteroboric" anatomies are believed to be more prone for non uniform stomatal closure while those with well connected intercellular spaces ("Homoboric") providing high gaseous mobility are not. Therefore patchiness of stomata may operate only in leaves with heteroboric leaf anatomy.

Mansfield et al. (1990) suggested that the uneven distribution of a substance moving in the xylem could result in local differences in transpiration or differences in resistance of the xylem conduits so that water flow is favored in some areas of the leaf. This may also lead to the patchy stomatal closure.

Effect of moisture stress on different mesophyll sub components

Progressive stress has been clearly shown to reduce the photosynthetic rates by altering the mesophyll ability to fix CO_2. The damage to the biochemistry of photosynthesis can be mediated through a damage caused either to the reactions associated with the thylakoid membrane system or to the stromal reactions associated with the carbon reduction cycle especially, the carboxylation reaction (Kaiser, 1987).

At present two conflicting views exist about major sites for a primary interaction of water deficit with the photosynthetic machinery. One view favors the thylakoid mediated light reactions of photosynthesis as a main target and the other argues for the dark reactions that occur in the aqueous phase of the chloroplast stroma as the main factor that gets affected under stress.

Recent reports pertaining to the effect of stress on the photosynthetic capacity of the mesophyll are reviewed here.

Effect of stress on carboxylation:

The process of carboxylation and therefore the CO_2 gradient for gas exchange is predominantly controlled by rubisco. Any environmental variable affecting carboxylation process operates through rubisco. The observed changes in the properties of RuBisCO in the leaves of slowly stressed plants of different species include the activation, total activity and concentration of the enzyme (Vu _et al._, 1987; Vu and Yelenski, 1988; Speer _et. al._, 1988).

Vu and Yelenski, (1988) showed that when grape vines were progressively stressed to -2 MPa leaf water potential, resulted in a 20% reduction in the activation state of RuBisCO. They opined that the water stress mediated non-stomatal inhibition of photosynthesis was primarily due to the decrease in the content and activation state of RuBisCO. However, they reported that the K_{cat} (25 moles of CO_2 per mole of enzyme per second) and $K_{m(CO_2)}$ (18.9 umole) remained almost similar in both well watered and stressed plants.

In a similar experiment with Soybean, Vu _et. al._ (1987) confirmed that non-stomatal inhibition of photosynthesis under drought stress is primarily from the reduction in the _in vivo_ activity and activation state of RuBisCO and also the V_{max} for carboxylase. They further concluded that the RuBP regeneration may not be the limiting factor under stress.

Mayoral et. al. (1981) working with stressed wheat and related wild species had earlier shown that the reduction in photosynthetic rates was associated with the reduction in the ability of RuBisCO and PEP carboxylase. They also recorded significant reduction in total protein content under low leaf water potentials.

Apart from the processes associated with rubisco, the other Calvin cycle reactions are also shown to be susceptible for stress effects through acidification of stroma. Berkowitz et. al., (1983) demonstrated that the inhibition of photosynthesis under stress condition was due to the stromal acidification in Spinach, Chlamydomonas and Aphalocapsa, a Blue Green Alga. Further Berkowitz and Gibbs (1983) showed that the stromal acidification due to osmotic stress resulted in the inhibition of enzyme activity. They inferred that Fructose 1-6, Bis Phosphatase was the most sensitive enzyme.

Effect of stress on photochemistry

Ben et. al., (1987) subjected Xanthium strumarium and Helianthus annuus to chronic and acute water stress and observed that CO_2 saturated rate of 'A' was more sensitive to stress. They concluded that these effects were due to the pre disposition of water stressed plants to inhibition of primary photochemistry (Bjorkman and Powells (1984). Similar conclusions were derived by Quick et. al., (1990).

Sharkey and Badger, (1982) suggested that RuBP regeneration capacity was reduced under water stress conditions. They found that photophosphorylation was more sensitive to stress than was the rate of electron transport in Xanthium stumarium. Caemmerrer (1981) and Forseth and Ehleringer (1983) also got similar results where electron transport was almost unaffected at leaf water potentials that completely inhibited photosynthetic rates.

More recently, Marco et. al., (1988) subjected hard wheat plants to different soil water potentials and attributed the reduction in assimilation rates to changes associated with the photo chemistry of the leaf under stress.

Lopez et. al., (1987) demonstrated in intact water stressed sunflower leaves that relatively modest decline in leaf water potential adversely affected photophosphorylation in situ. The effect of declining water potential on photo phosphorylation is large enough to constitute a major rate limitation to net photosynthesis. These data indicate that the inhibitory mechanism involves the catalytic activity of the coupling factor itself, a notion consistent with the in vitro studies of Younis et. al., (1979).

Transpiration induced stress was reported in Avicennia marina, Xanthium stumarium and several glycophytic species by Ball (1981). She concluded that the transpiration induced stress was similar to the stress effect envisaged by with holding irrigation water. She found that the assimilation rates were more affected specifically at higher CO_2 concentrations than at lower concentrations. There was a significant increase in quantum requirement. A similar increase in quantum requirement was also observed by Mohanty and Boyer (1976). An increase in the quantum requirement indicates a possible damage to the photo chemical reactions of the thylakoid membranes.

When moderately stressed or severely stressed plants were exposed to normal high light intensities of incident radiation, there was a lowered rate of electron transport as well as a reduced photo phosphorylation (Boyer, 1971; Keck and Boyer, 1974; Bjorkman and Powells, 1984; Mathews and Boyer, 1984). Such a damage to the thylakoid membranes of the chloroplasts were visualized by the chlorophyll a fluorescence technique also (Govindjee et. al., 1981; Bjorkman and Powells, 1984).

There are also other reports available explaining the possible damage to the electron transport system by moisture stress. But the recent reports using more sophisticated techniques of chlorophyll florescence proves that the thylkoid membranes and associated electron transport reactions are more resistant to stress. Scriber and Bilger (1987) mentioned that the rapid wilting of leaves at dim room light to about 50% RWC did not impair thylakoid energization. The observed absence of uncoupling in osmotically dehydrated intact chloroplasts or protoplasts is consistent with the impression that thylakoid membrane integrity was not affected by moderate water deficit (Kaiser, 1981b). On the other hand, Sharkey and Badger, (1982) an Turner and Welburn, (1985) showed that water stressed leaves have a drastically lowered ATP content. Also, the light induced increase in ATP levels in isolated intact chloroplasts was smaller in hypertonic than in isotonic media (Kaiser et. al., 1981b).

Effects of low light stress on photosynthesis

Low light intensities are yet another important abiotic stress condition often responsible for the reduced dry matter production. This reduction in dry matter accumulation has been well studied and attributed to the lowered assimilation rates. The low light stress effects are more pronounced during the kharif months.

Although the plants acclimatize to the low light conditions, many biochemical and physiological changes occur that result in the reduced assimilation rates. Such an argument gets support from the fact that the extent of response of `A' to high Ci is often less in plants grown under low light intensities. Both the initial slope of the $CO_2$ response curve and the `A' at saturated Ci were low in plants acclimatized to low light intensities (Caemerre and Farquhar, 1981; Taylor and Terry, 1984).

Photochemical as well as biochemical reasons have been attributed for the lowered assimilation rates under low light conditions. Light saturation point and photosynthetic capacities are shown to bee altered by growth light intensities. (Demming and Winter, 1988). AS a consequence of light adaptation changes in the leaf anatomy, the ultra structure of chloroplasts and the composition of chloroplast components occurs and thus alters the photosynthetic functional characteristics (Chow and Anderson, 1987; Prioul and Reyss, 1987; Chow et al., 1988). Therefore it can be concluded that alteration in chloroplast structure is the major factor contributing for the adaptation of the plants to different light intensities. The lack of response that is often noticed in low light acclimatized plants even at high light intensities with increase in Ci can be attributed to the intrinsic chloroplast characteristics, which were optimized for growth light conditions.

In a recent study at our center, Shivashankara (1991), quantified limitations of potential photosynthesis in low light acclimatized plants and concluded that photochemical and biochemical limitations were higher in low light grown plants. He also showed that the apparent quantum yield as well as the A_{max} at saturated Ci were lower indicating an inhibition of the photochemical and biochemical reactions of photosynthesis.

The reaction leading to the carboxylation of RuBP catalyzed by RuBisCO has also been shown to be sensitive to changes in light intensities. The activation state of the enzyme is strongly modulated by light intensity (Woodrow and Berry, 1988). A positive correlation between light intensity and RuBisCO activity was shown by Vu et al., (1983); Seemann et al., (1990).

Effect of nitrogen stress on photosynthesis

Proper nitrogen nutrition is essential for the adequate development of the photosynthetic systems including the energy transducing components (eg. thylakoid membranes), enzymes of the photosynthetic carbon reduction cycle (eg. RuBisCO) and enzymes of nitrogen metabolism (Farquhar et al., 1980).

Deficiency of this essential element has been clearly shown to affect plant growth and development. Leaves with higher nitrogen content have a greater maximum rate of net photosynthesis in bright light with limiting CO_2 supply than those deficient in nitrogen (Evans, 1983; Lawlor et al., 1987b).

Significant relationships between nitrogen content and photosynthetic rates have been shown in many species (Evans, 1983; Evans and Seeman, 1984). Makino et al., (1985) showed that with reduction in nitrogen nutrition assimilation rates significantly decreased in rice. Similar reduction in `A' with decreasing nitrogen levels were also recorded in wheat by Lawlor et al., (1987a) and Lawlor et al., (1989).

Recently, Heitholt et al., (1991) showed a significant reduction in enzyme content especially RuBisCO when nitrogen nutrition was decreased. They concluded that the observed reduction in assimilation rates in nitrogen stressed leaves was due to a reduction in the activity and activation state of RuBisCO.

In sunflower plants grown at different levels of nitrogen nutrition, Kumuda (1991) showed that the reduction in `A' was primarily due to a reduction in the total soluble protein and in the amounts of RuBisCO. However, the ratio of RuBisCO to total soluble protein did not show any significant changes under nitrogen stress.

MATERIAL AND METHODS

MATERIALS AND METHODS

Observed variations in 'A' between genotypes and species can be broadly attributed to stomatal diffusive characteristics (g_s) and variations in the capacity of mesophyll sub components (g_m) to fix carbon.

Crop plants constantly experience a randomly changing environment. Environmental changes generally result in a significant reduction in observed 'A' either by their effects on g_s or g_m. Hence a knowledge of the extent of limitation imposed by stomatal and mesophyll factors on observed 'A' is a prerequisite in selecting traits for crop improvement by photosynthesis. Since changing environmental variables also exert a significant degree of modulation of photosynthesis, assessing the limitations of 'A' under the most common abiotic stresses is also of great importance.

With these aspects in mind, a series of experiments were conducted to assess the A_{max} and limitations of photosynthesis under non-stress and abiotic stress conditions. Measuring the gas exchange traits was the predominant approach in the present investigation. All the gas exchange parameters were recorded using a portable photosynthesis system (ADC LCA 2) in the open system. Quantification of the relative limitations of photosynthesis was done based on the CO_2 response curves. A new gas exchange method was developed to calculate the specificity factor of rubisco. The other gas exchange approach to evaluate the stress effect on the mesophyll factors was to develop VPD response curves. The methodology adopted in the various experiments are explained in this chapter.

Description of the portable photosynthesis system

A portable photosynthesis system (IRGA) of the ADC, England LCA-2 was used for recording of gas exchange parameters. Since the instrument operates in an open system, it is possible to maintain steady state CO_2 concentration and relative humidity in the leaf chamber. The instrument is composed of four distinct units.

Air Supply Unit (ASU): It is a peristaltic pump that pumps air at a constant rate ranging from 200 to 600 $ml.min^{-1}$. Air is pumped through two desiccant tubes containing Magnesium perchlorate that readily absorbs moisture from the air. The air leaving these desiccant tubes is therefore essentially dry. A selenoid valve regulates the flow of air either into the Parkinson's leaf chamber or to the reference chamber of the gas analyzer.

Parkinson's Leaf Chamber (PLC): It accommodates a leaf area of 6.25 cm^2 and is equipped to measure the light intensity in the PAR range by a quantum sensor, relative humidity by a thermocouple and temperature of the air. This PLC with a leaf clamped is held perpendicular to the incident light. A butyl rubber tubing is used to carry air from the leaf chamber in to the leaf chamber analyzer.

Leaf chamber analyzer (LCA): This unit consists of an IRGA with two chambers. The reference chamber to which air is pumped straight from the ASU, and an analysis chamber to which air from the PLC is pumped. An actively photosynthesizing leaf would deplete CO_2 from the air and hence the analysis chamber CO_2 concentration would be less than that in the reference chamber. This differential CO_2 concentration is further used to compute the photosynthetic rates by the data logger.

Data logger: From the basic data measured by various units explained, the data logger computes the assimilation rate (`A'), stomatal conductance (g_s), leaf temperature, inter-cellular CO_2 concentration (Ci), etc..

Recording gas exchange parameters: The top fully expanded leaf was clamped to PLC and held perpendicular to the incident light. Relative humidity was maintained at a steady state level equal to the ambient RH to simulate a condition very similar to that of the ambient air. By altering the flow rates of dry air from the ASU, it is possible to maintain a desirable RH in the leaf chamber. Recording of data was done at saturated light intensities. Keeping in mind the possible diurnal variations of `A' and g_s, all observations were made between 9 AM and 12 noon on a bright sunny day.

CO_2 response curves

CO_2 response curves refer to the pattern of increase in assimilation rate of leaves with increase in ambient CO_2

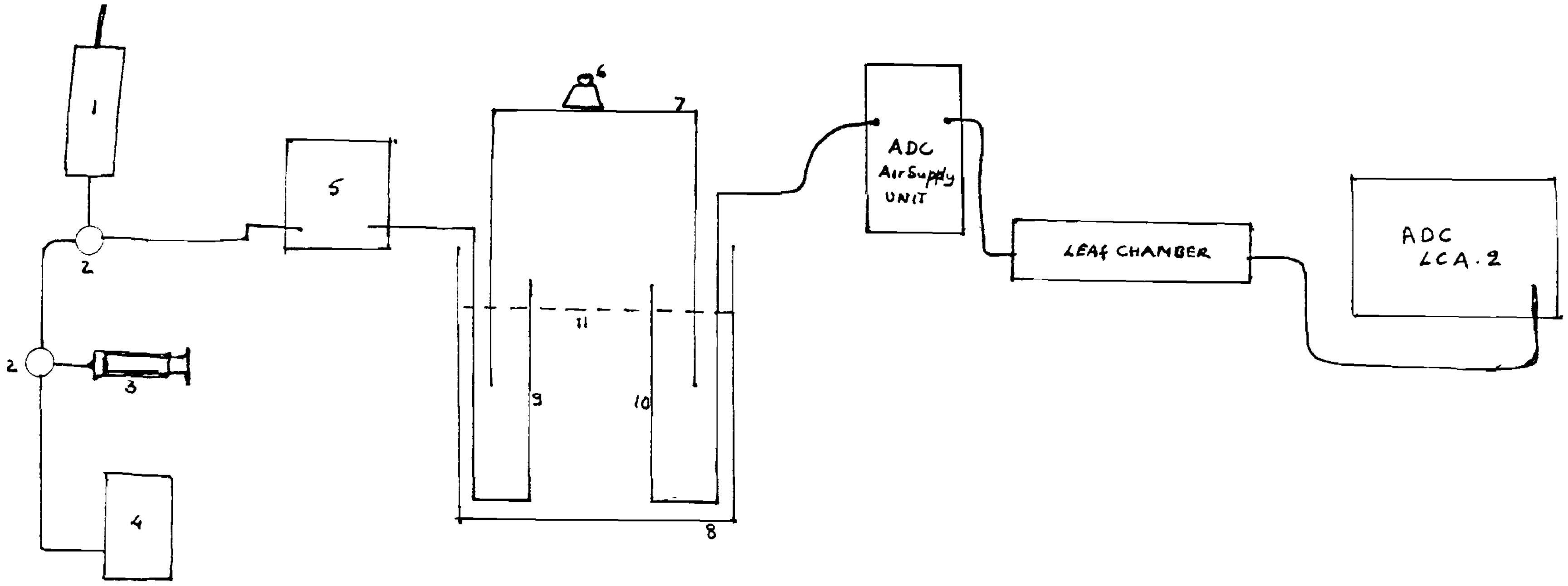


Fig. 1: Set up to obtain different CO_2 concentrations for CO_2 response curves using ADC LCA-2

1. CO_2 scrubber, 2. 3-way valve, 4. CO_2 source, 5. pump, 6. weight, 7. inverted drum, 8. plastic water bath, 9. inlet pipe, 10. outlet pipe, 11. water level.

concentration. For the development of such a CO_2 response curve, different CO_2 concentrations ranging from 50 to 800 ppm have to be developed.

Generation of different CO_2 concentrations

An indigenous and cheap technique was developed to generate different CO_2 concentrations (Sheshshayee, et al., 1992). This devise consists of a 25 lt. aluminum drum placed in its inverted position to float over water in a plastic bucket of 50 lt. capacity (Fig. 1). When filled with air, the drum floats over water in the inverted position and when air is removed, the drum moves down gradually thus proportionately decreasing the volume of air in the drum. An iron structure was provided to support the drum and also to facilitate its easy up and down movement. A copper tube was drawn along the sides of the bucket and brought up to the surface of water at the center of the bucket. Through this tube, air with known CO_2 concentration can be pumped into the inverted drum. And once it is full, air can be drawn out easily with out any alterations in the composition of the air maintained in the drum.

The drums were initially depleted by sucking out the air present in it. Further, air with either lower or higher CO_2 concentrations than the ambient air was pumped under pressure using an air pump.

To obtain CO_2 levels lower than the atmospheric concentration (330 ppm), the ambient air was pumped through a

KOH-Soda lime trap. Air coming out of this trap was free of CO_2. This CO_2 free air was proportionately mixed with air containing 330 ppm CO_2 to generate different concentrations of CO_2 lower than the ambient level.

The following procedure was adopted to obtain CO_2 concentrations higher than the atmospheric level. CO_2 was generated using a Kipp's apparatus by reacting 10 g. of $CaCO_3$ with 20 ml. of 1 N HCl. A known volume of this CO_2 was pumped into the drum using an air pump that pushed atmospheric air. Pumping of atmospheric air dilutes the CO_2 resulting in a known concentration of CO_2 higher than the ambient concentration. For instance, 15 ml. of CO_2 when diluted to 25 lt. in the aluminum drum, gives 600 ppm CO_2 gas mixture. By altering the CO_2 taken for dilution, different concentrations of CO_2 were generated. In all, six different concentrations, three above and three below the ambient concentration were maintained.

The CO_2 concentration was continuously monitored by the IRGA which forms a component of the portable photosynthetic system (ADC LCA 2). The generated CO_2 concentrations remained fairly constant for a period of over 20 minutes. Since the time of exposure of leaves to each concentration of CO_2 is not more than five to seven minutes, possible changes in the concentration of CO_2 does not impose any serious problems while measuring 'A'.

The drums with different CO_2 concentrations were connected seperately to the ASU.

Development of a CO_2 response curve

Assimilation rates were measured on the top fully expanded leaf (all observations were made at saturated light intensity unless otherwise mentioned). The selected leaf was clamped to the leaf chamber and held perpendicular to the incident light. Relative humidity in the leaf chamber was maintained at a steady state level similar to the ambient RH by manipulating the rate of flow of dry air from the ASU. Data were recorded after 'A' and g_s stabilized to a new steady state level.

Based on the measured 'A' and g_s, the inter-cellular CO_2 concentration (Ci) was computed using the following formula.

$$Ci = \{(g_c - E/2) \times C_c - A\}/\{g_c + E/2\}$$

where g_c = total conductance to CO_2 ($moles.m^{-2}.s^{-1}$)
E = transpiration rate ($moles.m^{-2}.s^{-1}$)
C_c = CO_2 concentration in the cuvette (ppm)
A = assimilation rate (μ $moles.m^{-2}.s^{-1}$)

The data logger of the portable photosynthesis system is equipped with these formulae and the instrument instantaneously calculates Ci and is stored in the memory. 'A' was plotted against Ci using a best fit polynomial equation of the nature $Y = aX^2 + bX + c$.

Quantification of relative stomatal and mesophyll limitations of assimilation rate

CO_2 response curves were generated to quantify the relative stomatal and mesophyll limitations of 'A' in the top fully expanded leaves of the crop plants. Linear resistance analysis of the CO_2 response curves as proposed initially by Farquhar and Sharkey (1982) was adopted to compute the relative stomatal limitations of `A'. This approach was further modified to quantify the relative mesophyll limitation of `A'. Brief explanation of the procedures are given below.

Quantification of relative stomatal limitation of `A'

CO_2 response curves were developed in the top fully expanded leaves (Fig. 2). The observed photosynthetic rate at saturated light intensity and ambient CO_2 concentration (330 ppm) was measured and denoted as `A' on the CO_2 response curve. The inter-cellular CO_2 concentration would be less than the ambient CO_2 level. This reduction in Ci compared to Ca is due to the diffusive resistances offered by stomatal as well as mesophyll factors.

If stomata were not to limit the diffusion of CO_2 to the inter-cellular spaces, in other words, at infinite g_s, one would expect that the CO_2 concentrations between ambient air and inter-cellular spaces would equilibrate at 330 ppm. The assimilation rate at this Ci would therefore be devoid of the limitation of `A' by stomatal conductance. If $g_s$ does not limit `A', the assimilation rate increases to a new steady state level, owing to the increase in Ci. It is denoted as A_o on the CO_2 response curve. It would hence be correct to assume that `A' is reduced from $A_o$ to `A' due to the limitations imposed by stomatal conductance. The proportion of decrease in

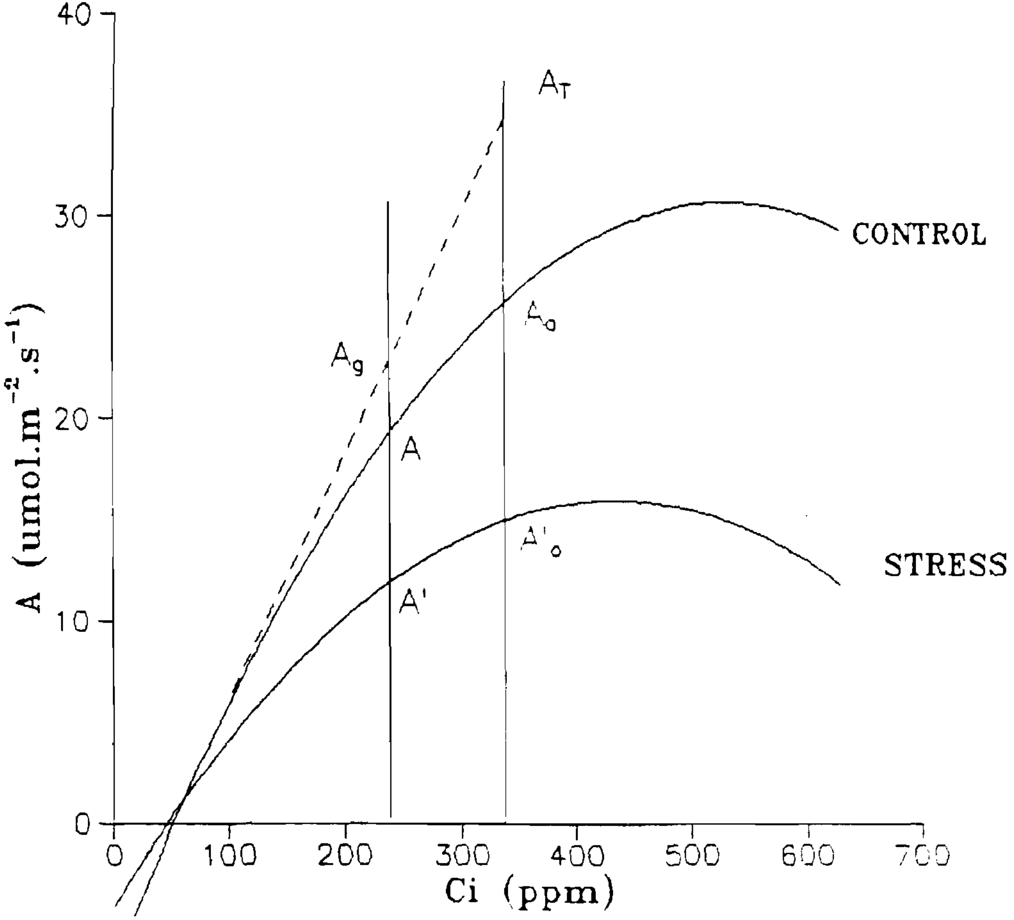


Fig. 2: Hypothetical CO_2 response curves under

assimilation rate from A_o to `A' can be taken as relative stomatal limitation expressed in percentage (l_s %).

$$l_s = \frac{A_o - A}{A_o} \times 100$$

where l_s = relative stomatal limitation of `A'
A_o = assimilation rate where stomatal factors are not limiting but mesophyll factors limit `A'
A = observed assimilation rate at ambient CO_2 and saturated light intensity.

Quantification of relative mesophyll limitation of `A'

At the initial region of the CO_2 response curve, the rate of increase in `A' per unit increase in Ci (dA/dCi) is maximum compared to any part of the curve. It is therefore assumed that the initial response of `A' to Ci represents the potential *in vivo* carboxylation efficiency of a leaf. If this carboxylation efficiency were to remain high at all inter-cellular CO_2 concentrations, the CO_2 response curve would be a linear function. However in reality, the response of `A' to Ci follows a hyperbolic function. A tangential extension of the linear region of the $CO_2$ response curve depicts a hypothetical response curve of `A' to Ci when mesophyll factors are not limiting assimilation rate.

If the stomata are imposing a limitation to `A', the Ci would be lower than the Ca. Therefore to estimate the assimilation rate when mesophyll factors are not limiting, a line perpendicular to the X axis was drawn at operating Ci (when Ca = 330 ppm). The point of inter section of this line and the hypothetical tangential line was denoted as A_g (Fig. 2). The assimilation rate decreased from A_g to 'A' due to the limitation imposed by the mesophyll factors. Thus the mesophyll limitation was computed in percentage as follows.

$$l_m = \frac{A_g - A}{A_g} \times 100$$

where l_m = relative mesophyll limitation of 'A'
A_g = potential assimilation rate that is not limited by mesophyll factors but limited by stomatal factors

Determination of _in vivo_ carboxylation efficiency (dA/dCi)

Unlike the stomatal conductance, there are no direct methods to measure CO_2 conductance in the mesophyll cells. It is extremely difficult to measure the mesophyll diffusive conductance to CO_2 transfer owing to a large number of factors determining mesophyll conductance (g_m).

Using the CO_2 response curves, a new method was standardized to determine the mesophyll conductance. It is often quoted that the response of 'A' to Ci is linear when Ci is extremely limiting. This linear portion of the CO_2 response curve is often considered as the _in vivo_ carboxylation efficiency (Farquhar and Sharkey, 1982). Since the carboxylation process actually controls the entry of CO_2 into the mesophyll, the measured carboxylation efficiency can be considered as an indirect estimate of the mesophyll conductance.

Two methods were standardized to calculate the initial response of `A' to Ci as an estimate of in vivo carboxylation efficiency.

The initial linear portion of the CO_2 response curve was extended by drawing a tangent to this portion. The slope of this straight line was measured and expressed as mesophyll conductance or in vivo carboxylation efficiency (Fig. 2).

The second method involved a mathematical derivation of the initial slope is as follows.

As mentioned earlier, the CO_2 response curves were fitted into a second degree polynomial function.

$$Y = aX^2 + bX + c \text{ ----------(1)}$$

where Y = `A'

X = Ci

Differentiating this equation we get,

$$dY/dX = 2aX + b \text{ ----------(2)}$$

Substituting a Ci value (at limiting concentration range) in equation (2), we get the rate of increase in `A' per unit increase in Ci. This differential coefficient was taken as an indication of the in vivo carboxylation efficiency (g_m).

Quantification of relative stomatal and mesophyll limitations of 'A' under abiotic stress conditions

CO_2 response curves were employed to apportion the relative stomatal and mesophyll limitations of 'A' in plants experiencing different abiotic stresses. CO_2 response curves were developed as explained earlier. The same approach was adopted for the quantification of limitations (Fig. 2).

Quantification of relative stomatal limitation of 'A' under stress

The assimilation rate of stressed leaves was measured and marked on the CO_2 response curve as A'. The assimilation rate at infinite stomatal conductance where Ci is expected to equilibrate with Ca was determined and marked on the CO_2 response curve as A_o'. This assimilation rate represents the possible assimilation rate when stomatal limitations were removed. Therefore, the assimilation rate in a stressed leaf decreased from A_o' to A' under stress due to stomatal limiting factors. Hence, the relative stomatal limitation of 'A' under an abiotic stress condition can be calculated by the following formula.

$$l_s = \frac{A_o' - A'}{A_o'} \times 100$$

where A_o' = potential assimilation rate when stomatal factor are not limiting but mesophyll factors are limiting under stress.

A' = observed assimilation rate under stress.

Quantification of relative mesophyll limitation of `A' under stress

It has been sufficiently documented in the literature that any abiotic stress affects assimilation rate by its effect on the mesophyll factors. As explained earlier, the initial slope of the CO_2 response curve serves as a tool to approximate the mesophyll conductance. Any reduction in this initial slope of CO_2 response curve under stress can be taken as an indication of the stress effect on the mesophyll factors. Thus assimilation rate measured at ambient CO_2 levels of a stressed leaf can be assumed to be reduced from the theoretically possible assimilation rate when mesophyll factors are not limiting `A' (i.e., A_g on the CO_2 response curve). The CO_2 response curve and the various points marked are depicted in fig 2.

The mesophyll limitation of `A' under an abiotic stress can be calculated from the following formula.

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

where A_g = potential assimilation rate of the control plant not limited by the mesophyll factors but limited by stomatal factors.

A' = observed assimilation rate under stress.

Estimation of stomatal and assimilation loop gains

From the calculation of relative limitations, it is evident that the mesophyll limits `A' more than the stomatal factors under abiotic stress conditions. The reduction in g_s can therefore be attributed to the feedback control of g_m on g_s. To assess this hypothesis the loop gain analysis proposed by Farquhar et al. (1980) was adopted.

Approach and methodology

There are two important loop gains, namely, stomatal loop gains and assimilation loop gains.

Stomatal loop gain (G_g): It is defined as the product of the physical gains and physiological gains of stomatal conductance.

$$G_g = \begin{array}{c}\text{Physical gain of}\\ \text{conductance}\end{array} \times \begin{array}{c}\text{Physiological gain}\\ \text{of conductance}\end{array} \qquad --- (1)$$

The physical gain of stomatal feedback loop is defined as the partial differential co-efficient of Ci with respect to g_s, when `A' and Ca are kept constant.

Therefore, $\delta Ci/\delta g_s = 1.6\ A/g_s$

Since Ci increases with an increase in g_s, when `A' and Ca are held constant the physical gain of stomatal feedback takes a positive value.

The physiological gain of the stomatal feedback loop is defined as the differential co-efficient of g_s with respect to Ci (dg_s/dCi). It is proved that g_s decreases when the Ci is increased. Therefore, dg_s/dCi takes a negative significance. The physiological gain of stomatal feedback can be obtained by taking the slope of the curve fitted for g_s against the Ci at the operating Ci (Where Ca = 340 ppm). Therefore equation (1) becomes,

$$G_g = (\delta Ci/\delta g_s) \times (dg_s/dCi)$$

Assimilation loop gain (G_A):

It is defined as the product of the physical gain and the physiological gain of the assimilation rate.

$$G_A = \text{Physical gain of assimilation} \times \text{Physiological gain of assimilation} \quad \text{--- (2)}$$

Physical gain of assimilation loop is defined as the partial derivative of Ci with respect to 'A' when Ca and g_s are kept constant. This is obtained by the following partial differential equation.

$$\delta Ci/\delta A = -1.6/g_s$$

Since, at a constant Ca and g_s, Ci decreases as 'A' is increased, the physical gain of assimilation loop assumes a negative value.

Physiological gain of assimilation loop is defined as the differential co-efficient of 'A' with respect to Ci (dA/dCi). This can be obtained by the slope at the operating Ci of the assimilation rate curve fitted against Ci. Since 'A' increases with increase in Ci, the physiological gain of assimilation loop takes a positive significance. Therefore, equation (2) becomes,

$$G_A = (\delta Ci/\delta A) \times (dA/dCi)$$

Quantification of the specificity factor of rubisco

The enzyme RuBISCO is known to catalyze the carboxylation of RuBP leading to photosynthesis as well as the oxygenation of RuBP leading to photorespiration. The net assimilation rates of the leaves is the ratio of the relative velocity of carboxylation and oxygenation. The *in vivo* velocities of these individual processes depend on the kinetic constant associated with the enzyme. The ratio of the kinetic constant of oxygenation and carboxylation reaction is further termed as the specificity factor. To assess the effect of moisture stress on the specificity factor of RuBISCO a gas exchange technique was adopted as explained by Brooks and Farquhar (1985).

Methodology

The ratio of carboxylation to oxygenation depends on the specificity factor of Rubisco, CO_2, and O_2 concentrations. This is given by the following equation,

$$v_c/v_o = \frac{V_c.K_o.\ [CO_2]}{V_o.K_c.\ [O_2]}$$

Where, v_c = Rate of carboxylation
v_o = Rate of oxygenation
V_c = V_{max} for carboxylation
V_o = V_{max} for oxygenation
K_c = Affinity constant for carboxylation
K_o = Affinity constant for oxygenation
$[CO_2]$ = Carbon dioxide concentration

$[O_2]$ = Oxygen concentration

The ratio of kinetic constants is called the specificity factor.

However at a given CO_2 and O_2 concentration, v_c/v_o entirely depends on the specificity factor of Rubisco. Carboxylation efficiency which to a large extent determines the `A', is controlled by Rubisco content, activation state and specificity factor at a given CO_2 and O_2 concentrations, it is essential to estimate the specificity factor of Rubisco to understand the regulatory role of RubisCO in photosynthesis.

There are different approaches to estimate the specificity factor. The traditional and useful method is to estimate the kinetic constants of RubisCO and to calculate the specificity factor under in <u>vitro</u> conditions. But a measurement of <u>in vivo</u> specificity factor would be more realistic under a prevailing environmental condition. Brooks and Farquhar (1985) proposed a novel technique for the estimation of specificity factor under in <u>vivo</u> conditions. The main assumption in this approach was that the specificity factor does not change with the level of activation or with light intensity. Using this assumption a model was developed for the calculation of the specificity factor.

Derivation of the model:

$$v_c/v_o = S \times \frac{[CO_2]}{[O_2]} \quad \ldots\ldots\ldots \quad (1)$$

where, S = Specificity factor

At compensation point, net assimilation rate is zero. Since one mole of CO_2 is released during photorespiration for every two moles of oxygen consumed. Hence the equation (1) becomes,

$0.5\ v_o/v_o = S.[CO_2]/\ [O_2]$, since $v_c = 0.5v_o$

Where the $[CO_2]$ is the Ci (Γ^*) (internal CO_2 concentration) at which v_c is equal to $0.5\ v_o$.

Upon simplification the equation becomes,

$$0.5 = S.(\Gamma^*)/\ [O_2]$$

or

$$S = 0.5.[O_2]/\ (\Gamma^*)$$

Γ^* can be arrived at using gas exchange techniques. This is the Ci at which initial response of three CO_2 response curves taken at three different light intensities meet. This point is less than the CO_2 compensation point since it avoids the CO_2 released from processes other than photorespiration. Γ^* and O_2 concentration should be expressed in molar units (μ moles).

Initial response of `A' to Ci was taken at three different light intensities using ADC-IRGA-LCA-2 model. Neutral polytene filters were used to alter light intensity. Low CO_2 was generated using KOH-sodalime. The linear model was fitted for the data. Internal CO_2 concentration at which all the three lines meet (Γ^*) was taken for the calculation of specificity factor.

VPD response curves

To evaluate the effect of moisture stress on mesophyll factors an attempt was made to enhance the Ci by increasing g_s at a given mesophyll capacity. This experiment was conducted with an hypothesis that if the mesophyll factors are damaged due to moisture stress, and when more CO_2 is provided by enhancing g_s, the excess substrate CO_2 cannot be utilized by the CO_2 fixing machinery. Thus `A' cannot increase. To test this hypothesis, g_s was increased by altering the Vapour pressure deficit (VPD) of the leaf.

The response of g_s to VPD has been clearly documented. The stomatal conductance increases with decrease in VPD. An attempt was made to increase the g_s by decreasing the VPD. The vapour pressure deficit was decreased by increasing the RH of the air around the leaf. It is possible to increase the RH of the air around the leaf in the portable photosynthesis system (ADC LCA 2) in the open system. As explained earlier, RH was increased by decreasing the rate of flow of dry air into the leaf chamber. RH in the leaf chamber was increased to different levels over and above that of the ambient RH by decreasing flow rates of dry air from the ASU. This resulted in increased g_s to different higher values.

Gas exchange data were recorded after the g_s stabilized to new steady state values. The assimilation rates and stomatal conductance values so obtained were plotted against VPD using a personal computer.

CO_2 sensitivity

To assess the mesophyll capacity for carbon assimilation, calculating the CO_2 sensitivity is yet another approach. the response of assimilation to an increased CO_2 concentration in the inter-cellular spaces can be considered as a reflection of the functional ability of the mesophyll factors. This response of `A' to increased Ci is generally refereed to as the CO_2 sensitivity.

The CO_2 sensitivity of a leaf was determined by measuring the assimilation rate ambient and saturated CO_2 concentrations. The proportion of increase in `A' from ambient to saturated CO_2 levels was considered as the CO_2 sensitivity using the following formula.

$$CO_2 \text{ sensitivity} = \frac{A_{660} - A_{330}}{A_{660}}$$

Photorespiration based on APS and TPS

Stress associated changes in photorespiration is one among the most important changes in the mesophyll. Evaluating the role of photorespiration as an important component of the mesophyll would be a plausible objective to understand a stress induced reduction in assimilation rates of crop plants.

In the present investigation, photorespiration was calculated based on the measurement of Apparent photosynthesis (APS) and Total photosynthesis (TPS). the apparent photosynthesis was measured, as explained earlier, at ambient CO_2 and O_2 concentrations. An air mixture of 330 ppm CO_2 and 2% O_2 was supplied to the leaf and the assimilation rate was measured using the portable photosynthesis system (ADC LCA). This low O_2 levels almost totally inhibits oxygenation making provision for an increased flux of RuBP towards carboxylation resulting in an increased assimilation rate. This higher assimilation rate was measured and denoted as true or total photosynthesis which is devoid of photorespiration. The difference between TPS and APS is therefore an estimate of photorespiration. The proportion of photorespiration of the total photosynthesis was estimated using the following formula.

$$PR = \frac{TPS - APS}{TPS} \times 100$$

Quantification of total leaf soluble protein

One gram of the leaf material was taken and ground with 5 ml. of tris-HCl buffer (pH 8.5) in a pestle and mortar. a pinch of EDTA and PVP was added to the leaf material before grinding. The ground material was centrifuged at 5000 g for 10 mins. at 4°C. The supernatant was used for estimating the total soluble protein.

Two ml. of the clear supernatant was taken and protein was precipitated by adding 2 ml. of 10 per cent TCA (Trichloro acetic acid). After centrifugation at 10000 g. for 10 min. the precipitate was redissolved in 5 ml. of 1 N NaOH. From this NaOH solution, 0.1 ml. of aliquot was taken and volume was made up to 1 ml. with distilled water. The total protein content was then estimated using FCR (Folin Ciocalteu Reagent) method (Lowry et al., 1951).

FCR method for protein estimation

To the NaOH aliquot, 5 ml. of reagent C was added and stirred vigorously for 10 min. Then 0.5 ml. of reagent D was added mixed immediately and incubated for 30 min. at room temperature. Optical density was recorded at 660 nm and the protein content was estimated using a standard curve developed earlier for Bovine serum albumin (BSA).

Preparation of reagents

Reagent A: 2% Na_2CO_3 in 0.1 N NaOH
Reagent B: 0.5% $CuSO_4.5H_2O$ in 1% sodium citrate.
Reagent C: 2 ml. of reagent B mixed with 100 ml. of reagent A just before use.
Reagent D: Folin Ciocalteu reagent.

Raising of plant material

Plant materials were raised in carbonized rubber containers (20cm X 10cm X 20cm). The pots were filled with 10 kg. of red sandy loam soil. Plants were thinned down gradually to maintain a final population of two plants per container.

Plants were grown at field capacity under normal solar radiation for a period of 35 to 40 days. Nutrients were supplied to the potted plants as per the recommendations in the package of practices. These plants were used for all the experiments. Different stresses were imposed on these plants as shall be explained under the respective experiments. All gas exchange parameters and CO_2 response curves were generated on the top fully expanded leaves of these plant species using a portable photosynthesis system (ADC LCA 2). Observations were recorded on a full sunny day between 9 AM and 12 noon.

Making use of these methodologies and approaches, experiments were conducted on the following three major aspects to meet the objectives of the investigation.

1. Assessment of A_{max} and apparent photosynthesis in sunflower and maize.
2. Quantification of relative limitations of `A' in a few C_3 and C_4 plants under non stress conditions.
3. Gas exchange parameters and quantification of relative limitations of `A' in plants experiencing various abiotic stresses.

A_{max} and apparent photosynthesis in sunflower and maize

To assess the potential of the chloroplast, an experiment was conducted with pot grown sunflower and maize plants. Plants were grown in plastic containers for 35 to 40 days. Care was taken to maintain only two plants per pot. Water and nutrients were supplied as per the requirements to grow the plants under non limiting input conditions at normal light intensities.

The following observations were recorded in this experiment.

1. Apparent photosynthetic rate (`A')
2. Assimilation rate at saturated Ci (A_{max})

Determination of A_{max}

Assimilation rates were recorded in the same leaf at ambient and elevated CO_2 concentrations (660 ppm) under saturated light intensities. The CO_2 concentration was increased in the leaf chamber of the portable IRGA by the indegenously developed CO_2 source (Sheshshayee et al., 1992) as explained earlier. The assimilation rate measured at this saturated CO_2 concentration was considered as A_{max}.

Quantification of limitations of photosynthesis in a few C_3 and C_4 species under non stress conditions.

To assess the gas exchange traits and to quantify the relative stomatal and mesophyll limitations of `A' in well watered plants, a pot culture experiment was conducted.

A. Gas exchange and limitations of `A' under non stress conditions.

The following species were grown in carbonized rubber containers as explained earlier for 35 to 40 days.

C_3 species,	C_4 species
sunflower (<u>Helianthus annuus</u>)	Sorghum (<u>Sorghum bicolor</u>)
soybean (<u>Glycine max</u>)	Maize (<u>Zea mays</u>)
cowpea (<u>Vigna unguiculata</u>)	Ragi (<u>Elusine coracana</u>)

The following observations were recorded.

1. Gas exchange parameters like 'A', g_s and Ci were recorded on the top fully expanded leaf using a portable photosynthesis system (ADC LCA 2) as explained earlier.

2. Based on the CO_2 response curves, relative stomatal limitation (l_s %) and mesophyll limitation (l_m %) were computed as explained earlier.

3. The <u>in vivo</u> carboxylation efficiency was assessed by measuring dA/dCi as explained earlier.

B. Effect of partial defoliation on gas exchange parameters

The major objective of this experiment was to change the g_s by altering the root to leaf area ratio. Our preliminary experiment conducted with sorghum indicated that stomatal conductance significantly increased by partial defoliation.

Imposition of partial defoliation

Sunflower, sorghum and soybean plants were grown in carbonized rubber containers for a period of 35 to 40 days at field capacity under saturated light intensities. Fifty percent of the leaves were randomly removed from the plants early in the morning (after sunrise). The following observations were recorded after four and 24 hours after defoliation.

Observations recorded

1. Gas exchange parameters using the portable IRGA
2. CO_2 response curves
   a. A v/s Ci curves
   b. g_s v/s Ci curves, as explained earlier.

Gas exchange and relative stomatal and mesophyll limitations of 'A' under different abiotic stresses

To evaluate the reduction in gas exchange traits and to quantify the relative limitations imposed by the stomatal and mesophyll factors on 'A', different abiotic stresses like moisture stress, low light stress and nitrogen deficiency, experiments were conducted on the following three aspects.

A. Quantification of limitations under different abiotic stresses.

B. Effect of enhanced g_s on 'A' in plants subjected to moisture stress

C. To evaluate the reasons for the high relative mesophyll limitations of 'A' under moisture stress.

A. Quantification of relative stomatal and mesophyll limitations of 'A' under different abiotic stresses

Photosynthetic traits are modulated to a large extent by the existing environmental factors. To assess the effect of a few important environmental factors on gas exchange parameters, an experiment was conducted with plants subjected to different abiotic stresses

a. Effect of moisture stress

To assess the effect of different degrees of moisture stress on gas exchange parameters and to quantify the limitations of photosynthesis, sunflower, cowpea and sorghum plants were subjected to moderate and severe moisture stress conditions.

Imposition of moisture stress

Sunflower, cowpea and sorghum plants were grown in well watered condition for 35 to 40 days in carbonized rubber containers. Different degrees of moisture stress were imposed on these plants as follows.

By altering the irrigation schedule, well watered plats were brought to 50 percent field capacity and grown in that condition for seven days. These sets of plants were designated as moderately stressed. Another set of well watered plants were brought down to 25 percent field capacity and grown in that condition for seven days. This way the plants were subjected to severe moisture stress.

Observations recorded

On the top fully expanded leaves of these species experiencing different degrees of moisture stress, the following observations were recorded.

1. Gas exchange parameters like `A', g_s and Ci were recorded using a portable photosynthesis system.

2. CO_2 response curves were generated and the relative stomatal and mesophyll limitations were quantified as explained earlier.

b. Effect of low light stress

To assess the effect of low light stress on the gas exchange parameters and to quantify the relative stomatal and mesophyll limitations of `A', sunflower and sorghum plants were grown under two low light intensities.

Imposition low light stress

With an objective to assess the extent of reduction in gas exchange traits and to quantify the relative limitations of `A' under low light conditions, sunflower and sorghum plants were subjected to different degrees of low light stresses as follows.

Sunflower and sorghum plants were grown at field capacity under natural light intensity in containers for 35 to 40 days. A set of these well grown plants were shifted to a poly house which decreases the light intensity uniformly by 40 per cent (light intensty maintained was 1000 μ moles.m^{-2}.s^{-1}) and grown in that light intensity for seven days.

Similarly another set of well watered plants were shifted to a very low intensity treatment of 500 μ moles.m^{-2}.s^{-1}. and grown in that light intensity for a period of seven days. These plants experienced a severe low light treatment.

Observations recorded

In the stressed sunflower and sorghum plants the following observations were recorded at the growth light intensities.

1. Gas exchange parameters like 'A', g_s and Ci were recorded using a portable IRGA as explained earlier.

2. CO_2 response curves were generated in the respective growth light intensity and the relative stomatal and mesophyll limitations were quantified.

c. Effect of nitrogen stress

Nitrogen deficiency stress is yet another common abiotic stress known to reduce assimilation rates. To evaluate the effect of nitrogen stress on gas exchange parameters and to quantify the limitations of 'A', sunflower plants were subjected to nitrogen stress.

Imposition of Nitrogen deficiency

To assess the effect of nitrogen deficiency on photosynthetic traits, sunflower plants were grown in rubber containers with low nitrogen nutrition. These plants were supplied with only 50 percent of the recommended dose of fertilizer nitrogen (50 kg/ha) both during basal application and top dressing. In another set of pots sunflower plants were grown with full doses of fertilizer nitrogen (50 kg/ha) to serve as control.

Observations recorded

1. Gas exchange parameters like 'A', g_s and Ci
2. CO_2 response curves were generated at saturated light intensities and the relative stomatal and mesophyll limitations were quantified as explained earlier.

Loop gain analysis

In the present investigation, the reduction in g_s was much more than the reduction in g_m. To test whether g_m controlled g_s, a Loop gain analysis was done using the gas exchange data obtained for moisture stress and light stress as explained earlier.

B. Effect of enhanced g_s on `A' in plants subjected to moisture stress

The results of the previous experiments clearly suggested that though the stomatal conductance decreased under stress, the stomatal limitation on `A' either did not change or showed a marginal change in all the abiotic stresses. To further probe into these aspects, the stomatal conductance was enhanced by altering the VPD.

VPD response curves

The response of `A' and g_s to changing vapour pressure deficit was monitored using a portable photosynthesis system as explained earlier.

C. Assessing the reasons for a high mesophyll limitation of photosynthesis in plants subjected to moisture stress

The data of earlier experiments clearly suggested that the relative mesophyll limitation of `A' significantly increased under any abiotic stress. It was also evident that though $g_s$ decreased under stress, the stomata did not offer any significant limitations on `A' under stress. An experiment was conducted to assess the possible reasons of a high mesophyll limitations of 'A' in sunflower and cowpea plants subjected to moisture stress.

Imposition of moisture stress

Sunflower, cowpea and maize plants were subjected to moisture stress by maintaining 35 to 40 days old well watered plants at 50 percent field capacity for seven days. The following parameters were recorded at the end of the stress period.

1. Relative water content of the leaves.
2. Gas exchange parameters
3. CO_2 sensitivity
4. Photorespiration based on APS and TPS measurements
5. Total protein content
6. Specificity factor of rubisco based on gas exchange techniques

Relative Water Content (RWC)

To assess the moisture status of the leaf, relative water content was estimated. The procedure adopted was as follows.

Leaf punches were obtained from stressed and control leaves. After recording the fresh weight of the punches, they were immersed in water in a petri plate and kept in darkness for 24 hours. And the leaf punches were kept in darkness to avoid a possible increase in dry matter due to photosynthesis. After 24 hrs. of incubation in darkness, the leaf punches were carefully blotted using a filter paper and the turgid weight was recorded. The leaf punches were oven dried at 70° C for 4 to 5 days and dry weight was recorded. The relative water content was determined using the following formula.

$$\mathrm{RWC} = \frac{\text{Fresh wt.} - \text{Dry wt.}}{\text{Turgid wt.} - \text{Dry wt.}} \times 100$$

Gas exchange parameters

The gas exchange parameters like `A,' g_s and Ci were recorded in the top fully expanded leaf of stressed plants. All gas exchange data were recorded using a portable photosynthesis system (ADC LCA 2) as explained earlier.

CO_2 sensitivity

Reduction in mesophyll capacity for carbon assimilation is often determined by studying the response of `A' to increase in Ci. To assess the effect of moisture stress on the mesophyll capacity, CO_2 sensitivity was measured as explained earlier.

Photorespiration

Photorespiration has been documented as an important parameter that mediates a stress induced inhibition of photosynthesis. To assess the role of photorespiration in reducing `A' in water stressed sunflower and cowpea, assimilation rates were measured at 21 percent and 2 percent O_2 and 330 ppm CO_2 concentrations. The details of the approach of quantifying the PR is explained earlier.

Total protein content

When plants experience moisture stress, reduction in the total soluble protein content is a generally observed phenomenon. To test the reduction in total soluble protein content of the stressed leaf, sunflower and cowpea plants were subjected to moisture stress as explained earlier. The total soluble protein content of the leaves was quantified using the Follin Ciocoleu method.

Specificity factor of rubisco based on gas exchange technique

Reduction in the *in vivo* carboxylation efficiency is one of the most important factors resulting in the inhibition of photosynthesis under moisture stress. The factors associated with rubisco such as its content, activation state, specific activity and constants of enzyme kinetics have been shown to be important determinants of carboxylation. With an objective to assess the ratio of the kinetic constants of carboxylation and oxygenation, the specificity factor of rubisco was measured based on a simple gas exchange technique. The detailed methodology of the procedure adopted is explained earlier.

RESULTS

RESULTS

Photosynthesis is finely regulated by both intrinsic plant and environmental factors. Under controlled environmental conditions, the diffusive characteristics of stomata and mesophyll regulate the rate of carbon assimilation by the leaves. Hence, the regulation of photosynthesis is broadly studied under stomatal and mesophyll factors. Various environmental factors affect assimilation rate (\`A') by their differential effects on stomatal and mesophyll factors. Therefore, the major emphasis of the present investigation was to assess and quantify the limitations of \`A' imposed by stomatal and mesophyll factors.

Since stomatal conductance (g_s) and mesophyll conductance (g_m) vary greatly between species and also within a species, the relative limitation on \`A' imposed by stomata (ls %) and by mesophyll factors (lm %) are specific to species. Because of a considerable control of g_s and g_m by constantly changing environment, the ls and lm are dependent and specific to changes in the environmental factors also. These limiting factors, therefore, reduce the potentiality of carbon assimilating machinery of the leaf. A series of experiment were conducted with the following broad objectives.

1. To assess the potential carbon assimilation rates of chloroplast in a few species.
2. To quantify the relative limitations of \`A' in a few C_3 and C_4 species under non-stress conditions.

3. To quantify the relative limitation of 'A' under different abiotic stress conditions.

4. To evaluate a few mesophyll subcomponents under moisture stress

A_{max} and apparent photosynthesis in sunflower and maize

Measuring the maximum photosynthetic rate (A_{max}) at saturated CO_2 concentrations of a leaf is one of the approaches to assess the potential capacity of the leaf to fix carbon. A_{max} was quantified by measuring 'A' at saturated inter-cellular CO_2 concentration in sunflower and maize as explained earlier.

Assimilation rates were measured at saturated and ambient CO_2 concentrations in 30 to 40 day old sunflower and maize plants. The associated gas exchange data are presented in table 1.

Both sunflower and maize leaves showed a significant increase in assimilation rates at saturated Ci. In sunflower A_{max} was 91 percent higher than 'A' recorded at ambient CO_2 concentration. Whereas, maize recorded a 37 percent increase in 'A' when Ci was at saturated levels. The data clearly indicate that the chloroplasts have a significantly higher potential to fix carbon both in C_3 and C_4 plants. Although the C_4 plants have an intrinsic CO_2 saturating mechanism operating, still respond to increase in CO_2 supply. Therefore substrate availability forms an important limitation for assimilation under ambient conditions. The diffusive conductances through the

Table 1: A_{max} and apparent photosynthesis (A) in sunflower and maize

Crop	A	A_{max}	% increase
Sunflower	20.64	39.5	91.37
Maize	25.50	35.0	37.25
CD (p=0.05)	1.05	2.3	

stomata, hence, could act as a prominent limiting factor in well watered plants. However, at a given lower inter- cellular CO_2 concentrations, the inadequate functioning of the mesophyll components could also be partly responsible for the lower assimilation rates of crop plants.

Quantification of limitations of photosynthesis in a few C_3 and C_4 species.

With an objective to assess the gas exchange parameters at ambient CO_2 and to quantify the relative stomatal and mesophyll limitations to the apparent assimilation rate ('A'), a pot culture experiment was conducted with the following C_3 and C_4 crop species. Sunflower (Helianthus annuus.L); Cowpea (Vigna unguiculata.L) and Soybean (Glycine max.L) all C_3 species. Sorghum (Sorghum bicolor.L); Maize (Zea mays.L) and Finger millet (Elusine coracana.G) all C_4 species. The results obtained are presented in this chapter.

A. Gas exchange parameters and limitations of 'A' under non stress conditions

Plants were grown in battery containers under well watered condition. Nutrients were supplied by appropriately calculating the required fertilizers as recommended by the package of practices. After 30-40 days of growth, the top fully expanded leaves were used to record the gas exchange parameters with a Portable IRGA (ADC model LCA-2) in the open system. The data are presented in table 2.

Assimilation rate (‘A’):

The C_3 plants showed lower assimilation rates (‘A’) than the C_4 plants. But Sunflower recorded an ‘A’ of 20.64 μ moles $CO_2.m^{-2}.s^{-1}$ which was not much different from that of Sorghum which recorded 20.00 μmoles.$m^{-2}.s^{-1}$ (Table 1). Soybean showed the least ‘A’ (13.5 μ moles.$m^{-2}.s^{-1}$) where as Maize had the highest of 25.5 μ moles.$m^{-2}.s^{-1}$.

Stomatal conductance (‘g_s’):

The stomatal conductance for water vapour was relatively low in C_4 species. However, only Sunflower showed the maximum ‘g_s’ of 729 mmoles.$m^{-2}.s^{-1}$. where as Cowpea and Soybean recorded 420 mmoles.$m^{-2}.s^{-1}$ and 340 mmoles.$m^{-2}.s^{-1}$, respectively (Table 2). The C_4 species that are known to have lower stomatal conductance recorded 284 mmoles.$m^{-2}.s^{-1}$ and 340 mmoles.$m^{-2}.s^{-1}$, respectively in Sorghum and Maize (Table 2)

Inter-cellular CO_2 concentration (Ci)

All the C_3 species had significantly higher Ci at ambient CO_2 levels than the C_4 plants. Maize recorded the minimum Ci of 140 ppm as against 260 ppm in Sunflower followed by 240 ppm in Soybean.

Ratio of ‘A’ to Ci (A/Ci):

The assimilation to inter-cellular CO_2 concentration is often interpreted as the efficiency of mesophyll components (chloroplast efficiency for carbon fixation). The C_4 plants had

Table 2 : Gas exchange parameters in a few C_3 and C_4 species

Crop	‘A’	‘g_s’	Ci	A/Ci	A/g_s	A_{max}	Ci*
Sunflower	20.64	729	260	0.097	0.029	39.5	75
Cowpea	14.78	420	200	0.074	0.035	23.5	60
Soybean	13.50	340	240	0.056	0.039	27.0	49
Sorghum	20.00	284	160	0.125	0.070	34.0	15
Maize	25.50	340	140	0.182	0.075	35.0	10
Finger millet	18.60	-	200	0.093	-	36.0	12
CD (p=0.05)	4.32	50.8	35.1				5.6

Ci* = CO_2 compensation point (ppm)

significantly higher A/Ci ratios compared to the C_3 plants indicating that the C_4 plants have higher efficiency of carbon fixation than the C_3 plants at a given substrate level. Maize recorded the highest A/Ci ratio of 0.182 μ moles.$m^{-2}.s^{-1}.ppm^{-1}$ followed by Sorghum (0.125 μ moles.$m^{-2}.s^{-1}.ppm^{-1}$). Cowpea and Soybean had low A/Ci ratios of 0.074 and 0.056, respectively.

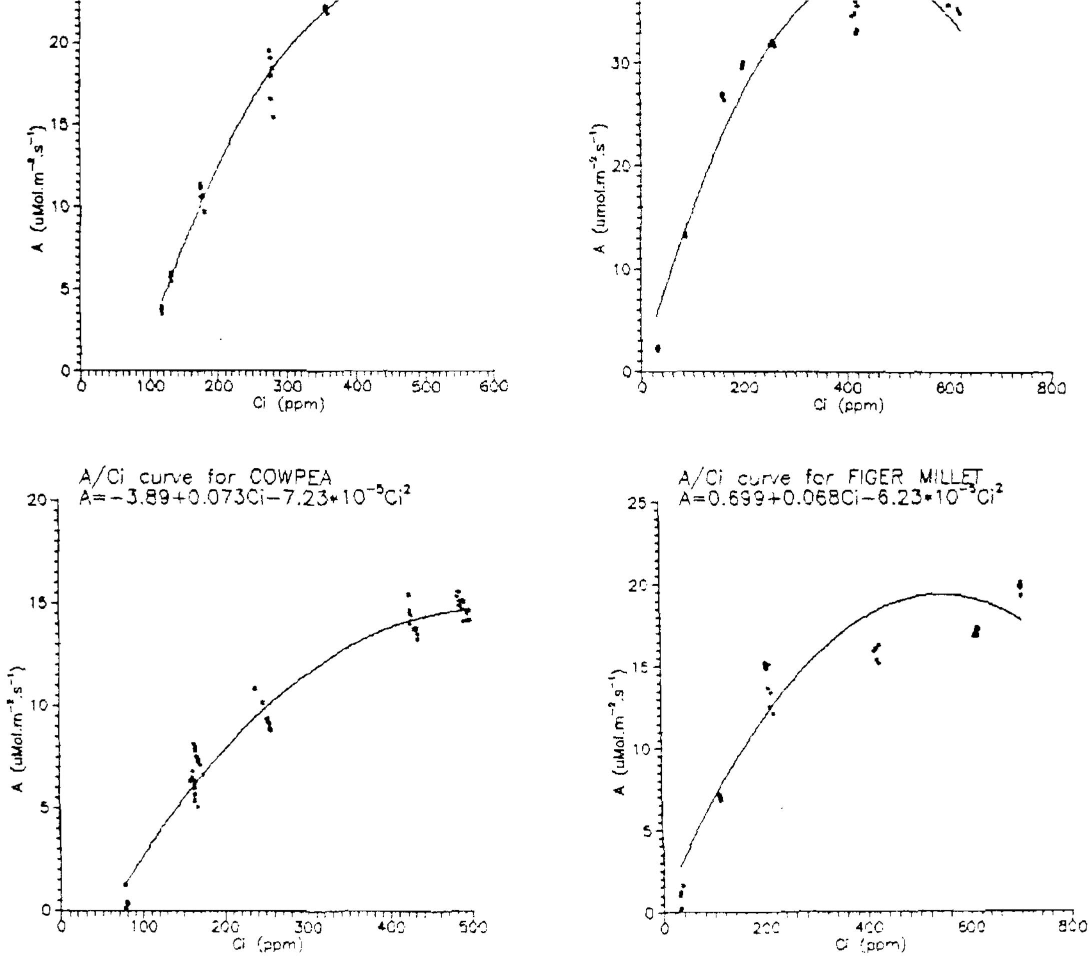
It is evident from the data presented in table 2 that the C_4 plants have higher rates of assimilation per unit internal CO_2 concentration as well as higher assimilation rates per unit stomatal conductance (A/g_s). Though Sunflower had higher `A', compared to other C_3 plants, it had the lowest A/g_s ratio indicating lower water use efficiency.

Quantification of relative stomatal and mesophyll limitations of `A' based on CO_2 response curves

The response of assimilation rate to increasing concentrations of inter-cellular CO_2 was recorded in the above mentioned crop species. The data are illustrated in figures 3 and 4.

The response pattern of `A' to increasing Ci was similar in all the crop plants tested. Assimilation rate in all the species increased almost linearly at the lower CO_2 concentrations (low Ci). Further increase in Ci resulted in a curvilinear response and at a particular Ci, the response became asymptotic.

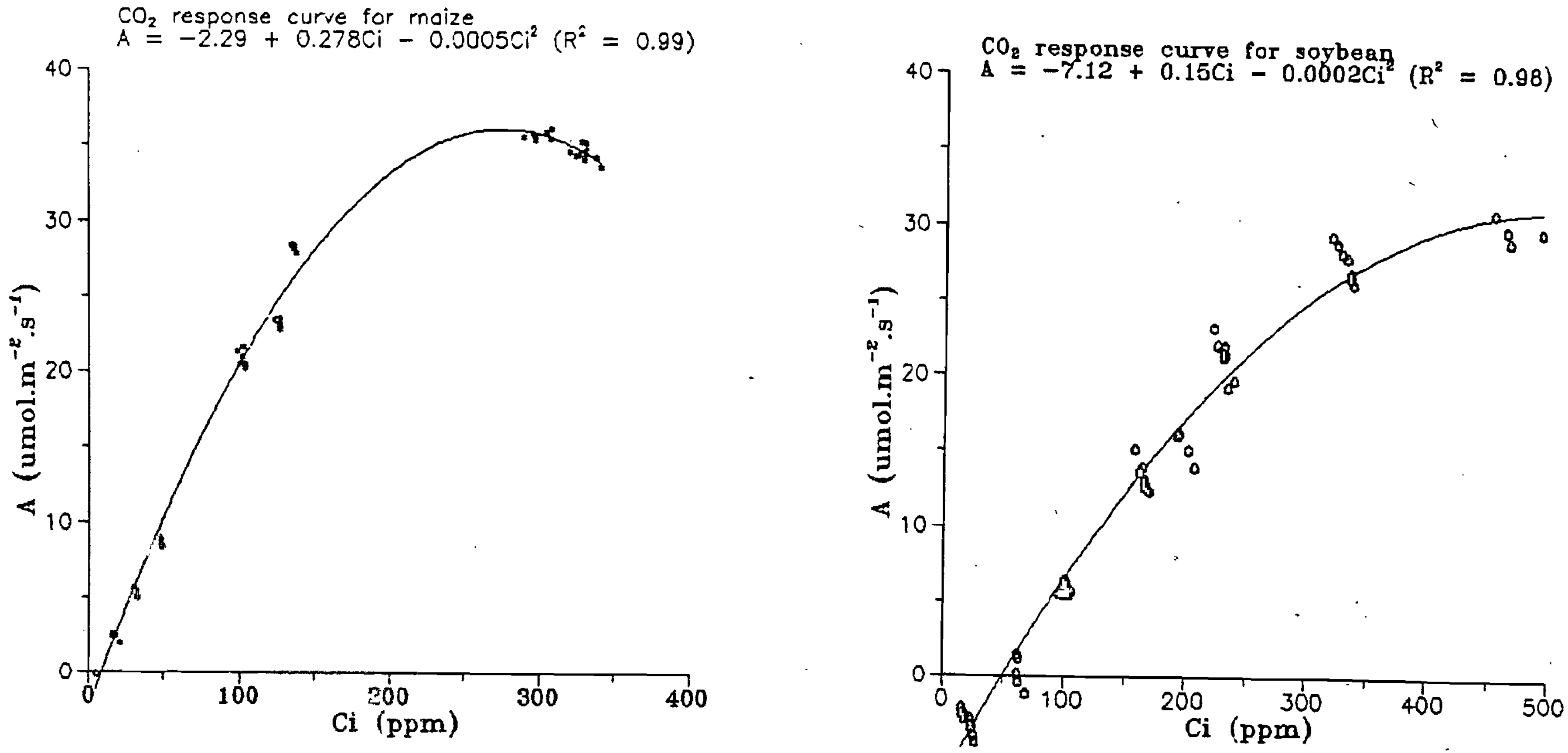
Though the pattern of response of `A' to Ci was similar in



A/Ci curve for COWPEA
$A=-3.89+0.073Ci-7.23*10^{-5}Ci^2$

A/Ci curve for FIGER MILLET
$A=0.699+0.068Ci-6.23*10^{-5}Ci^2$

Fig. 3: CO_2 response curves under non stress conditions


CO_2 response curve for maize
$A = -2.29 + 0.278Ci - 0.0005Ci^2$ ($R^2 = 0.99$)

CO_2 response curve for soybean
$A = -7.12 + 0.15Ci - 0.0002Ci^2$ ($R^2 = 0.98$)

Fig. 4: CO_2 response curves under non stress conditions

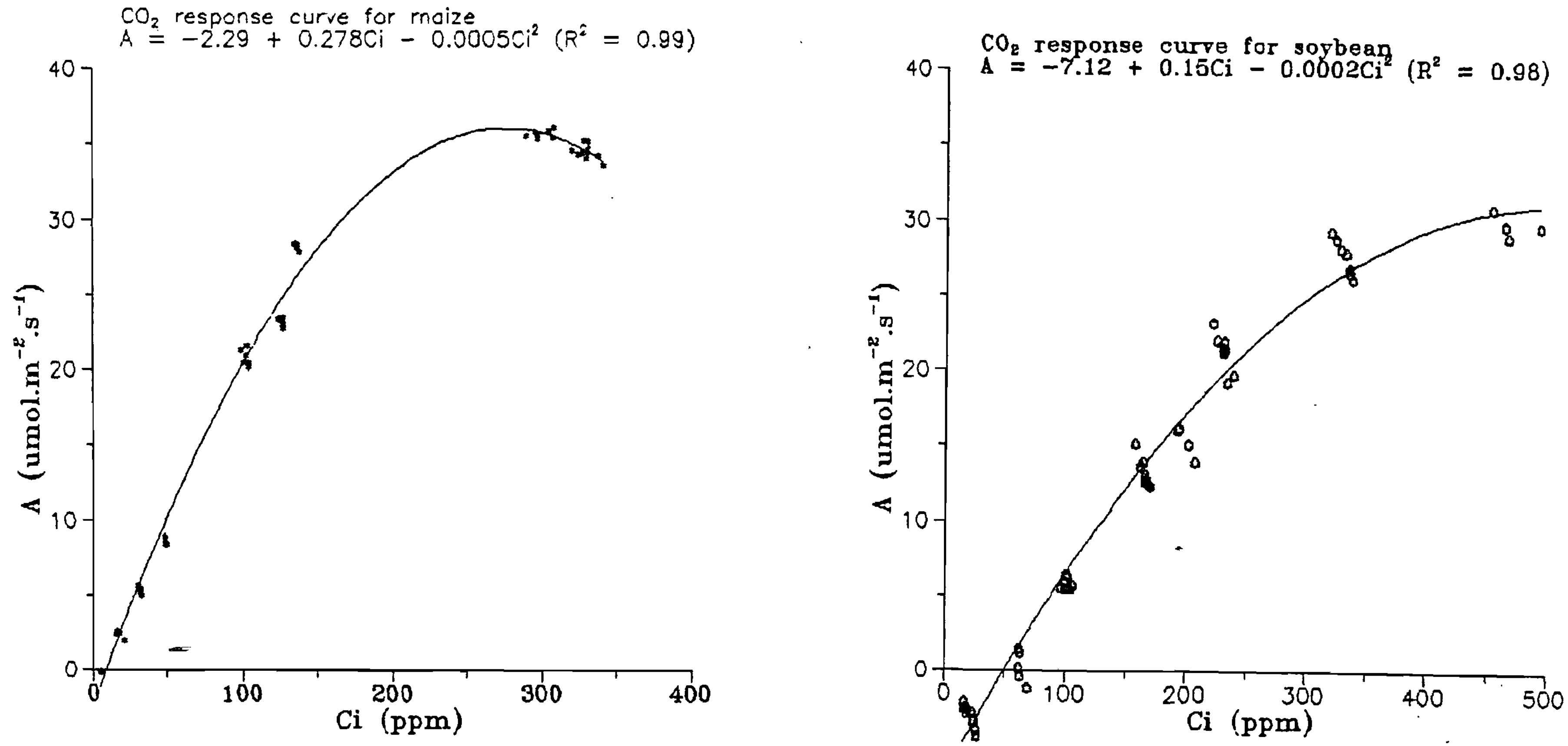


Fig. 4: CO_2 response curves under non stress conditions

all the species, there were, however, significant differences among them.

The CO_2 compensation point (The Ci at which the photosynthetic consumption of CO_2 and photorespiratory release of CO_2 were equal), showed significant variation between C_3 and C_4 plants. The C_4 plants had lower CO_2 compensation point ranging from 12 ppm in Finger millet to 15 ppm in Sorghum. C_3 plants, on the other hand, that equire higher concentrations of CO_2 to get a positive gain in carbon fixed typically had higher T. Sunflower recorded the maximum CO_2 concentration point indicating high rate of photorespiratory loss of fixed carbon (Table 2).

In vivo carboxylation efficiency (dA/dCi)

The rate of increase in `A' per unit increase in Ci, that is, the differential coefficient of `A' with respect to Ci (dA/dCi), is often measured to arrive at the *in vivo* carboxylation efficiency of the leaf. The dA/dCi of the C_4 plants was significantly higher compared to the C_3 plants. The C_3 plants showed an average of 0.0757 μmoles $CO_2.m^{-2}.s^{-1}.ppm^{-1}$, ranging from 0.072 in sunflower to 0.080 in soybean (Table 3). On the other hand maize had the highest rate of increase in `A' per unit increase in Ci of 0.250 followed by 0.110 in sorghum. The data clearly suggest that the mesophyll conductance of the C_4 plants is always higher than the C_3 plants (Table 3).

Table 3 : Relative stomatal and mesophyll limitations of 'A' under non stress condition

Crop	A	A_o	A_g	A_T	ls(%)	lm(%)	dA/dCi slope	dA/dCi pol. eqn.
Sunflower	20.64	25.70	22.8	33.0	19.60	8.9	0.072	0.106
Cowpea	14.78	17.25	17.5	22.0	14.32	15.54	0.075	0.079
Soybean	13.50	19.00	16.0	24.0	28.95	15.63	0.080	0.079
Sorghum	20.00	28.30	22.2	36.2	28.70	10.10	0.110	0.123
Maize	25.50	35.00	32.0	-	26.76	20.31	0.250	0.227
F.millet	18.60	24.40	21.5	32.3	23.80	13.50	0.098	-
CD (p=0.05)	4.6	-	-	-	3.47	3.35	0.005	-

Relative stomatal and mesophyll limitations

Development of CO_2 response curves is an important prerequisite for the computation of relative limitations of 'A'. The relative stomatal and mesophyll limitations to the observed photosynthetic rates were computed as proposed by Farquhar and Sharkey (1982) with certain modifications (for a detailed description of the methodology refer Material and Methods). The computed data are presented in Table 3.

Quantification of limitations revealed that in all the species included in this experiment, the stomata imposed a higher degree of limitation to 'A' in relation to the limitation imposed by the mesophyll factors under well watered conditions.

Except in Maize and Cowpea, in the other species tested, the stomatal limitations were twice as great as the relative mesophyll limitation. Though Sunflower had higher stomatal conductance, the stomatal limitation was 19.6% relative to the mesophyll limitation that was only 8.9%. The relative stomatal and mesophyll limitations were respectively, 28.95% and 15.63% in Soybean; 14.32% and 15.54% in Cowpea and 28.7% and 10.10% in Sorghum.

B. Effect of partial defoliation on gas exchange parameters

It is evident from the data reported in the previous experiment that the well watered plants experience a higher limitation to photosynthesis from the stomatal factors. Therefore any increase in the stomatal conductance under well watered condition should result in an increase in the photosynthetic rate. With this hypothesis, an effort was made to enhance the `g_s' by altering the root to leaf area ratio. The root to leaf area ratios were altered by randomly defoliating the plants. The results obtained are explained below.

The response of `A' and `g_s' to partial defoliation was monitored in Sunflower, a high `g_s' type and Sorghum and Soybean, both low g_s types. The data are presented in table 4.

It is evident from the data that defoliation increased the stomatal conductance in all the three species. There was also a simultaneous increase in assimilation rates. The increase in `A' was more pronounced in Soybean and Sorghum. Sorghum recorded a 17.03 percent increase in `A' and 28.99 percent increase in `$g_s$'. Where as in Soybean, `A' and `$g_s$' increased by 40.13% and 40.08 percent, respectively. However, sunflower recorded a marginal increase of 13.5 percent in `g_s'.

The data suggest that under non-stress conditions, the assimilation rates can be significantly enhanced by increasing the stomatal conductance for CO_2 transfer. This was more pronounced in species that intrinsically had low `g_s'.

CO_2 response curves were developed in these species to evaluate the effect of defoliation on the mesophyll factors. The data are illustrated in Figs. 5, 6 and 7. Sunflower did not show any variation in `A' either in the initial linear region or

Table 4 : Effect of defoliation on gas excange parameters in a few C_3 and C_4 species

	Control			Defoliated		
	'A'	g_s	Ci	'A'	g_s	Ci
Sunflower	17.52	1012.39	285	18.38	1534.14	296
Sorgum	23.04	385.90	216	27.77	543.50	222
Soybean	9.46	623.40	295	15.80	1053.40	295
CD ($p=0.05$)	5.17	127.00	35	6.00	101.2	28

interaction : 'A'= 2.07
g_s= 127
Ci= 28

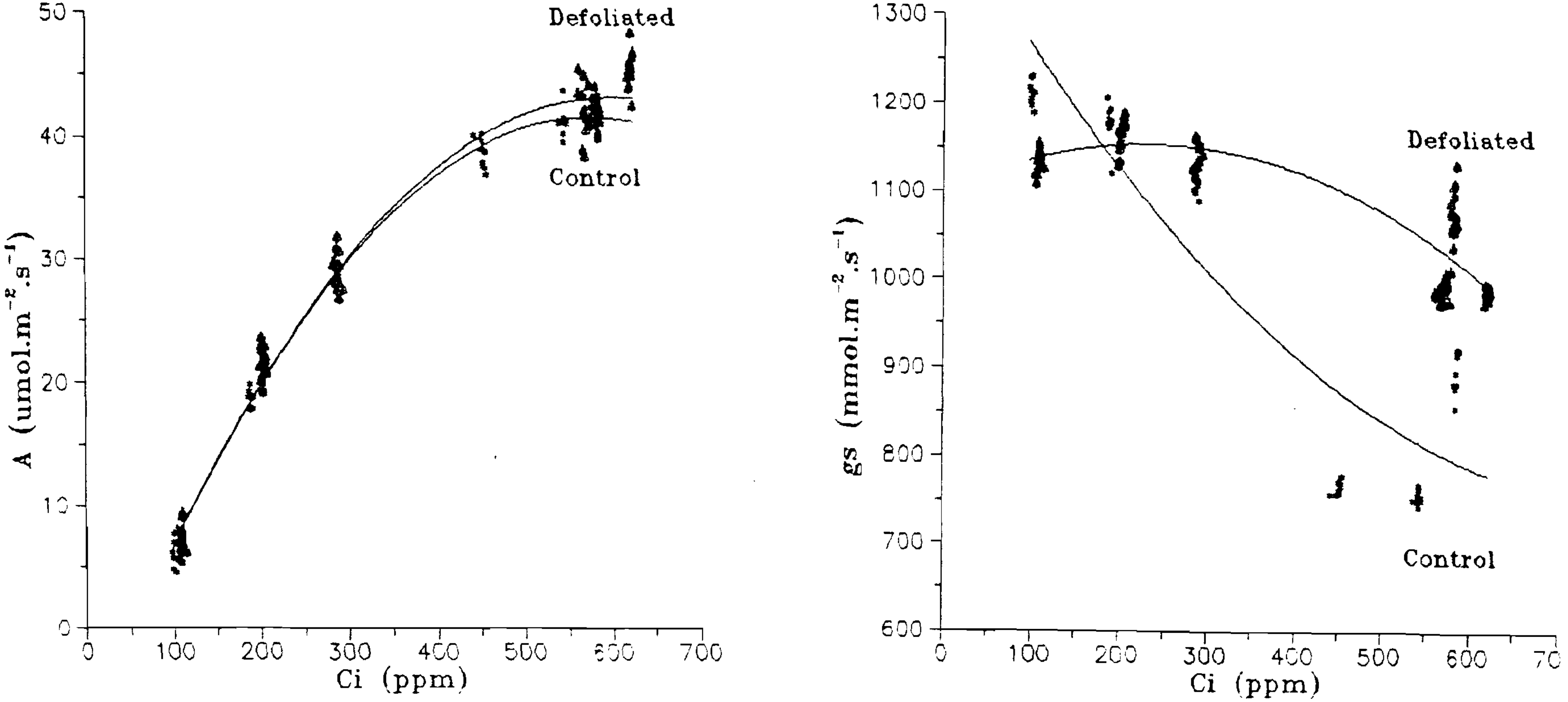


Fig. 5: Effect of partial defoliation on gas exchage parameters in sunflower.

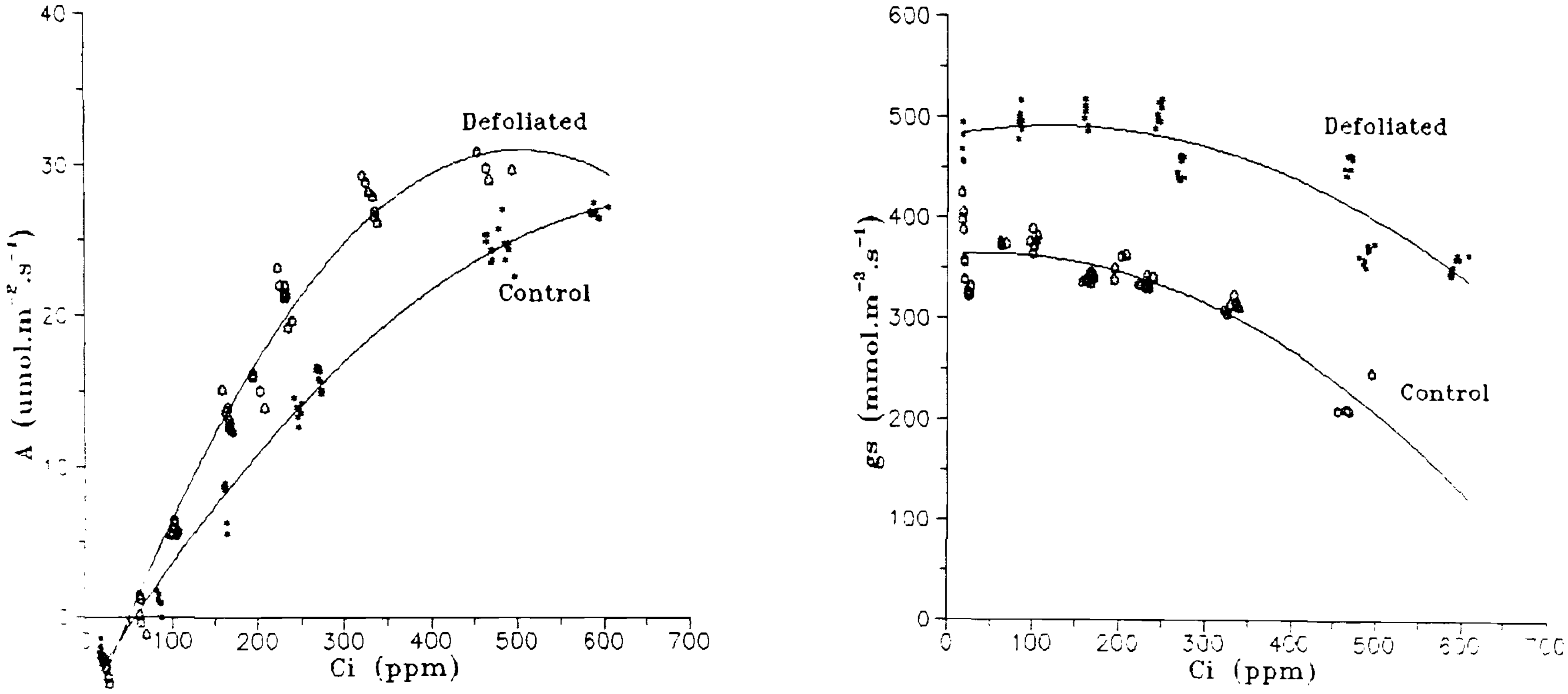


Fig. 6: Effect of partial defoliation on gas exchage parameters in soybean.

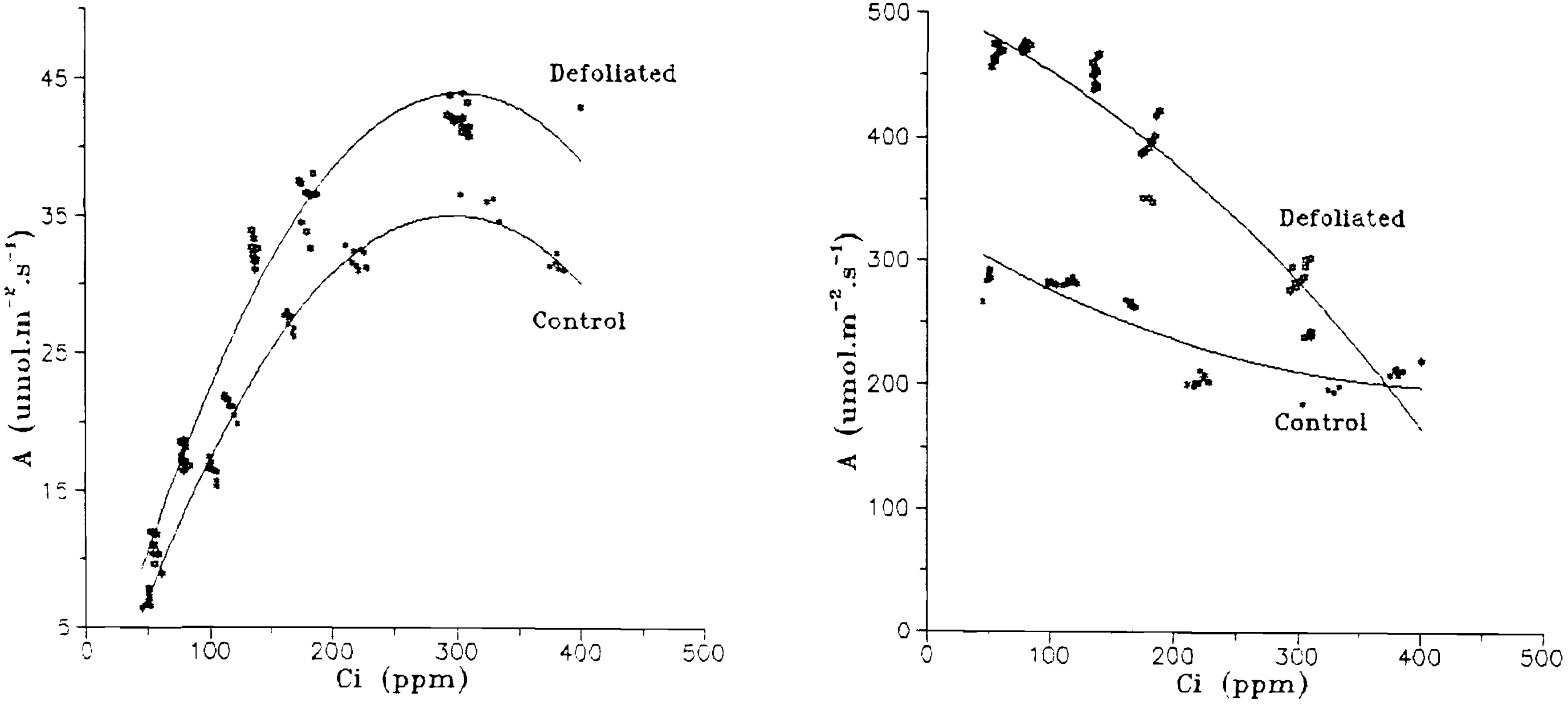


Fig. 7: Effect of partial defoliation on gas exchage parameters in sorghum.

in the CO_2 saturated region between defoliated and control plants. On the other hand, Sorghum and Soybean showed increases in both initial slope and CO_2 saturated rate of assimilation rate. The in vivo carboxylation efficiency as reflected by dA/dCi increased from 0.215 to 0.255 in sorghum and from 0.080 to 0.140 in Soybean. The data further reiterated that an increase in 'g_s' in species that have an intrinsically low stomatal conductance will result in an increased photosynthetic rate. It became evident from the CO_2 response curves that defoliation brought about certain changes in the mesophyll component of photosynthesis, hence resulting in an increased initial slope (dA/dCi) as well as in the CO_2 saturated rate of photosynthesis.

From the data of these experiments, following conclusions can be made.

1. Chloroplasts have significantly higher capacity for carbon fixation in both C_3 and C_4 plants.
2. Substrate availability is an important limitation to photosynthesis. Hence g_s is an important component.
3. Under well watered conditions, major limitation was imposed on 'A' by stomatal factors.
4. Increasing g_s by partial defoliation resulted in a corresponding increase in 'A'. Defoliation treatment also improved the mesophyll factors.
5. Variations in 'A' between species could be due to the inadequate functioning of the mesophyll to utilize the low CO_2. A linear relationship between in vivo carboxylation efficiency (dA/dCi) and 'A' exists between species ($r = 0.9$).

Gas exchange and relative stomatal and mesophyll limitations of 'A' under different abiotic stresses

Crop plants often experience a number of biotic and abiotic stresses that hamper the crop growth and productivity. Among many abiotic stresses, drought stress, nutrient stress and low light stress are the most common and important ones. It has been clearly established that the non availability of any one or more of these variables can significantly reduce the plant productivity. Reduction in 'A' under stress could be due to a reduction in gs or gm. Quantifying the traits that are affected under stress will help to identify genotypes with stability in these traits. The measurement of the gas exchange traits can hence be adopted as a potential tool for the assessment of the photosynthetic rates and the effects of abiotic stresses on the machinery of photosynthesis.

A. Quantification of limitations under different abiotic stresses

Photosynthesis in crop plants are broadly regulated by the stomatal diffusive characteristics and the mesophyll ability for the utilization of CO_2. Assessment of the relative reductions in stomatal conductance and mesophyll ability for carbon fixation paves way for a better understanding of factors affecting 'A' in plants experiencing abiotic stresses. With an objective to estimate the photosynthetic characteristics and also to assess the relative limitations imposed by the stomata and the mesophyll factors, Sunflower, Cowpea and Sorghum plants were subjected to various abiotic stresses. The results obtained are presented in this chapter.

a. moisture stress

By regulating the irrigation schedule, 30 to 40 days old pot grown sunflower, cowpea and Sorghum plants were brought to 50 percent field capacity and maintained at that water regime for seven days. This degree of stress was called moderate stress. To another set of pots, irrigation was altered in such a manner to bring the soil to 25 percent of field capacity and maintained for seven days to generate severely stressed plant material. Gas exchange parameters were measured and CO_2 response curves were generated on the top fully expanded leaf. The data for the gas exchange parameters are presented in table 5.

Gas exchange parameters

Assimilation rate in Sunflower decreased by 42.75 percent and 79.14 percent under moderate and severe moisture stress condition, respectively. The `A' under moderate stress was 11 $\mu$ moles.$m^{-2}$.$s^{-1}$ and 4.2 $\mu$ moles.$m^{-2}$. $s^{-1}$ under severe stress. These rates were significantly less compared to the unstressed control that recorded an `A' of 20.09 μ moles.m^{-2}.s^{-1}. The stomatal conductance for water vapour (`g_s') decreased by 71.22 percent from 788 mmoles.m^{-2}.s^{-1} in control to 277 mmoles.m^{-2}.s^{-1} in plants experiencing a moderate stress. Under severe stress g_s decreased to 187.6 mmoles.m^{-2}.s^{-1} (76.22% of control).

The inter-cellular CO_2 concentration (Ci), recorded an

Table 5 : Gas exchange parameters in plants subjected to various degrees of moisture stress

Stress level	`A'	g_s	A/g_s	Ci	A/Ci
Sunflower					
Control	20.09	788	0.025	263	0.079
Mild stress	11.50	277	0.051	271	0.042
Severe stress	4.19	187	0.029	283	0.023
CD (p=0.05)	7.00	48	-	15	-
Sorgum					
Control	13.23	265	0.051	150	0.090
Mild stress	12.15	155	0.091	165	0.087
Severe stress	6.84	88	0.077	196	0.035
CD (p=0.05)	5.20	39	-	19	-
Cowpea					
Control	14.78	418	0.035	200	0.051
Mild stress	9.71	154	0.063	216	0.045
CD (p=0.05)	5.3	50	-	10	-

increase for Sunflower plants experiencing moisture stress. The Ci increased from 263 ppm in control to 283 ppm under severe stress condition.

Assimilation rate decreased from 13.25 μ moles.m^{-2}.s^{-1} in control to 6.84 μ moles.m^{-2}.s^{-1} in severely stressed Sorghum plants. However the moderate stress treatment did not seem to show any marked reduction in 'A' in Sorghum. Assimilation rate decreased by only 8.3 percent under moderate water stress. The 'g_s', on the other hand, decreased from 265 mmoles.m^{-2}.s^{-1} in control to 155 mmoles.m^{-2}.s^{-1} under moderate stress which was 58.47 percent of control. The stomatal conductance further decreased to 88 mmoles.m^{-2}.s^{-1} (66.61 percent reduction from control) when plants experienced a severe moisture stress. Assimilation rate per unit stomatal conductance, a qualitative measure of the water use efficiency, increased under moderate stress to 0.091 μ moles.$mmole^{-1}$ from 0.051 μ moles.$mmole^{-1}$ in control. Under severe stress, although the A/g_s ratio decreased to 0.077 μ moles.$mmole^{-1}$, it was higher than that in the control. A similar trend was also seen in Sunflower and Cowpea. This indicates an increase in the water use efficiency when the plants are subjected to a moderate water stress.

The inter-cellular CO_2 concentration (Ci), increased from 150 ppm in Sorghum grown in well watered condition to 165 ppm under moderate stress and 196 ppm in severe moisture stress condition.

There was a significant reduction in both 'A' and 'g_s' in

Cowpea grown in different degrees of moisture stress. 'A' decreased by 34.3 percent from 14.78 μ moles.$m^{-2}.s^{-1}$ in control to 9.71 μ moles.$m^{-2}.s^{-1}$ in moderately stressed Cowpea plants. Though a C_3 plant, Cowpea had an intrinsically low 'g_s' of 418 mmoles.$m^{-2}.s^{-1}$ under control which decreased to 154 mmoles.$m^{-2}.s^{-1}$ under moderate stress. The 'g_s' under stress was 63.08 percent of control. As in other two species, the A/g_s ratio increased under stress in Cowpea also (Table 5). The Ci in Cowpea decreased compared to control to 215 ppm under moderate moisture stress condition.

Relative limitations of 'A' under moisture stress

With an objective to assess the relative limitation to photosynthesis by the stomatal and mesophyll factors in plants experiencing different degrees of moisture stress, CO_2 response curves were developed. The A/Ci curves of the three species experiencing different degrees of moisture stress are illustrated in Figs. 8, 9, 10. Relative stomatal and mesophyll limitations were computed from the CO_2 response curves and the data are presented in table 6.

The slope of the initial linear response of 'A' to Ci (dA/dCi) decreased significantly under stress. The dA/dCi decreased markedly from 0.057 in control to 0.024 in sunflower plants experiencing moderate and severe moisture stress, respectively. Corresponding to the reduction in dA/dCi, the relative limitation imposed by the mesophyll factors (lm%) on assimilation rate increased significantly (Fig. 8 and Table 6).

The relative mesophyll limitation to assimilation in sunflower plants were 51.27 percent in moderate stress and 82.85 percent in severe stress condition. However,the mesophyll limitation was only 11.44 percent in well watered plants.

Contrastingly, the relative stomatal limitation was the maximum in well watered sunflower plants (21.21%). As the intensity of the moisture stress increased, there was a gradual reduction in the limitation to assimilation rate offered by the stomatal components. The relative stomatal limitation decreased to 14.81 percent under moderate stress and to 6.89 percent under severe stress conditions.

Similar trends were also seen in Sorghum and Cowpea (Table 6 and Figs. 9 & 10). Sorghum showed a significant reduction in dA/dCi from 0.052 in control to 0.046 and 0.031 in moderately and severely stressed plants, respectively. A corresponding increase in `l_m' was noticed from 3.64 percent in control to as high as 51.27 percent in severely stressed plants.

The limitation imposed by the stomata to the apparent photosynthetic rate was high in unstressed Sorghum plants (35.36%). However, as the intensity of the stress increased, there was no significant change in the relative stomatal limitation in Sorghum (Table 6). In cowpea, the rate of change of `A' with respect to Ci (dA/dCi) decreased from 0.079 in control to 0.068 under stress. The `l_m' as witnessed in the other two crops, increased significantly from 13.8% to 42.88 percent under stress. Although,the stomatal limitation in

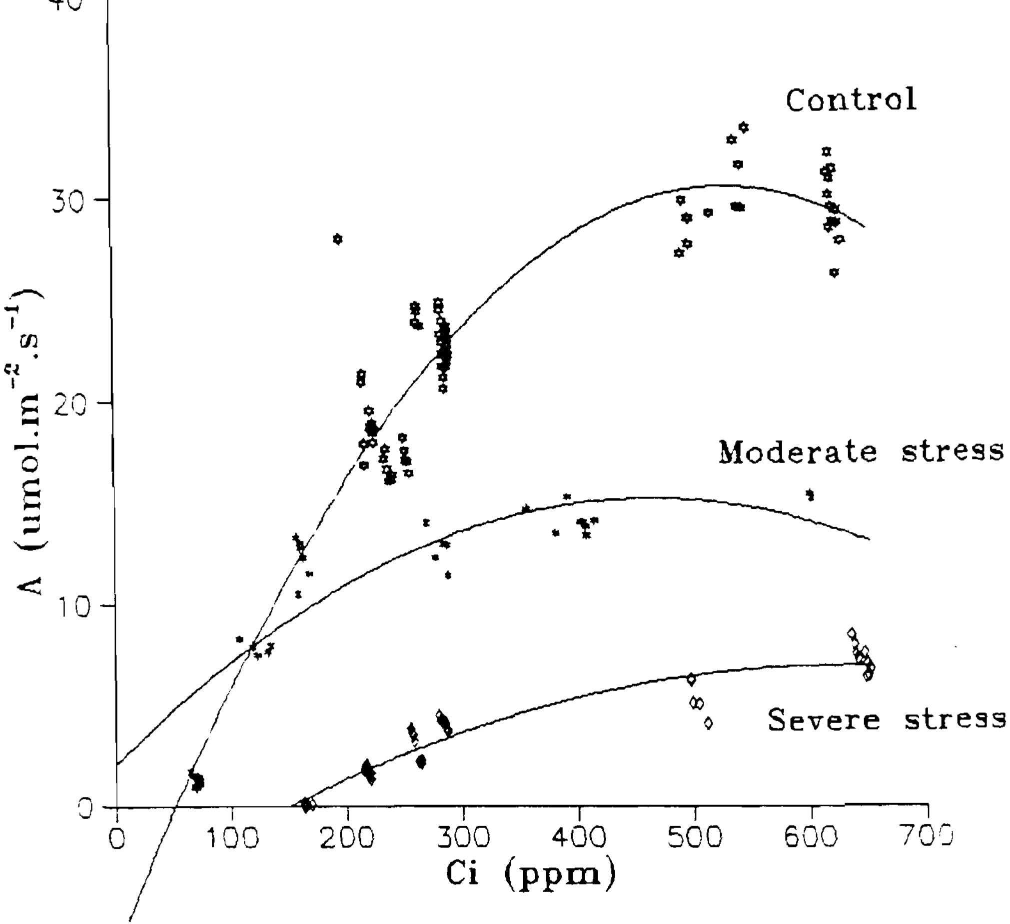


Fig. 8: Effect of different degrees of moisture stress on assimilation rates in sunflower.

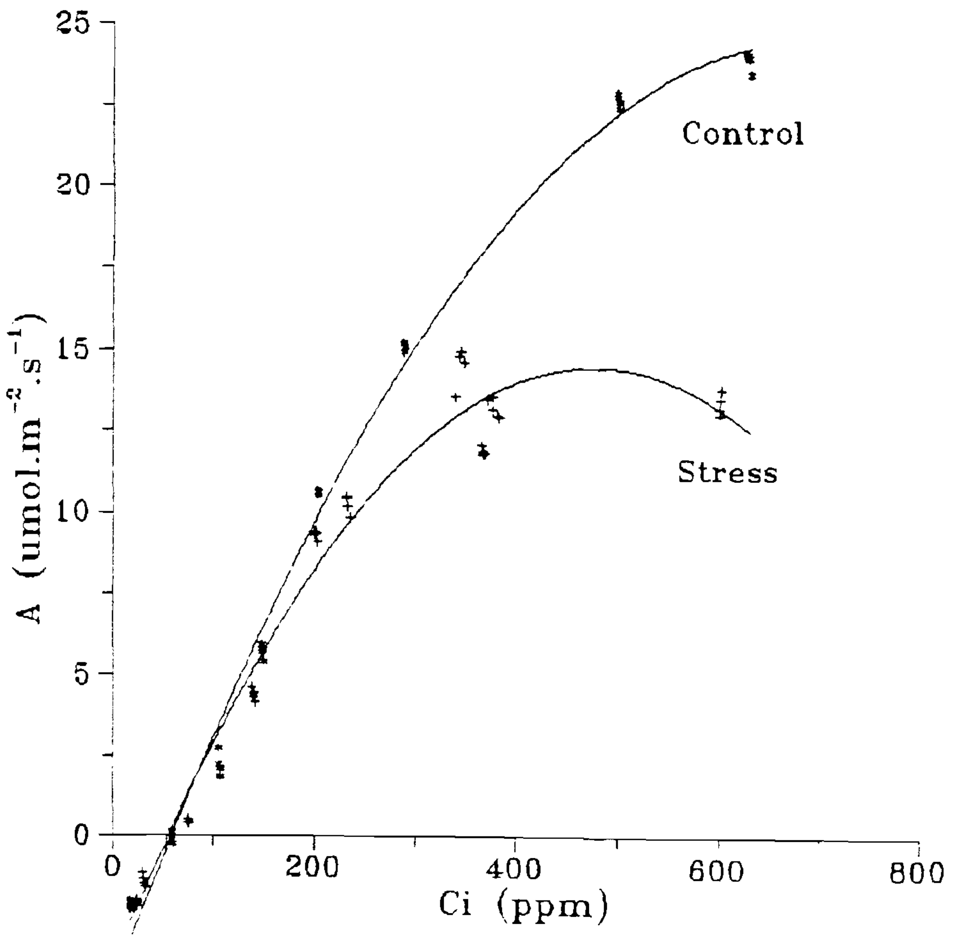


Fig. 9: Effect of different degrees of moisture stress on assimilation rates in cowpea.

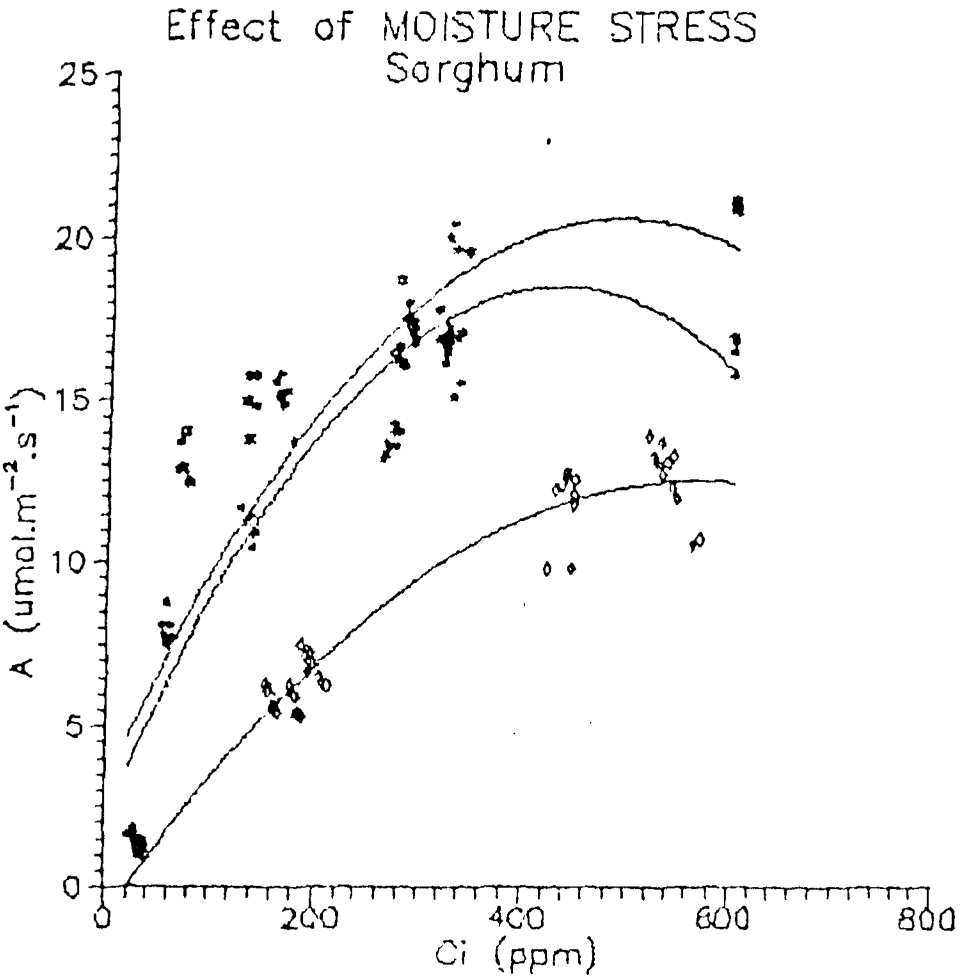


Fig.10: Effect of different degrees of moisture stress on assimilation rates in sorghum.

Table 6 :Relative limitations of 'A' in plants subjected to various degrees of moisture stress

	A	A_o	A_g	ls(%)	lm(%)	dA/dCi
Sunflower						
Control	20.09	25.5	23.6	21.21	11.44	0.057
Mild stress	11.50	13.5	23.6	14.81	51.27	0.021
Severe stress	4.19	4.5	23.6	6.89	82.25	0.024
CD (p=0.05)	7.00	-	-	7.39	10.19	0.008
Sorghum						
Control	13.25	20.5	13.75	35.36	3.64	0.052
Mild stress	12.50	18.2	13.75	31.32	9.09	0.046
Severe stress	6.70	10.6	13.75	36.79	51.27	0.031
CD (p=0.05)	5.20	-	-	3.1	10.9	0.010
Cowpea						
Control	14.78	17.0	17.00	13.80	13.80	0.079
Mild stress	9.71	13.0	17.00	25.3	42.88	0.068
CD (p=0.05)	5.3	-	-	9.8	12.59	0.006

Cowpea registered an increase under stress, the bulk of the limitation to 'A' was due to the mesophyll factors which was evident from a larger increase in 'l_m'.

From the data on the gas exchange parameters and relative limitations of 'A' computed from the CO_2 response curves in plants experiencing various degrees of moisture stress, the following inferences can be drawn.

1. Both 'A' and g_s decreased in plants subjected to moisture stress in all the species.
2. Under moisture stress condition, predominant limitations for photosynthesis was imposed by the mesophyll components.
3. As the intensity of the stress progressed, there was no marked change in ls % where as, l_m % increased correspondingly.
4. The <u>in vivo</u> carboxylation efficiency (dA/dCi), exhibited a significant reduction as moisture stress progressed.
5. Therefore, though g_s dramatically decreased under stress, reduction in 'A' was more due to the reduction in g_m.
6. The greater reduction in g_s than g_m under moisture stress, clearly indicates that g_m exerted a feed-back control on stomatal factors.

b. Low light stress

Sunflower and Sorghum plants were grown under normal light intensity for 30 to 40 days. One set of plants were transferred to low light intensities of 1000 μ moles.m^{-2}.s^{-1} and another set to 500 μ moles.m^{-2}.s^{-1} and acclimatized for a period of seven days prior to recording observations on gas exchange parameters. The gas exchange rates were recorded in the respective growth light intensities. Assimilation rates were compared with control plants which were grown under normal high light intensities. The data are presented in Table 7.

Gas exchange parameters

Low light acclimatized sunflower plants showed significant reductions in photosynthetic rates. `A' decreased from 20 $\mu$ moles.m$^{-2}$.s$^{-1}$. in high light to 14.75 $\mu$ moles m$^{-2}$. s$^{-1}$ in plants acclimatized to 1000 $\mu$moles.m$^{-2}$.s$^{-1}$ The reduction in `A' was 26.25 percent in moderately low light intensities whereas the reduction was 67.5 percent in plants acclimatized to very low light intensities. The stomatal conductance of Sunflower plants decreased from 940 mmoles.m^{-2}.s^{-1} in high light to 620 mmoles.m^{-2}.s^{-1} and 340 mmoles.m^{-2}.s^{-1} in plants acclimatized to 1000 μ moles.m^{-2}.s^{-1} and 500 μ moles.m^{-2}.s^{-1} light intensities, which constituted 34.04 percent and 63.83 percent reduction, respectively.

Assimilation per unit stomatal conductance (A/g_s) did not reveal any marked variation under low light intensities. However the A/g_s ratio marginally increased to 0.024 compared to 0.021 under control. It decreased to 0.019 when plants were acclimatized to very low light intensity of 500 μ moles.m^{-2}.s^{-1}

Table 7 : Gas exchange parameters in plants subjected to various degrees of light stress

Stress level	'A'	g_s	A/g_s	Ci	A/Ci
Sunflower					
Control	20.00	940	0.021	220	0.091
Mild stress	14.75	620	0.024	290	0.051
Severe stress	6.50	334	0.019	300	0.022
CD (p=0.05)	4.08	198	-	17	-
Sorgum					
Control	21.00	271	0.077	170	0.124
Mild stress	19.00	188	0.101	151	0.125
CD (p=0.05)	3.39	72	-	20	-

The inter-cellular CO_2 concentration (Ci), increased from 220 ppm in high light to 290 ppm at 1000 μ moles.$m^{-2}.s^{-1}$ and 300 ppm at 500 μ moles.$m^{-2}.s^{-1}$ light intensity. Such an increase in Ci was noticed under moisture stress conditions also. The 'A' per unit Ci (A/Ci ratio), progressively decreased from 0.091 in high light to 0.051 and 0.022, respectively in the two low light intensities. The reduction in the A/Ci ratio indicates a possible damage to the carboxylation efficiency of the chloroplasts (Table 7).

Sorghum plants also showed a similar trend in response to low light intensities (Table 7). 'A' decreased by 26.19 percent from 21 μ moles.$m^{-2}.s^{-1}$. in high light condition to 19.0 μ moles.$m^{-2}.s^{-1}$ in low light (1000 μ moles.$m^{-2}.s^{-1}$). Stomatal conductance also decreased by 30.63 percent. As in sunflower, since 'A' and g_s decreased by similar proportions in Sorghum, the ratio of 'A' and g_s showed only a marginal increase in low light intensities. However, the A/Ci ratio in Sorghum plants grown in 1000 μ moles quanta.$m^{-2}.s^{-1}$ light intensity did not show any significant reduction compared to plants grown high light intensities of 2000 μ moles.$m^{-2}.s^{-1}$.

Relative limitations of 'A' under low light conditions

The relative stomatal and mesophyll limitations were computed based on the CO2 response curves developed in low light acclimatized sunflower and Sorghum plants (Figs. 11 & 12). The data are presented in Table 8.

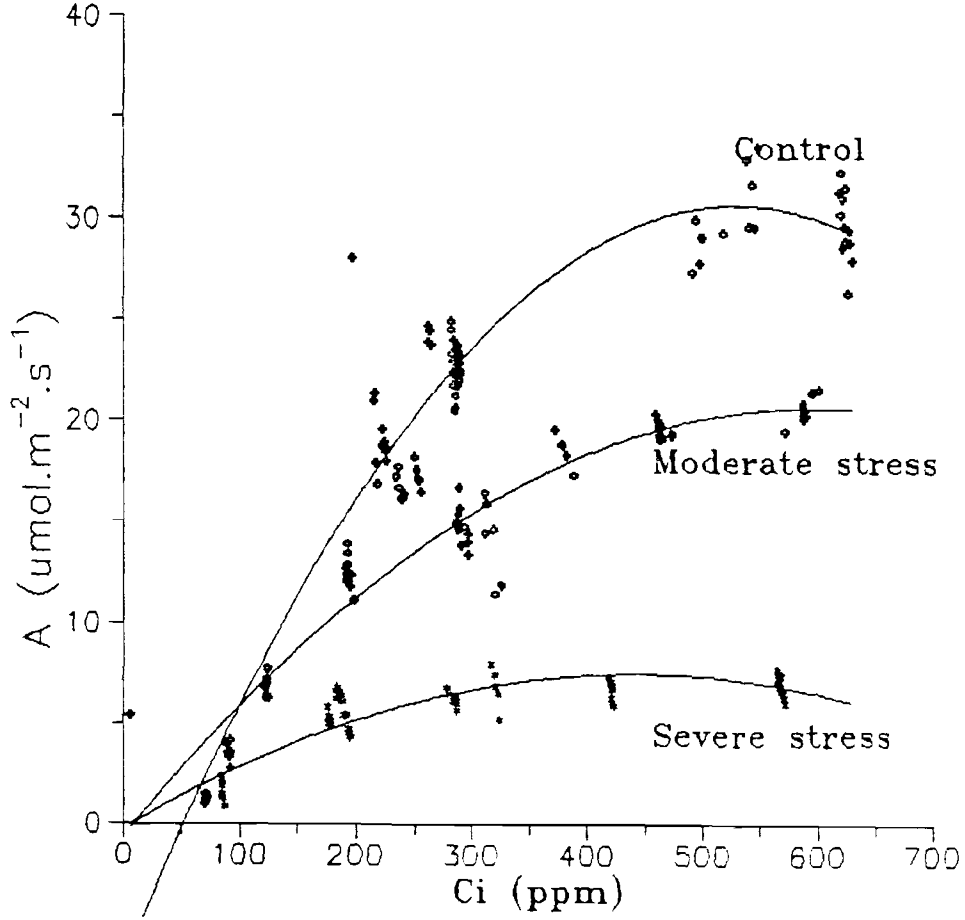


Fig.11: Effect of different degrees of low light stress on assimilation rates in sunflower.

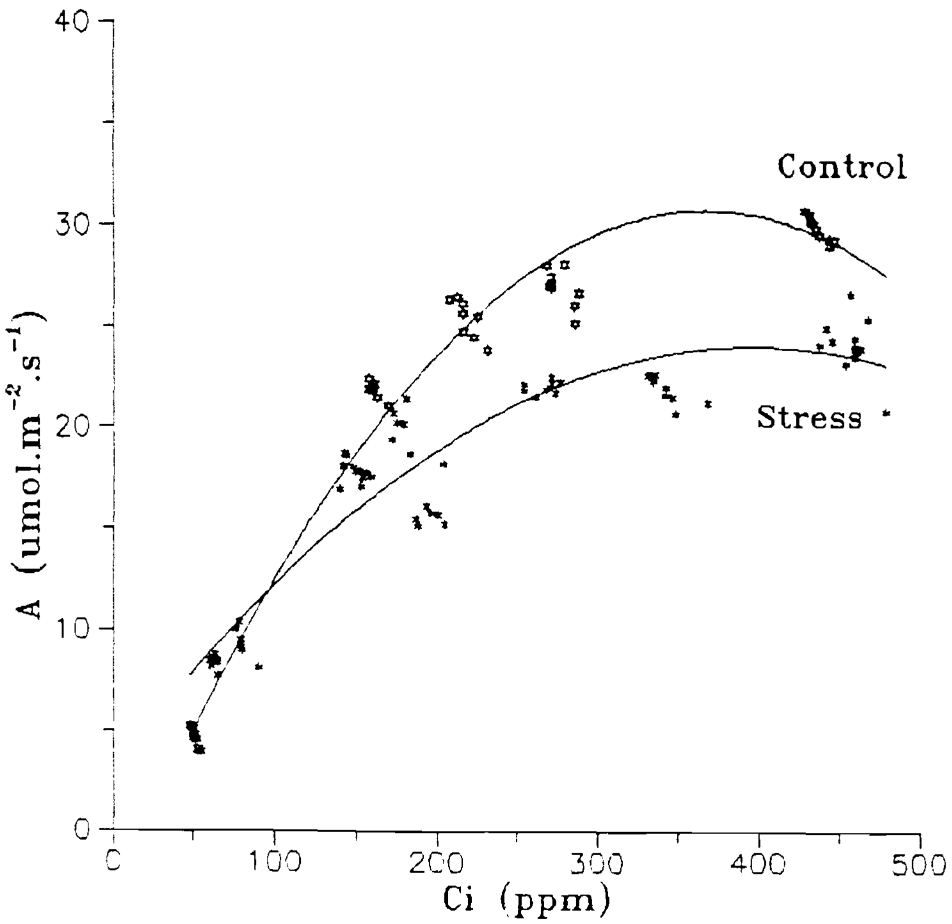


Fig.12: Effect of different degrees of low light stress on assimilation rates in sorghum.

Table 8 :Relative limitations of 'A' in plants subjected to various degrees of light stress

	A	A_o	A_g	ls(%)	lm(%)	dA/dCi
Sunflower						
Control	20.00	24.5	23.0	18.37	12.77	0.085
Mild stress	14.75	16.0	23.0	7.81	35.87	0.036
Severe stress	6.50	7.0	23.0	7.14	71.74	0.011
CD (p=0.05)	4.08	-	-	3.6	6.8	0.012
Sorgum						
Control	21.00	30.5	23.50	31.15	10.64	0.108
Mild stress	19.00	23.5	23.50	34.00	19.15	0.054
CD (p=0.05)	3.39	-	-	4.2	5.2	0.009

In Sunflower, the stomatal limitation of ‘A’ was relatively more compared to the mesophyll limitation in plants grown in normal high light intensities. The stomata imposed 18.37 percent limitation where as mesophyll contribution to the limitation was only 12.77 percent in high light grown sunflower plants. When the plants were grown in reduced light intensities, the limitation shifted predominantly towards the mesophyll factors. The relative mesophyll limitation increased to 35.87 percent at 1000 μ moles.m^{-2}.s^{-1} and further increased to 71.74 percent in very low light intensities. On the other hand the ‘l_s’ decreased to 7.81 percent in plants acclimatized to 1000 μ moles.m^{-2}.s^{-1} and further decreased to 7.14 percent in plants grown in 500 μ moles.m^{-2}.s^{-1} light intensity

The initial slope of the CO$_2$ response curve (dA/dCi) was 0.085 for sunflower plants grown at high light intensities. The dA/dCi decreased significantly to 0.036 and 0.011 when plants were shifted to lower light intensities. This indicated a reduction in the *in vivo* carboxylation efficiency under sub optimal light intensities (Fig. 11).

A similar trend was noticed in Sorghum plants also. Low light significantly increased the relative mesophyll limitation of ‘A’ from 10.64 percent in control to 19.55 percent in plants grown at 1000 μ moles.m^{-2}.s^{-1} The relative stomatal limitation, on the other hand, marginally increased from 31.75 percent in control to 34% in low light. The concomitant decrease in dA/dCi from 0.108 to 0.054 under stress reiterated the reduction in carbon fixing ability (Fig 12).

From the experiments conducted with sunflower and Sorghum plants acclimatized to different low intensities, the following inferences can be made.

1. With decrease in light intensity, 'A' and g_s decreased in both the species.
2. On quantifying the relative limitations of 'A', it became evident that mesophyll factors imposed greater limitations where as the stomatal components were important in high light intensities.
3. The <u>in vivo</u> carboxylation efficiency (dA/dCi), decreased progressively with reduction in the light intensity.

c. Nitrogen stress

Sunflower plants were grown with recommended doses of fertilizer Nitrogen as prescribed in the package of practices. Another set of potted plants were maintained with only 50% of the recommended Nitrogen to develop plant material stressed for nitrogen. Gas exchange parameters were recorded in 30 to 40 days old plants. Care was taken to supply the other essential nutrients as prescribed in the Package of Practices. The data are presented in Table 9.

Gas exchange parameters

Assimilation rate decreased significantly by 17.31 percent under stress compared to the control plants that showed an 'A'

Table 9 : Gas exchange parameters in plants subjected to nitrogen stress

	'A'	g_s	A/g_s	Ci	A/Ci
Sunflower					
Control	26.00	633.94	0.041	261	0.099
Stress	21.50	600.05	0.036	272	0.079
CD (p=0.05)	2.5	96.5	-	10	-

Table 10: Relative limitations of 'A' in plants subjected to nitrogen stress

	A	A_o	A_g	ls(%)	lm(%)	dA/dCi
Control	26.00	31.0	34.0	16.13	23.53	0.085
Stress	21.55	24.0	34.0	10.42	36.76	0.069
CD (p=0.05)	-	-	-	5.9	6.4	0.008

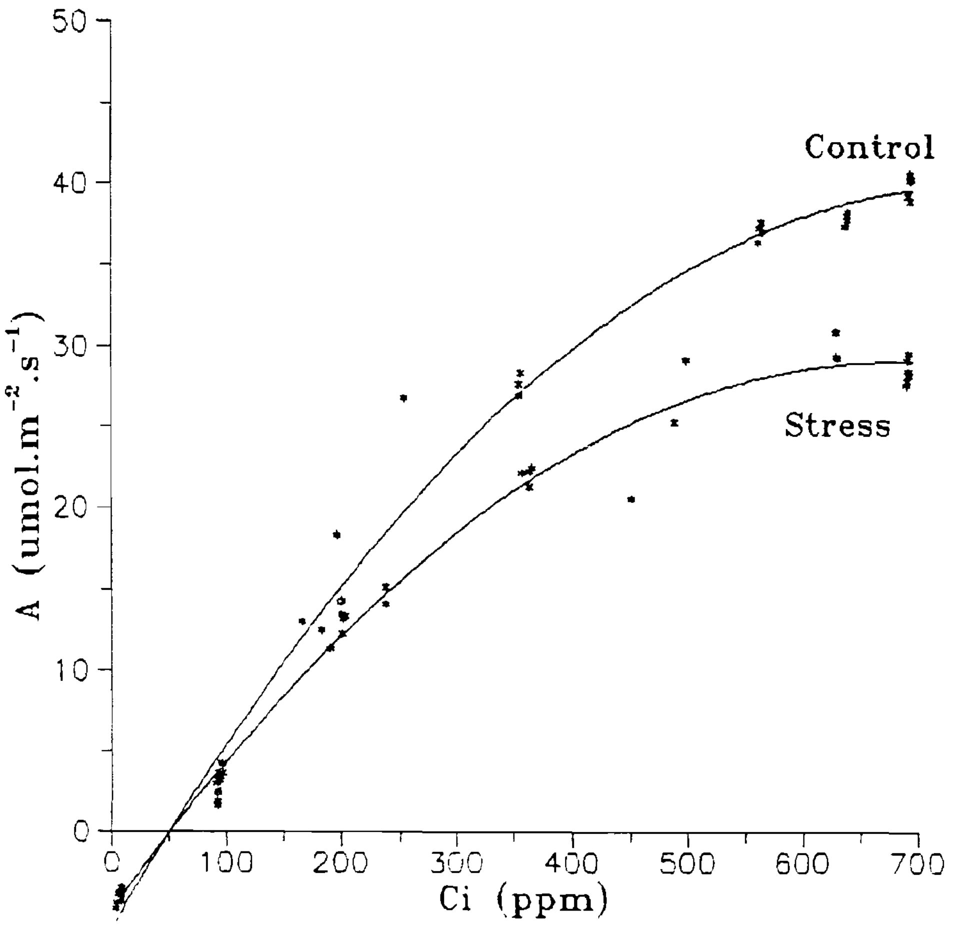


Fig.13: Effect of nitrogen stress on assimilation rates in sunflower

of 26.0 μ moles.$m^{-2}.s^{-1}$. The stomatal conductance on the other hand, decreased marginally by 5.34 percent under stress. Therefore the assimilation rate per unit stomatal conductance slightly decreased from 0.041 in control to 0.036 in plants experiencing nitrogen stress. indicating a reduction in the water use efficiency. The Ci in nitrogen stressed leaves increased to 272 ppm from 261 ppm in control plants. The A/Ci ratio which was 0.099 in control declined to 0.079 in nitrogen stress condition.

Relative limitations to 'A' under nitrogen stress

As explained in the other two abiotic stresses, CO_2 response curves were developed in plants grown under control conditions as well as in plants grown in nitrogen deficiency. Based on these CO_2 response curves, the relative stomatal and mesophyll limitations were computed. The data are presented in table 10 and the CO_2 response curves are illustrated in Fig. 13.

The relative mesophyll limitation increased under nitrogen stress from 23.53 percent in control to 36.76 percent. The limitation imparted by the stomatal factors, on the other hand, decreased under stress (Table 10). The assimilation rates of sunflower plants grown in control conditions experienced a relative stomatal limitation of 16.13 percent. The l_s slightly decreased to 10.42 percent in nitrogen stress. Thus mesophyll factors play an important role in limiting 'A' in nitrogen deficient plants as seen in other abiotic stresses. A decline in the rate of increase in `A' with respect to Ci (dA/dCi), further supports the importance of the mesophyll factors. The slope of the initial part of the CO_2 response curve which was 0.085 in control condition decreased to 0.069 under nitrogen deficiency. This indicates a reduction in the carboxylation efficiency of such plants.

The salient findings of the experiment with nitrogen stress can be summarized as follows.

1. Though both `A' and $g_s$ decreased under stress, the reduction in `A' was more pronounced.
2. Relative mesophyll limitation of `A' significantly increased under stress
3. dA/dCi also showed a considerable reduction under stress.

From the data on the gas exchange parameters and the quantification of limitations from the co2 response curves in plants experiencing various abiotic stresses, the following findings can be high lighted.

1. Under any abiotic stress, both `A' and g_s decreased.
2. The limitation imposed by the mesophyll factors significantly increased as the stress progressed. Where as the limitation offered by stomata did not change markedly
3. Mesophyll conductance as measured by dA/dCi showed a significant and corresponding reduction under any abiotic stress.

4. It can be, therefore, concluded that in all abiotic stresses the limitation imposed by g_s is marginal and major limitation was from the mesophyll factors.

5. The reduction in g_s was more than the reduction in `A' in many cases indicating a feed back control of g_s by g_m.

Assessment of stomatal and assimilation Loop Gains

Assimilation rate, stomatal conductance and Ci are intricately inter related. When gs increases, Ci also increases resulting in an enhanced `A'. If `A' remains high, consumes the substrate and results in the reduction of Ci. On the other hand, any reduction in `A' results in an increase in Ci. This causes a feed back stomatal closure. Such a loop of interaction among these three parameters can be studied by analysing the loop gains of assimilation and conductance.

It was noticed that under abiotic stresses, g_s decreased to a greater extent compared to the reduction in g_m. To test the hypothesis that stomata are under a feed back control from mesophyll factors, the gas exchange parameters and CO_2 response curves developed in plants experiencing abiotic stresses were used to assess the assimilation and stomatal loop gains.

The results of the loop gains are presented in Table 11 The data indicate that the stomatal loop gains are more than the assimilation loop gains under stress suggesting a feedback control of g_m on g_s.

Table 11: Stomatal assimilation loop gains in sunflower and sorghum under various abitic stresses.

	moisture stress		Light stress	
	G_g	G_A	G_g	G_A
Sunflower				
Control	-0.021	-0.111	-0.0072	-0.145
Mild stress	-0.108	-0.029	-0.0610	-0.093
Severe stress	-0.030	-0.196	-0.0360	-0.052
Sorghum				
Control	-0.057	-0.314	-0.086	-0.638
Mild stress	-0.380	-0.475	-0.335	-0.459
Severe stress	-0.628	-0.560	-	-

B. Effect of enhanced g_s on 'A' in plants subjected to moisture stressed plants

Moisture stress resulted in a reduction in both 'A' and g_s. To test whether the reduction in 'A' was in fact due to a reduction in the mesophyll capacity to fix CO_2, an attempt was made to enhance g_s without altering the mesophyll capacity. Assimilation rate should respond to changes in g_s if stomatal conductance regulates 'A'. It is a well established fact that stomata responds to changes in VPD. At lower VPD values, g_s increases. Such an increase in g_s would hence increase the CO_2 concentration at the carboxylation site, providing a higher substrate concentration for the photosynthetic process. With this hypothesis, the g_s of the stressed plants were selectively increased by appropriately decreasing the VPD of the leaf in the leaf chamber of the portable photosynthesis system (ADC LCA-2).

Since sunflower recorded the highest reduction in g_s under stress, an attempt was made to increase the g_s of this crop only. The data are illustrated in fig. 14.

The stressed sunflower leaf at 24 mbars VPD recorded a g_s of 360 mmoles. At a lower VPD value of 12 mbars, g_s was significantly increased to 800 mmoles which was equal to that of the unstressed plant. 'A' was recorded after the g_s stabilized to a new higher value. The assimilation rate which was 16.5 μ moles.$m^{-2}.s^{-1}$ in stressed plants did not show remarkable increase when g_s was enhanced over two times (fig. 14). This lack of increase in 'A' of stressed plants despite a significant

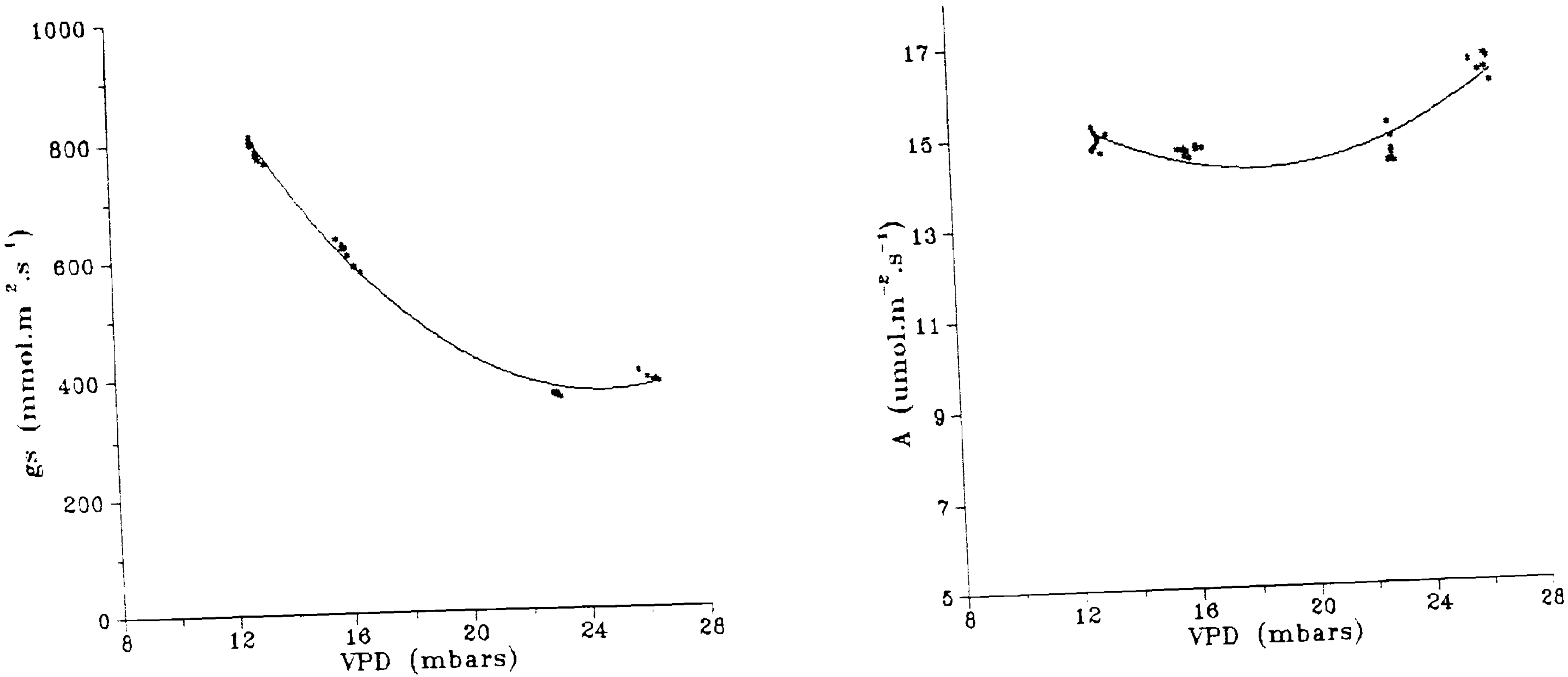


Fig.14: Effect of enhanced g_s on 'A' in sunflower plants subjected to moisture stress.

increase in g_s and hence Ci, clearly suggests a diminished capacity of the mesophyll factors to utilize the enhanced substrate levels.

C. Assessing the reasons for a high mesophyll limitation of 'A' in plants subjected to moisture stress

It is evident from the results presented earlier that in plants experiencing abiotic stress, 'A' did not show marked increase even at saturated inter-cellular CO_2 concentration (Ci). The co2 response curve developed under several abiotic stresses revealed a significant reduction in dA/dCi, a measure of the *in vivo* carboxylation efficiency and a concomitant increase in l_m. These data clearly suggested a possible direct inhibition on the mesophyll components of photosynthesis. To examine the reasons for this increase in mesophyll limitation, an experiment was conducted by subjecting sunflower, cowpea and maize plants to moisture stress. Leaf water status, gas exchange characteristics, leaf protein content was determined. CO_2 sensitivity, photorespiration and specificity factor of rubisco, as a measures of mesophyll components were also determined.

Relative Water Content (RWC):

Sunflower, Cowpea and Maize plants were subjected to moisture stress by growing the plants at 50 percent field capacity for seven days. The relative water content of the top fully expanded leaf was determined at the end of the stress period (table 12).

Table 12: Relative water content of plants subjected to moisture stress

Crop	Control	Stress
Sunflower	72.4	52.3
Cowpea	74.0	57.4
Maize	84.0	66.7
CD (p=0.05)	3.8	5.1
Interaction	10.1	

The relative water content of well watered sunflower, cowpea and maize were 72.4, 74.0 and 84.0 percent, respectively. Water stress reduced the RWC of the plants significantly to 52.3 % in sunflower, 57.4 % in Cowpea and 66.7 % in Maize.

Gas exchange parameters:

Both assimilation rate (`A') and stomatal conductance (`g_s') decreased under stress in all the three species. Assimilation rate decreased from 20.08 μ moles.$m^{-2}.s^{-1}$ in well watered sunflower to 12.76 μ moles.$m^{-2}.s^{-1}$ under stress. The stomatal conductance, on the other hand showed a reduction of 76.07 percent in stressed sunflower. The `A' at saturated Ci (A_{max}) also showed a significant reduction under stress (Table 13)

Similarly, cowpea also showed a significant reduction in `A' under stress. `A' was 9.52 μ moles.$m^{-2}.s^{-1}$ in stressed cowpea which was 34.83 percent less than the control. Stomatal conductance decreased from 418 mmoles in control to 154.32 mmoles under stress. A_{max} decreased significantly from 23 μ moles.$m^{-2}.s^{-1}$ in well watered plants to 14.5 μ moles.$m^{-2}.s^{-1}$ in plants experiencing moisture stress.

Maize followed a similar trend in all the parameters studied. The plants grown in well watered condition recorded an `A' of 25.19 $\mu$ moles.$m^{-2}.s^{-1}$ and a `g_s' of 270 mmoles.$m^{-2}.s^{-1}$. Under stress, `A' and `g_s' decreased to 9.97 μ moles.$m^{-2}.s^{-1}$ and 123.46 mmoles, respectively. The reduction in `A' and `g_s'

Table 13: Gas exchange parameters of plants subjected to moisture stress

	'A'	g_s	A_{660}	dA/dCi	CO_2 sensitivity
Sunflower					
Control	20.08	728	36.0	0.144	0.793
Stress	12.76	174	15.5	0.079	0.215
CD (p=0.05)	3.9	126	6.5	0.009	-
Cowpea					
Control	14.61	418	23.0	0.079	0.574
Stress	9.52	154	14.5	0.068	0.523
CD (p=0.05)	5.4	93	5.9	0.006	-
Maize					
Control	25.19	270	35.0	-	0.389
Stress	9.97	123	12.73	-	0.277
CD (p=0.05)	5.9	71	6.9	-	-

under moisture stress was 60.42 percent and 54.28 percent, respectively. The A_{max} showed a significant reduction under stress. `A' at 660 Ci was 35 μ moles.m^{-2}.s^{-1} in control. A_{max} decreased under stress to 12.73 μ moles.m^{-2}.s^{-1} (Table 13).

Though the reduction in g_s was more than the reduction in `A', the present data (table 13) suggests that the reduction in `A' under moisture stress was more due to the stress effect on mesophyll factors of photosynthesis. The rate of increase in `A' per unit increase in Ci (dA/dCi), considered as an indirect reflection of carboxylation efficiency, recorded a significant reduction in sunflower and cowpea subjected to moisture stress (Table 13). This further supports the conclusion that mesophyll factors are affected during stress.

CO_2 sensitivity under moisture stress

Quantifying the co2 sensitivity of photosynthesis is yet another approach to assess whether the reduction in `A' under stress is due to mesophyll factors or not. $CO_2$ sensitivity is the response of `A' to increased CO_2 concentrations (saturated CO_2). The data on the CO_2 sensitivity presented in table 13, clearly suggest that `A' did not increase even when Ci was saturated. Sunflower showed the highest reduction in CO_2 sensitivity. The well watered sunflower plants showed a CO_2 sensitivity of 0.793 that decreased to 0.215. Though CO_2 sensitivity in cowpea decreased under stress, the reduction was marginal. It decreased from 0.574 in control to 0.523 under stress. Maize on the other hand also recorded reduction in co2 sensitivity from 0.389 in well watered plants to 0.277 under moisture stress.

These data clearly indicate a damage to the mesophyll factors. Further experiments were conducted to study a few subcomponents of mesophyll under moisture stress condition.

Effect of moisture stress on Photorespiration (PR):

Photorespiration was determined by inhibiting the oxygenation process by providing low oxygen (2%) in the gas mixtures used for the measurement of gas exchange characteristics.

Well watered Sunflower plants showed a significant increase of 30.31 percent in assimilation rate at 2% oxygen. `A' increased from 20.08 μ moles.m^{-2}.s^{-1} at 21% oxygen (ambient air) to 28.81 μ moles.m^{-2}.s^{-1} at 2% oxygen. Interestingly on the other hand, there was a very marginal increase in assimilation rate at 2% O_2 in water stressed sunflower plants (5.8%). The apparent photosynthesis (APS) was 12.76 μ moles.m^{-2}.s^{-1} at 21% oxygen and TPS increased only slightly to 13.55 μ moles.m^{-2}.s^{-1} at 2% oxygen (Table 14).

Cowpea showed an increase of 21.36 percent in `A' under control condition. Assimilation rate increased from 14.61 μ moles.m^{-2}.s^{-1} at 21% oxygen to 18.58 μ moles.m^{-2}.s^{-1} at 2% oxygen. The water stressed Cowpea plants showed an APS of 9.52 μ moles.m^{-2}.s^{-1} at 21% oxygen. The total photosynthesis (TPS) at 2% oxygen, devoid of photorespiration, increased to 13.86 μ

Table 14: Effect of moisture stress on photorespiration

	APS $(A_{21\%\ O_2})$	TPS $(A_{2\%\ O_2})$	PR (%)
Sunflower			
Control	20.08	28.81	30.31
Stress	12.76	13.55	5.80
CD ($p=0.05$)	3.12		
Cowpea			
Control	14.61	18.58	21.36
Stress	9.52	13.86	31.31
CD ($p=0.05$)	3.9		
Maize			
Control	25.19	26.62	5.38
Stress	9.97	10.75	7.26
CD ($p=0.05$)	7.3		

moles.$m^{-2}.s^{-1}$ which constituted a 31.31 percent increase suggesting a role of PR in the inhibition of photo- synthesis under moisture stress conditions in cowpea (Table 14)

It is an established fact that the C_4 plants do not have a significant amount of PR. In accordance, Maize failed to show any significant increase in assimilation rates at 2% oxygen either in control (5.38%) or in the leaves experiencing moisture stress (7.26%).

Total protein content

The total soluble protein content was estimated following Lowry's method. The mean total protein content of the three species is presented in table 15.

There was a significant reduction in the total soluble protein in all the three species studied under moisture stress. Well watered Sunflower plants had 22.5 mg of protein. g^{-1} FW of leaf. The protein content in Cowpea and Maize were respectively, 19.38 mg.g^{-1} FW and 23.75 mg.g^{-1} FW. The corresponding total soluble protein in sunflower, cowpea and maize plants experiencing moisture stress were 15.81 mg.g^{-1} FW, 10.0 mg.g^{-1} FW and 13.13 mg.g^{-1} FW, respectively. The reduction in 'A' under stress corresponded well with the reduction in the total soluble protein content.

The reduction in the total soluble protein seems to be the major mesophyll factor responsible for the decline in the assimilation rate of leaves experiencing moisture stress. To

Table 15: Effect of moisture stress on total soluble proteins and the efficiency of assimilation expressed per unit protein

	Control		stress	
	protein	A/protein	protein	A/protein
Sunflower	22.50	4.12	15.81	4.14
Cowpea	19.38	3.63	10.00	3.98
Maize	28.75	4.82	13.13	3.42
CD (p=0.05)	3.01	0.98	2.96	0.91
CD Interaction A/Protein		0.21		

further clarify whether the efficiency of assimilation has also decreased under stress, `A' was expressed per unit soluble protein. The data are presented in table 15.

The ratio of assimilation per unit soluble protein recorded a significant difference between the species. Maize recorded the maximum ratio of 4.82 μ moles g^{-1} protein.s^{-1} followed by sunflower with 4.11 μ moles.g^{-1} protein.s^{-1} compared to 3.63 μ moles/g protein/s in cowpea. The variations in the assimilation rate between these crop species correlated well with the ratio of assimilation per unit soluble protein. Thus the low assimilation rate in cowpea can be attributed to the protein content of the leaf.

However, though assimilation rate expressed per unit protein was altered under stress in sunflower and cowpea, the changes were not statistically significant. The data clarified that the reduction in `A' under moisture stress was more due to the reduction in the protein content per se rather than its efficiency in sunflower and cowpea. On the other hand, `A' decreased by 60 percent in maize which could be attributed to both reduction in protein content and its efficiency for carbon assimilation. The `A' per unit protein decreased to 3.42 μ moles.g^{-1} protein (Table 15).

A high reduction in the total soluble protein could also mean a significant reduction in the most important enzyme protein, namely, RuBisCO. In the absence of data regarding the changes in the content of RuBisCO under moisture stress, an attempt was made to assess the specificity factor following gas exchange methods.

Specificity factor of RuBisCO:

The enzyme Ribulose 1-5, Bis phosphate Carboxylase/ Oxygenase is a very essential protein that occupies a pivotal position in photosynthesis. The enzyme content, its activity and activation state and the kinetic constants associated with the enzyme catalysis are the most important characteristics that determine the photosynthetic efficiency of crop plants. Since the enzyme RuBisCO can catalyze a productive carboxylation of RuBP and a wasteful oxygenation of the same substrate, the apparent net exchange of CO_2, therefore depends on the relative velocities of carboxylation and oxygenation of RuBP. The ratio of the V_{max}/k_m of carboxylase and oxygenase explains the net carbon gained by plants. This ratio is often referred to as the 'Specificity factor' of RuBisCO. A higher specificity factor implies better carboxylation and vice-versa.

A gas exchange technique for the measurement of the specificity factor as explained by Brooks and Farquhar (1985) was adopted with certain modifications (See Materials and Method). The specificity factor was calculated for Cowpea and Sunflower subjected to moisture stress. The data obtained are presented in table 16 and illustrated in Figs. 15 and 16.

There was a marked reduction in the specificity factor of Cowpea RuBisCO under stress. The specificity factor showed a

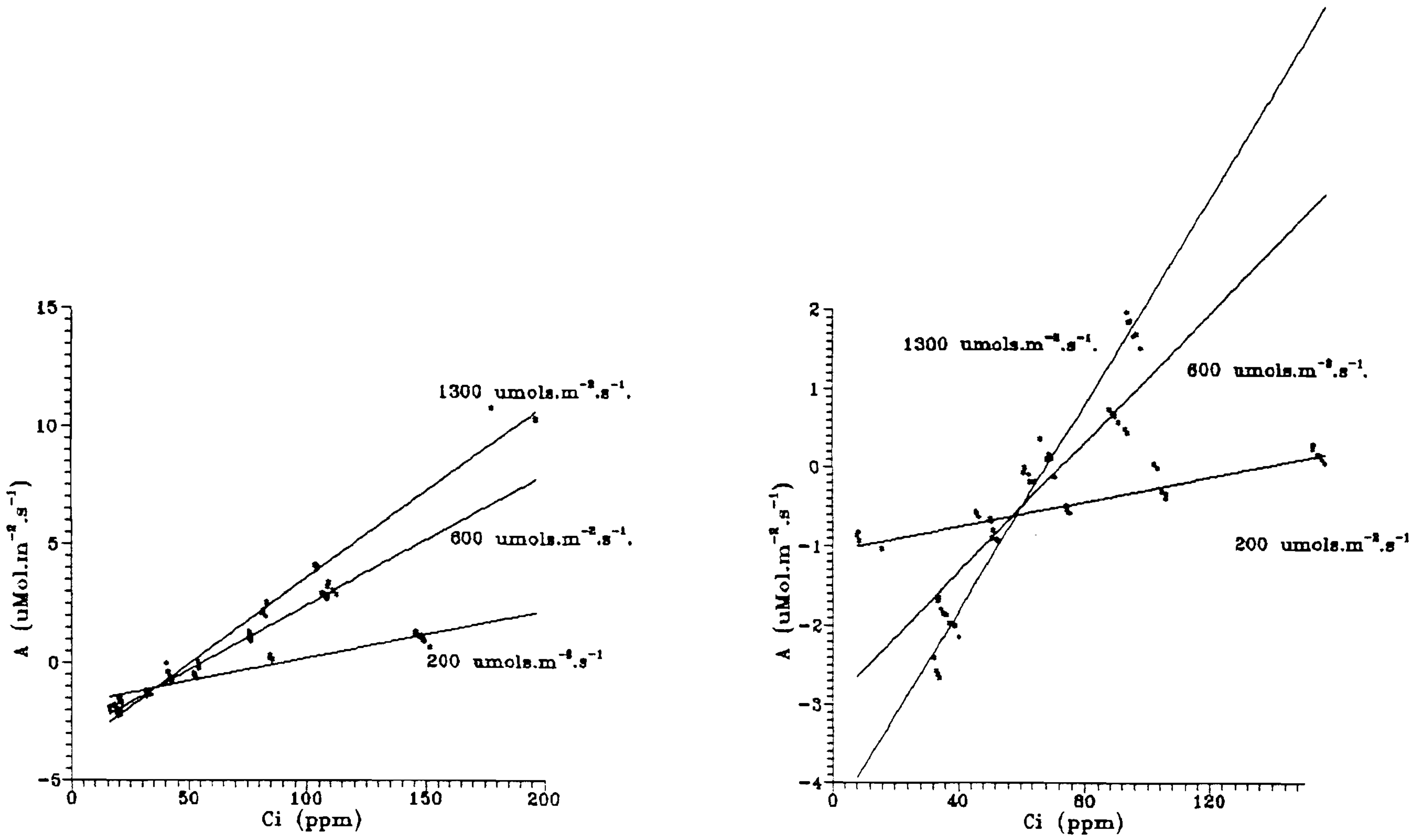


Fig.15: Initial slope of the CO_2 response curve to compute the specificity factor of RuBisCO in cowpea.

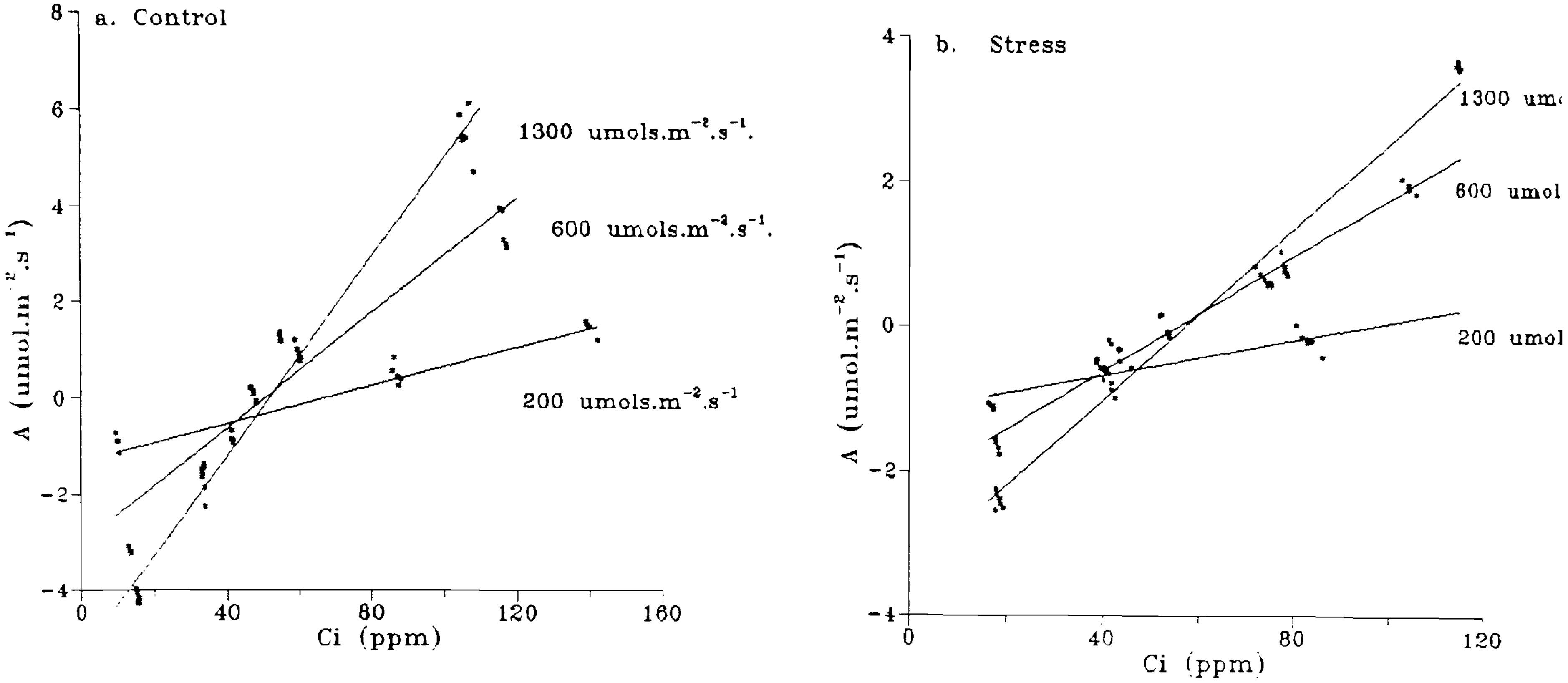


Fig.16: Initial slope of the CO_2 response curve to compute the specificity factor of RuBisCO in sunflower.

Table 16: Effect of moisture stress on the specificity factor of RuBisCO in cowpea and sunflower

	Control	Stress
Sunflower	86.09	84.25
Cowpea	110.00	66.00
CD ($p=0.05$)	12.1	10.6
Interaction	17.2	

value of 110 in control plants that decreased to 66 in plants experiencing moisture stress. On the contrary, there was no significant reduction in the specificity factor in Sunflower RuBisCO under moisture stress. The specificity factor for Sunflower under control was 86.09 that decreased only to 84.25 under stress indicating either a proportionate change in Carboxylase and Oxygenase activities or no change at all under stress.

From the data generated in this experiment, the following important inferences can be drawn.

1. The reduction in assimilation rate under moisture stress is predominantly due to a reduced mesophyll conductance as evidenced by a decrease in dA/dCi.

2. Reduction in total protein content seems to be an important mesophyll factor determining `A' in stress.

DISCUSSION

DISCUSSION

Significant increase in grain yield in recent years is attributed mainly to optimization of agronomic inputs (Gifford, 1987), maximizing light interception by optimising canopy architecture (Nelson 1988) and enhancing partitioning efficiency of crop plants (Austin et al, 1980). It has been recognized that a plateau has already been achieved in these aspects in most of the crops, especially in cereals. Therefore, further improvements in canopy photosynthesis is possible only by enhancing the unit leaf area photosynthetic rates. Though considerable degree of species and genotypic variation exists in single leaf photosynthetic rates (Fischer et al., 1981; Mohan and Hobbs, 1981; Hobbs and Mohan, 1985; Austin et. al 1986; Shankar et al., 1990; Nanjareddy, 1992), progress made in improving photosynthesis has not been rapid and encouraging.

Owing to the complex multigenic control of photosynthesis, conventional breeding for high photo- synthetic rate has not met with greater success. In recent years, research effort is being concentrated on evaluating various sub components that regulate or limit photosynthetic rates. If a few important limiting factors are identified, incorporation of these factors to enhance assimilation rates by either conventional breeding technique or through molecular biological techniques would enable us for a quicker and assured success in improving photosynthesis.

Though essentially a biochemical process, photosynthesis can be regarded as a diffusive process also (Kriedman & Downton, 1981). The movement of CO_2 from the air into the carboxylation site and its assimilation experience a number of diffusive resistances that are broadly classified under stomatal and non-stomatal factors. The non-stomatal or mesophyll factors, though a highly complex phenomenon, determine the diffusive gradient for CO_2 transfer. Once CO_2 is made available at the carboxylating site, further fixation requires, electron transport, RuBisCO, Calvin cycle enzymes, end product synthesis and its export.

There is considerable variation between the potential `A' when $CO_2$ is made available at the carboxylating site and the `A' often realized at ambient CO_2. Assessment of potential `A' ($A_{max}$) and apparent photosynthesis will give some lead to identify the important limitations imposed for achieving potential `A'.

Therefore, one of the objectives of the present investigation was to assess A_{max} and apparent photosynthesis, in sunflower and maize plants.

A_{max} and APS in Sunflower & Maize

To assess the potential of the chloroplast to fix CO_2, an experiment was conducted to quantify the A_{max} of Sunflower and Maize plants.

`A' at saturated CO_2 was significantly high in both species. The increase in 'A' from ambient CO_2 to saturated CO_2 was very high in Sunflower, compared to Maize. Increase in 'A' was 91 percent in sunflower and in Maize it was 37 percent (Table 1). This clearly inferred that the chloroplasts have very high ability to fix carbon if substrate CO_2 is not limiting. Although the C_4 plants have evolved an efficient CO_2 concentrating mechanism in the Bundle sheath cells, photosynthesis positively responded to the increase in substrate CO_2 levels.

Any increase in the substrate levels in the mesophyll cells will increase carboxylation apart from significantly decreasing the oxygenase limitation. Therefore, the CO_2 diffusive processes assume grater importance under ambient CO_2 levels. In a recent study, Shivashankara, (1992), based on light response curves in sunflower and cowpea, concluded that the primary limitation to 'A' comes from CO_2 diffusive characteristics and photorespiratory limitations.

However at a given CO_2 level, the rate of carboxylation efficiency further depends on the mesophyll characteristics especially the factors associated with RuBisCO and also other mesophyll traits like ATP and NADPH generation. In this context, it is essential initially, at a given CO_2 level, to quantify the extent of limitation imposed by CO_2 diffusive processes and the intrinsic mesophyll factors.

One of the major objectives of the present investigation was to develop a new approach to quantify the relative stomatal and mesophyll limitations of `A' under both non stress and abiotic stress conditions.

Partitioning the stomatal and mesophyll limitations of `A'

With the intention of partitioning the limitations imposed on `A' many approaches have been developed and standardized in the recent past. Most of the approaches developed have been based on the following two methods.

1. Resistance analysis
2. Path dependent or sensitivity analysis

Resistance analysis was originally proposed by Gaastra in 1959, which was based on the assumption that photosynthetic response to CO_2 would increase in a linear fashion. Recently Farquhar and Sharkey (1982) slightly modified the assumption and pointed out the possibility of an over estimation of stomatal limitation by linear resistance analysis. Instead of the originally proposed linear resistance analysis by Gaastra, (1959). Farquhar and Sharkey (1982) proposed that at infinite stomatal conductance, theoretically Ca and Ci would equilibrate. The Assimilation rate measured at Ci = 330 ppm would therefore represent an `A' that is not limited by stomatal factors. This method has been widely used for quantifying stomatal limitation only.

More recently, Prioul et al., (1984), and Jones (1985) developed a path dependent method to quantify the stomatal and mesophyll limitations of photosynthesis. The path of decline in photosynthesis due to an environmental variable can be quantified using this method. The sensitivity of either stomata or mesophyll to the changing environmental variables is considered in this methodology. The reduction in `A' of control leaf to a lower value under stress could be due to the reduction in `A' first due to stomata and then to the reduction in mesophyll. This method is also used when the `A' is assumed to be reduced first due to a reduction in mesophyll followed by the reduction in stomatal conductance (Fig.17). Assmann (1988) gave a detailed evaluation of these methods. The path dependent method of quantifying limitations to `A' is based on the sensitivity analysis. If assimilation can be described as a function of various parameters such as

$$`A' = f(g, H, T........),$$

then, the sensitivity of `A' to any one of the variables can be obtained by finding out the rate of change in `A' per unit change in that variable when other variables are held constant. In other words, the sensitivity can be described as a partial derivative with respect to that variable.

Though this procedure can be adopted for quantifying stomatal and mesophyll limitations of photosynthesis, the method is useful only to explain a reduction in `A' due to an environmental variable. But it is not possible to apportion the limitation of photosynthesis of an observed `A' under a given condition. For instance, it is not possible to quantify the relative stomatal and mesophyll limitations of `A' of an unstressed leaf.

The path dependent method can describe only the relative stomatal and mesophyll limitations of a stress induced decline in `A'. The resistance analysis can quantify only the stomatal limitations. However it is essential to quantify both stomatal and mesophyll limitations for a given situation even under non stress condition. In view of this, a new method was developed to quantify the relative stomatal and mesophyll limitations based on the linear resistance techniques.

Quantification of mesophyll limitation - A new approach

The stomatal limitation was computed both under non stress and abiotic stress conditions as explained by Farquhar and Sharkey (1982). Based on the assumption of the linear resistance analysis, the response of `A' to Ci is maximum at low internal CO_2 concentrations. Thus, it is assumed that the carboxylation response to increasing CO_2 concentration is maximum at the initial region. This initial response of the CO_2 response curve (dA/dCi) is often considered as the carboxylation efficiency or sometimes as the mesophyll conductance. This linear response becomes curvilinear and asymptotic with further increase in Ci beyond that of the ambient level. This plateauing of the response has been attributed to the lack of an equal capacity for RuBP regeneration and therefore the mesophyll inability to utilize CO_2 (Caemmerer and Farquhar, 1981; Farquhar and Sharkey, 1982).

In the method proposed for quantifying mesophyll limitation, it is assumed that if mesophyll factors were not to limit `A' then the initial linear region of the $CO_2$ response curve would not have become curvilinear. Hence an extrapolation of this linear region will represent a A- Ci relation that is expected when mesophyll factors do not limit `A'. At operating Ci if observed `A' is less than the extrapolated point based on the initial slope of the $CO_2$ response curve, suggests that mesophyll factors limit `A'. At operating Ci, if mesophyll factors were not limiting, the assimilation rate should have been at the same point as on the extrapolated line (point A_g in Fig.2). In cases where observed `A' is less than $A_g$ it is assumed that mesophyll factors limit `A'. The relative mesophyll limitations are quantified as follows.

$$l_m\ (\%) = \frac{A_g - A}{A_g} \times 100$$

This method sufficiently explains the limitations offered by stomatal and mesophyll factors on `A' at a given condition. This method can be conveniently used for computing limitations of stressed and unstressed plants.

Quantification of mesophyll limitations under stress

It has been shown that the stress induced reduction in `A' is primarily due to a reduction in the mesophyll conductances. Thus under any abiotic stress condition the initial slope of the $CO_2$ response curve significantly decreases resulting in the inhibition of photosynthesis. Therefore it was assumed that the reduction in `A' under stress was due to a reduction in the mesophyll conductance as evidenced by a decrease in the initial slope of the CO_2 response curve. This reduction in dA/dCi would therefore correspond to the increasing mesophyll limitation. Hence, for the computation of the mesophyll limitation of `A' under abiotic stresses, the linear initial slope of the unstressed leaf was considered.

The observed assimilation rate (A') under stress is a result of both stomatal and mesophyll limitations operating at ambient CO_2 levels. Based on the assumption made in this approach, if mesophyll limitations were not operating, the assimilation rate of the stressed leaf should have been on the extrapolated line (the point A_g in Fig. 2). Hence it is argued that the observed reduction in assimilation rate under stress from a possible A_g to A' is a result of the mesophyll limitations. Thus the mesophyll limitation of `A' under stress can be computed from the following formula.

$$l_m\ (\%)\ \text{stress} = \frac{A_g - A'}{A_g} \times 100$$

Quantification of relative limitation of `A' under non stress condition in a few C_3 and C_4 species

To assess the gas exchange parameters in relation to their potential, and to quantify the relative stomatal and mesophyll limitations of `A', an experiment was conducted with a few C3 and C4 species.

There was a significant variation in `A' and g_s between the crop plants tested (Table 2). Computation of A_{max} in these crops further indicated that in both C_3 and C_4 plants the chloroplasts have higher capacity for carbon assimilation.

Based on the CO_2 response curve, the relative stomatal and mesophyll limitations were quantified in these C_3 and C_4 plant (Table 3).

In all the species studied, stomata seemed to impart highest limitation to `A' under well watered conditions. Of the total limitations, stomatal factors shared the highest contribution in limiting `A'. In all the plant species except cowpea, stomata contributed over 60% to the total limitation of photosynthesis.

Stomata have been clearly shown to limit `A' under well watered condition by several workers (Jones, 1986; Perumal et al., 1989; James Jacob, 1988; Xu & Xu, 1989; Ephrat et. al 1990; James & Lawlor, 1991; Heitholt 1991).

Both among C_3 and C_4 species the plant that had a high g_s showed higher `A' (Table 2). Such a positive relationship between `A' and g_s has been reported by many workers (Wong et al., 1979). Though such a positive relationship between `A' and g_s was noticed, still stomatal limitation was more than the relative mesophyll limitation even in plants with higher g_s. For instance, sunflower that recorded the highest g_s of 729 mmole, had a high stomatal limitation compared to its relative mesophyll limitation.

Since in all the species observed 'A' was much less than A_g it infers that mesophyll factor also limit 'A'. This was further confirmed by the fact that the calculated <u>in vivo</u> carboxylation efficiency based on the initial slope of the CO_2 response curves shows significant positive correlation with observed assimilation rates ($R^2 = 0.8$). Therefore, though the l_s (%) was high in all the crop plants, mesophyll also had a significant control over 'A' at ambient CO_2.

Effect of partial defoliation on gas exchange character

Since stomatal factors imposed the maximum limitations on 'A', an attempt was made to enhance g_s and study 'A' in a few crop plants. The root to shoot ratio was enhanced by partial defoliation to increase the g_s in pot grown sunflower, soybean and sorghum.

Stomatal conductance increased significantly in all defoliated plants within 4 hrs. of treatment and was persistent even after 24 hrs. (Table 4). This increase in g_s resulted in a concomitant increase in 'A' as well in sorghum and soybean but not in sunflower. Sunflower that intrinsically had higher g_s compared to the other two crop species, although the stomatal conductance increased significantly, there was no increase in 'A'.

The results clearly indicate that increase in g_s and thus minimizing l_s(%) or CO_2 diffusive limitation substantially increased 'A' in soybean and sorghum. However, it is not clear and no explanation can be offered why 'A' did not increase in sunflower with increase in g_s.

The CO_2 response curves developed in these species indicated that both the initial slope and the A_{max} increased in soybean and sorghum. The increase in 'A' at saturated Ci is a clear indication of the involvement of a mesophyll factor due to defoliation. If only stomata were responsible for controlling 'A', it would not have increased at saturated Ci in defoliated plants. On the other hand the increase in the initial slope of the CO_2 response curve (dA/dCi), further reiterates the possibility of an increase in the mesophyll conductance (in vivo carboxylation efficiency) when plants were subjected to partial defoliation.

Such increase in 'A' by defoliation was also observed by Wareing et al. (1968); Neals et al., (1971); Thorne and Koller, (1974); Peat and Kramer, (1981). More recently Caemmerer and Farquhar, (1984) observed an increase in dA/dCi and CO_2 saturated 'A' when Phaseolus plants were defoliated. Since dA/dCi correlated well with carboxylase activity, they concluded that defoliation enhanced carboxylation efficiency. Earlier working with the same plant material, Jenkins and Woolhouse, (1981) had demonstrated an increase in in vitro electron transport rate in primary leaves after defoliation. They suggested that the increase in electron transport rate was most likely the consequence of an increase in the amounts of intermediate carriers of the electron transport chain. They also opined that the electron transport capacity to regenerate RuBP changes almost proportionally with RuBisCO.

In the present investigation also soybean and sorghum recorded increases in A_{max} and dA/dCi. However, it is necessary to probe into the factors responsible for such increases in mesophyll factors within such a short period.

These set of observations clearly indicate the possibility of further improving the observed assimilation rates by enhancing the substrate level at a given ambient CO_2 concentration by enhancing the diffusive conductance of CO_2 transfer. Yet another factor that could be attempted to improve the assimilation rates at ambient CO_2 is to enhance the in vivo carboxylation efficiency of the plants. The in vivo carboxylation efficiency showed a significant relationship ($r = 0.9$) with 'A' in well watered plants (Fig. 18). This suggested that the mesophyll factors also have a considerable control over assimilation rate. Enhancing 'A' via an improvement in the mesophyll factor would, therefore, prove more beneficial under limited water conditions as it can help in conserving moisture in the plants and result in a more efficient use of water for dry matter production.

Gas exchange parameters and relative limitations of 'A' under different abiotic stresses

Despite the fact that photosynthesis is controlled to a significant extent by the genetic make up of the plants, the *genotype and environment interaction complicates* the phenotypic

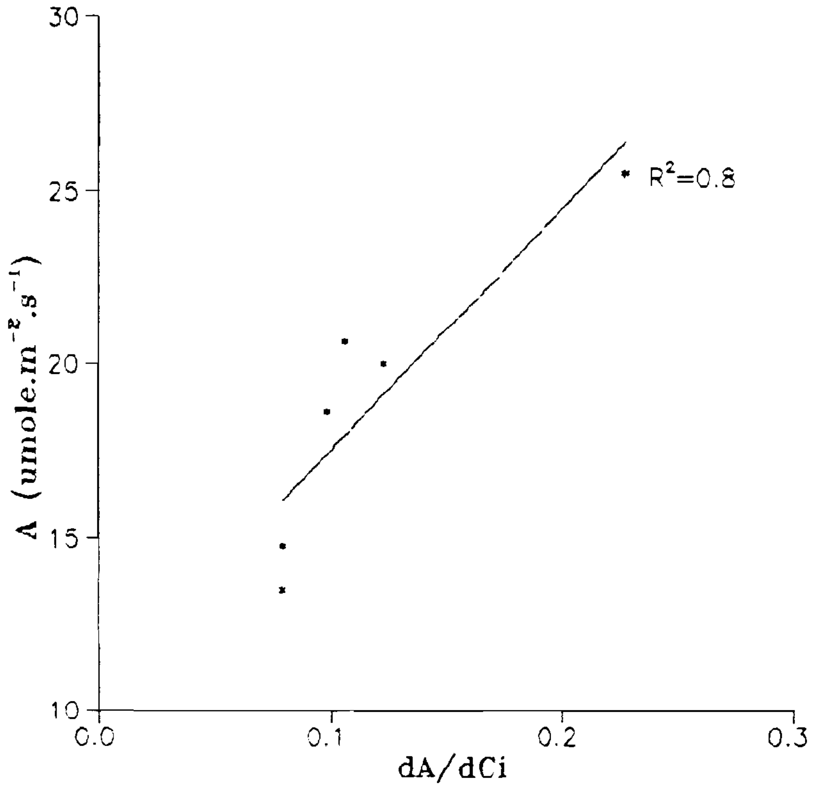


Fig.17: Relatioship between 'A' and dA/dCi in a few crops under non stress condition.

expression of photosynthesis. The gene expression and intrinsic regulation of photosynthesis is modulated to a large extent by the environmental factors. The existing environmental factors such as moisture in soil and leaf, light intensity, temperature and humidity of air and nutrient status of the soil are a few of the important environmental variables determining assimilation rates.

A large body of literature has clearly established the close coupling of the environmental variables with photosynthesis. It has also been shown that under any stress environment, `A' significantly decreases.

The stress associated reduction in `A' has been shown to be mediated through their effects either on the stomatal diffusive characteristics or on the chloroplast metabolism (Kaiser, 1987; Chaves, 1991; Jacob and Lawlor, 1991; Gimenez, et al., 1992). Though not unequivocally accepted, there are strong arguments supporting the fact that the stress induced reduction in `A' is due to mesophyll factors more so than the stomatal factors.

Stress induced reduction in assimilation rates have been amply attributed to a reduction in the mesophyll capacity for carbon assimilation. Mesophyll limitations have been shown to be responsible for the reduction in photosynthesis under moisture stress (Johnson et al., 1987; Kaiser, 1987; Chaves, 1991; Gimenez et al., 1992), deficient nitrogen or phosphate supply (Lawlor et al., 1989; Heitholt et al., 1991; Jacob and

Lawlor, 1991; 1992). Low light stress has also been shown to reduce `A' through a reduction in the mesophyl factors.

However, There is no quantitative data in the literature to prove that the mesophyll factors are getting affected under stress since there are no approaches to quantify mesophyll limitation under abiotic stresses.

Yet another important objective of the present investigation, therefore, was quantification of relative stomatal and mesophyll limitations in plants subjected to different abiotic stresses. Pot grown sunflower, sorghum and cowpea plants were subjected to moisture stress, low light stress and nitrogen deficient condition and gas exchange parameters were measured from which the limitations were computed.

In the present investigation data were generated to prove that the major limitation to `A' abiotic stresses was due to the influence mesophyll factors. The following gas exchange associated parameters prove the point that the mesophyll factors were affected.

1. Increase in Ci
2. Decrease in dA/dCi
3. Reduction in A_{max}
4. Quantification of limitations (l_s and l_m)

1. Increase in Ci

Both 'A' and g_s decreased significantly under all abiotic stresses imposed (Tables 5, 7 and 9). Though the assimilation to unit stomatal conductance increased under moderate stress, the ratio tended to decrease when plants were subjected to severe stress conditions. The calculated inter cellular CO_2 concentration (Ci) increased with the intensity of stress (Tables 5, 7 and 9). An increase in Ci is possible when 'A' decreases faster than the stomatal conductance. An increased Ci implies a lack of utilization of substrate CO_2 even when it is provided at the carboxylation site. Such an increase in Ci under stress indicates a possible damage to the mesophyll factors rendering it inefficient in utilizing the available substrate CO_2.

2. Decrease in dA/dCi

CO_2 response curves were developed in the plants experiencing abiotic stresses. The initial slope of these CO_2 response curves (dA/dCi) was considered as the in vivo carboxylation efficiency. A significant linear relationship was seen between observed assimilation rate and dA/dCi under non stress condition and thus it was asserted that dA/dCi regulated the variation in 'A' (Fig. 18). The relationship between dA/dCi and 'A' was also examined in stressed leaves.

The initial slope of the CO_2 response curve (dA/dCi) progressively decreased with increase in the intensity of stress

(Figs. 8 to 13). When the relationship between observed assimilation rate and dA/dCi was plotted (Fig. 19), it revealed a positive interaction ($r = 0.62$) suggesting that the in vivo carboxylation efficiency determines `A' even under stress.

3. Reduction in A_{max}

Yet another parameter often considered to assess the effect of stress on mesophyll factors is to quantify the A_{max} at saturated substrate levels. Less increase in `A' at saturated CO_2 implies a reduced capacity of the chloroplast to fix CO_2 thus resulting in a lowered A_{max}. It was evident from the CO_2 response curves that the assimilation rates did not increase to larger extends under stress. The A_{max} recorded at saturated Ci progressively decreased with the intensity of the abiotic stress (Figs. 8 to 13). The reduction in A_{max} and the lowered initial slope of the CO_2 response curves clearly suggested a possible damage to the mesophyll factors of photosynthesis.

Quantification of limitations

Since the earlier experiments showed an indication of a reduction in mesophyll ability for carbon assimilation, an attempt was made to quantify the relative stomatal and mesophyll limitation of `A' under abiotic stresses. As explained in the material and methods, $CO_2$ response curves were adopted for the quantification of limitations. Quantification of the relative limitations of `A' revealed an increase in the mesophyll limitations of photosynthesis as the stress progressed. This

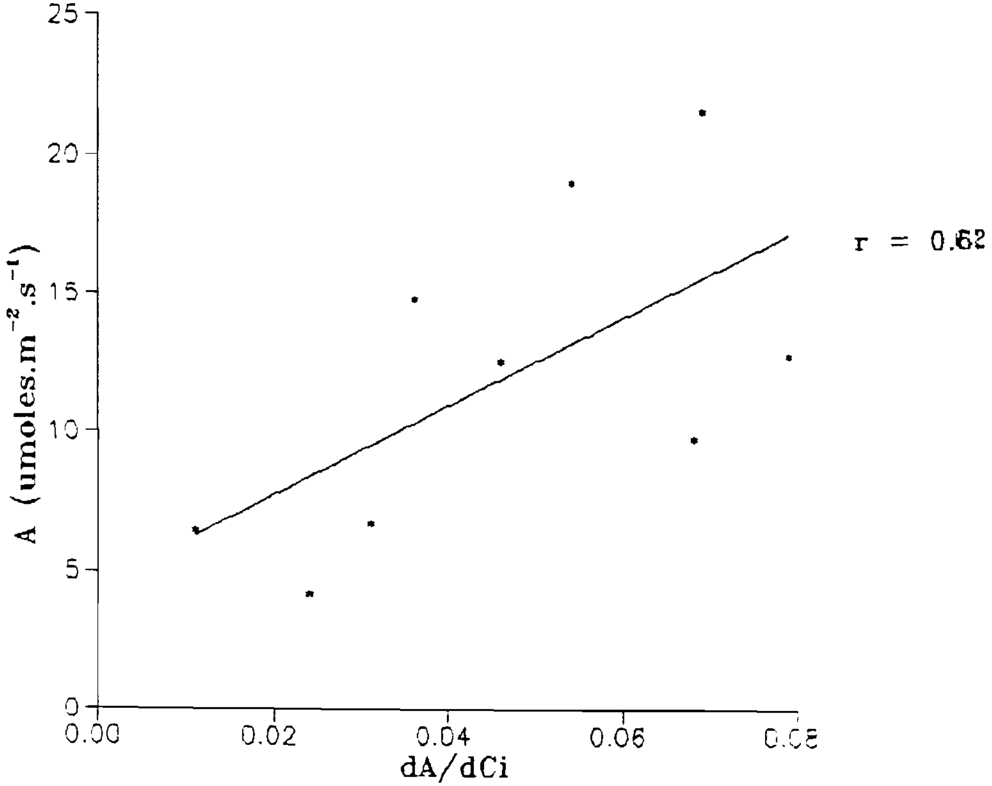


Fig.18: Relatioship between 'A' and dA/dCi in a few crops under abiotic stress condition.

type of increase in the mesophyll limitations of `A' was noticed under all the abiotic stresses studied (Table 6, 8 and 10). Stomatal limitation was the major factor in the total limitation of `A' under non stress condition in all the species. For instance in sunflower stomatal limitation constituted 65 percent of total limitation under non stress condition. Where as its contribution progressively decreased with increase in the intensity of moisture stress to a very low value of 8 per cent when severe stress was imposed. On the other hand, mesophyll limitation progressively increased to 92 per cent in the same plants (Table 17). Such a trend was noticed in sorghum and cowpea also. As the intensity of the abiotic stress increased, the limitations of photosynthesis shifted significantly towards mesophyll factors. These experiments clearly demonstrated that the predominant limitations to photosynthesis were imposed by the mesophyll factors when plants experience abiotic stress conditions.

Patchy stomata - An objection to mesophyll limitations

Although several workers have reported an increase in mesophyll limitations of `A' under abiotic stresses based on gas exchange measurements, such conclusions are being suspected owing to the discovery of a heterogeneous stomatal closure on the leaf surface in plants subjected to moisture stress.

Owing to this `patchiness' of stomatal closure, the validity of stress induced inhibition of photosynthesis by mesophyll factors have been questioned by some recent studies by

Table 17: Percent of l_s and l_m of total limitations under abiotic stress

	moisture stress		Light stress		Nitrogen stress	
	% l_s	% l_m	% l_s	% l_m	% l_s	% l_m
Sunflower						
Control	65	35	59	41	41	59
Moderate stress	22	78	18	82	22	78
Severe stress	8	92	9	91	-	-
Sorghum						
Control	91	9	75	25		
Moderate stress	77	23	50	50		
Severe stress	42	58				
Cowpea						
Control	50	50				
Moderate stress	37	63				

$$\% \, l_s = \frac{l_s}{l_s + l_m} \times 100 \qquad \% \, l_m = \frac{l_m}{l_s + l_m} \times 100$$

Sharkey and Seeman, (1989) and an initial report by Downton et al., (1988). Sharkey and Seeman, (1989) reported that, in bean plants subjected to water deficits, heterogenous ('patchy') stomatal closure was evident. Only patches of the leaves on water stressed plants were photosynthetically active. Using autoradiography analysis of $^{14}CO_2$ fed leaves, they deduced that stomata in portions of these leaves were closed and at these patches the internal CO_2 was thought to be at compensation point and 'A' was zero. The study by Downton et al., (1988) demonstrated that the patchy $^{14}CO_2$ uptake under moisture stress is due to the heterogenous stomatal closure and not to the differences in the effect of water potentials on different areas of the leaf. Terashima et al., (1988) explained that patchy stomatal closure affects the CO_2 response curves generated from area-based stomatal conductance measurements. Thus the calculated parameters from such a CO_2 response curve would be invalid.

Recently, Gunasekara and Berkowitz, (1992) subjected spinach, wheat and bean plants to different regimes of water stress. Based on their comparison of the autoradigraphs of $^{14}CO_2$ fed leaf laminae of the stressed and well watered plants, concluded that the water stress induced patchy stomatal closure was not found to be a general response. Not all stress regimes induced patchiness, nor will all plants demonstrate this response to water stress.

More over the occurrence of patchy stomatal closure has only been demonstrated on a microscopic scale, and it has been shown to be a random phenomenon (Downton <u>et al</u>., 1988; Mansfield, 1990).

When large areas of a leaf (more than 2 cm^2) are used for gas exchange measurements, the apparent photosynthetic rates and stomatal conductance represent average values over that area. Thus avoiding the possibility of an error component creeping into the analysis of A-Ci relationship. The occurrence of patchy stomatal closure has been reported only in moisture stressed leaves. Since identical inferences in terms of a larger mesophyll limitation were drawn under other stresses also, it can be concluded that stomatal closure in patches do not hamper the inferences drawn from gas exchange analysis. However more detailed evaluations of the occurrence of patchy stomata and its significance has to be more clearly understood.

Loop Gain analysis

In the present investigation it was noticed that the stomatal conductance decreased significantly under stress. However, the stomatal limitations did not show any significant variation. The reduction in assimilation rate under moisture stress was predominantly attributed to a large increase in the mesophyll limitation of `A'. Similar results where mesophyll limitations were mainly responsible for the stress induced reduction in `A' was also reported by Jacob, (1988); Jacob and Lawlor, (1991). These observations clearly indicated that the stomatal conductances were in a feed back control by mesophyll factors.

The stomatal movement has been clearly shown to be regulated by the inter cellular CO_2 concentration (Mott, 1988). The control of stomatal movement by the inter cellular CO_2 concentration is an attractive hypothesis to efficiently regulate transpirational loss of water, especially under moisture stress conditions.

To evaluate the feed back control of g_s by g_m, a loop gain analysis originally proposed by Farquhar et al., (1980) was adopted. The data generated on CO_2 response curves at different abiotic stresses were used for this analysis.

The data presented in table 11 clearly indicate that the stomatal loop gain (G_g) was higher than the assimilation loop gain (G_A) in all the stress levels. However, significant increase in stomatal loop gain was observed under moisture stress. A higher stomatal loop gain indicated that stomata were more strongly coupled with the changes in 'A' and Ci than the coupling of assimilation rate with changes in g_s and Ci. This type of a loop gain analysis clearly established that the stomata were under a feed back control by mesophyll factors. Therefore, it is acceptable that though stomatal conductance decreased to a greater extent than the reduction in assimilation rate, the stomatal limitation of photosynthesis did not record any significant increase under moisture stress.

Effect of increased g_s on 'A'

To evaluate and apportion the reasons for the reduction in assimilation rates between stomatal and mesophyll factors, an attempt was made to enhance the stomatal conductance by altering the Vapour Pressure Deficit (VPD) in moisture stressed sunflower plants (Fig.14)

By this approach stomatal conductance was increased from a very low value to a value as high as that recorded in well watered plants. It was noticed that there was no concomitant increase in 'A' with increase in g_s. This clearly suggested that stomatal factors were less responsible for a stress induced reduction assimilation rate. The lack of increase in 'A' when more substrate CO_2 was provided to the mesophyll cells suggested that the mesophyll metabolism was not capable of utilizing this extra substrate. This clearly suggest a damage to the functional efficiency of the system. Such a lack of increase in 'A' when g_s was enhanced further supports the earlier finding that predominant limitations to assimilation comes from mesophyll factors under moisture stress.

From various experiments conducted with plant species subjected to different abiotic stresses, the following inferences could be drawn.

1. Under any abiotic stress, both assimilation rate and stomatal conductance decreased.

2. in vivo carboxylation efficiency as measured by the initial

slope of the CO_2 response curve (dA/dCi) showed a significant reduction under stress.

3. With increase in the stress level, the stomatal limitation remained unchanged where as the mesophyll limitation substantially increased indicating a damage to the mesophyll metabolic processes.

4. The stomatal limitation did not significantly change despite a larger reduction in stomatal conductance.

5. The larger reduction in g_s compared to the reduction in g_m (dA/dCi) suggested that the stomata were in a feed back control from mesophyll. This fact became more evident from the Loop Gain analysis.

6. It is therefore confirmed that the stomata may not offer any limitation under abiotic stresses, suggesting that the role of stomata is mainly to control the rate of water loss.

Effects of moisture stress on various mesophyll components of photosynthesis

The predominant limitation to photosynthesis came from the mesophyll factors as evidenced by a significant increase in relative mesophyll limitation under all the abiotic stresses studied.

Though initially g_s may pose a limitation, progressive stress has clearly been shown to reduce assimilation rates by altering the mesophyll ability to fix CO_2. The damage to the overall process of photosynthesis can be mediated through an inhibition caused either to reactions associated with the thylakoid membranes or to the biochemical reactions that occur in the stroma (Kaiser, 1987). Controversies persist regarding the importance of these two factors under moisture stress conditions.

One group of workers favoring the effects on stromal biochemistry as the primary target of inhibition of 'A' under moisture stress have emphasized the reduction in total carboxylase activity. Vu <u>et al</u>., (1987); Vu and Yelenski, (1988); Speer <u>et al</u>., (1988) showed a reduction in the amount and activation state of RuBisCO. Reduction in the enzyme activity was shown to be due to the acidification of stroma (Berkowitz, 1983; Berkowitz and Gibbs, 1983).

Another group of workers have shown a simultaneous inhibition of photochemical reactions under moisture stress. Ben <u>et al</u>., (1987); Bjorkman and Powells, (1984) and more recently, Quick <u>et al</u>., (1990) suggested that water stress predisposed the plants for photo-inhibition. Photophosphorylation has been shown to be more susceptible to moisture stress than electron transport rates. Thus a reduction in ATP synthesis can result in a significant reduction in 'A' (Caemerer, 1981; Sharkey and Badger, 1982; Forseth and Ehleringer, 1983 and Lopez <u>et al</u>., 1987). Although there are a few reports indicating the reduction in electron transport rate, recent reports using more sophisticated techniques of chlorophyll fluorescence prove that electron transport is relatively resistant to stress (Schriber and Bilger, 1987).

With an objective to assess the possible reasons for this increase in the relative mesophyll limitation, several mesophyll factors like CO_2 sensitivity, photorespiration, specificity factor and soluble protein were examined in plants subjected to moisture stress.

The observed reduction in the <u>in vivo</u> carboxylation efficiency (dA/dCi) in our experiments could have been due to a reduction in either photochemical reactions or biochemical reactions of the stroma. However, in this study, a few aspects associated with RuBisCO were examined.

CO_2 sensitivity

The lack of increase in 'A' at saturated Ci, measured as the CO_2 sensitivity (Table 13) decreased significantly under moisture stress in all the species studied. Such reductions in CO_2 sensitivity of photosynthesis signify a reduction in either photochemical or other biochemical reactions. However, the lack of increase in A_{max} implies a reduced capacity to regenerate RuBP either due to a reduced capacity of the photochemical reactions to synthesize ATP and NADPH (Caemerer and Farquhar, 1981; Farquhar and Sharkey, 1982).

Although insensitivity of photosynthesis to decreased oxygen concentrations have been considered as an indication of the occurrence of end product inhibition on photosynthesis, lack of CO_2 sensitivity of assimilation can also serve the purpose to a certain extent. Therefore, the reduction in assimilation rates under moisture stress could also be due to coupled inhibition in triose phosphate utilization leading to Pi regeneration limitation (James Jacob, personal communication).

Photorespiration

Changes associated with the specificity factor leading to an increased oxygenation could also be an important reason for the reduction in 'A' under moisture stress. To examine this aspect, photorespiration was estimated in moisture stressed sunflower, cowpea and maize.

The data generated on PR in the present investigation revealed a contrasting behavior in sunflower and cowpea experiencing moisture stress (Table 14). When cowpea showed an increase in the contribution of PR to the total photosynthesis, in sunflower, there was a significant reduction in PR under stress. Though sunflower had a significantly high PR in control (30 per cent, table 14), it decreased to 5.8 per cent under stress. Where as, in cowpea, PR increased from 21 per cent in control to 31 per cent under moisture stress.

Sunflower plants also exhibited a larger reduction in CO_2 sensitivity under stress compared to cowpea or maize. Thus it is possible that the factors supporting carbon assimilation in sunflower at the given water status might have been damaged to a larger extent. Photosynthesis does not respond to decreased concentrations of oxygen when RuBisCO experiences a feed back control from lack of Pi recycling. Therefore it would not be erroneous to consider that photosynthesis in sunflower experienced a strong feed back limitation compared to cowpea (James Jacob, personal communication)

RuBBisCO characteristics

The other important factor that influence the reduction in the *in vivo* carboxylation efficiency are those parameters associated with RuBisCO. The observed changes in the properties of RuBisCO in leaves of slowly stressed plants of different species include the activation state, total activity, enzyme content and the kinetic constants of catalysis (Vu *et al.*, 1987; Speer *et al.*, 1988; Vu and Yelenski, 1988).

Specificity factor of RuBisCO

Theoretically a knowledge of the kinetic constants like K_m and V_{max} of carboxylase and oxygenase activities sufficiently define the rates of carboxylation and oxygenation at a given CO_2 and O_2 concentrations. A ratio of these kinetic constants of carboxylase and oxygenase is referred to as the specificity factor. At a given RuBisCO content and its activation state, carboxylation that largely determines 'A' at a given concentration of CO_2 and O_2, is controlled by the specificity factor.

Based on a gas exchange technique proposed by Brooks and Farquhar, (1985), the ratio of the kinetic constants was determined in the intact leaves of stressed and well watered sunflower and cowpea. The data presented in table 16 indicated a significant reduction in specificity factor of cowpea RuBisCO.

But no significant changes were noticed in the stressed sunflower leaves.

However, cowpea that recorded a larger increase in PR also showed a significant reduction in specificity factor. This might have been due to a larger variation of kinetic constants of oxygenase under stress in this species. But no satisfactory explanation can be offered for the lack of any remarkable reduction in the specificity factor of RuBisCO in sunflower.

Total soluble protein content

Total soluble leaf protein content recorded significant reduction under stress in all the three species (table 15). This reduction in the total soluble protein correlated well with the reduction in the assimilation rates of these crop species. The ratio of assimilation to unit soluble protein was estimated with an intention to assess the efficiency of assimilation (Table 15). The ratio of `A' per unit protein failed to record any significant change in sunflower and cowpea. However, the ratio significantly decreased in maize under moisture stress.

The larger reduction in total soluble protein and a good association between protein content and assimilation rate suggest that the reduction in `A' under stress could have been primarily due to a reduction in total soluble protein content of the leaf.

Since the specificity factor also decreased under stress in cowpea along with a significant reduction in the protein content, one must expect a much larger reduction in A/P ratio. However, the stress induced changes in the RuBisCO content and further the proportion of RuBisCO of the total soluble protein is less understood. In the absence of proper data on RuBisCO content and its activation state, it would be difficult to draw any inferences regarding the role of `A' per unit protein in stressed leaves. Nevertheless, it can be concluded that the reduction in assimilation rate under stress in all the species studied could be primarily due to a large reduction in the total leaf soluble protein content.

Conclusions

The following major conclusions can be made from the various experiments conducted in the present investigation.

1. The chloroplasts have very high potentials for carbon assimilation
2. Availability of substrate CO_2 and photorespiration seem to be the major limitations of `A' under non stress conditions.
3. Quntification of limitations based on the CO_2 response curves - an improvised method over the existing ones- suggested a larger stomatal limitation of `A' under well watered conditions.
4. However, mesophyll factors also seem to regulate the variation in `A' among species or genotypes as evidenced by a significantly positive correlation between observed assimilation rates and the calculated <u>in vivo</u> carboxylation efficiency based on the CO_2 response curves (dA/dCi).
5. But under any abiotic stress condition, mesophyll factors appeared to limit `A' to larger extent though g_s decreased to

greater extends.

6. Larger reduction in g_s than 'A' under abiotic stress conditions indicate that stomata are regulated by a feed control exerted by the mesophyll factors.

7. Under moisture stress conditions, the major reason for the reduction in 'A' was associated with the carboxylation efficiency (dA/dCi).

8. Further analysis of the sub components of mesophyll indicated that the reduction in total soluble protein as an important determinant of 'A' under moisture stress.

9. Possibilities that changes in specificity factor of RuBisCO and its content as important factors resulting in the reduction in assimilation rate under stress were also evidenced.

Further detailed evaluation of the causal effects in the inhibition of photosynthesis under stress conditions associated with RuBisCO in terms of the activation state and its concentration in the cells is necessary for a better understanding of the mechanisms.

More detailed analysis of the factors associated with the variations in in vivo carboxylation efficiency and the factors that determine A_{max} would be worth while in attempts for crop improvement via increasing assimilation rates of leaves.

SUMMARY

SUMMARY

The past several decades of experience in crop improvement has established that any further increase in productivity has to be through an increase in the assimilation rates of crop plants. Although several workers have identified significant genotypic variations in assimilation rates, this variation has not been successfully exploited for crop improvement owing to the complex multigenic control of photosynthesis. The phenotypic expression of photosynthesis is largely regulated by the existing environmental variables. Hence it becomes essential to evaluate the intrinsic as well as the environmental regulation of photosynthesis for a better understanding of the limitations of photosynthesis.

In the present investigation, several experiments were conducted with the major objective to assess the potential of chloroplast and to quantify the relative stomatal and mesophyll limitations of assimilation rate both under non stress and a few abiotic stress conditions. Further experiments were conducted to understand the possible reasons for a larger mesophyll limitation of photosynthesis under moisture stress condition.

Measurements of gas exchange traits using a portable photosynthesis system (ADC LCA-2), was the predominant approach in this investigation. The following experimental approaches and methodologies were adopted to meet the objectives of the investigation.

1. Quantification of A_{max} at saturated CO_2
2. Quantification of relative stomatal and mesophyll limitations of 'A' based on CO_2 response curve by suitably modifying the linear resistance analysis of well watered and in plants experimenting several abiotic stress.
3. Assimilation rates measured at normal (21%) and at low (2%) oxygen concentrations were measured to arrive at the photorespiratory losses.
4. CO_2 sensitivity as an indication of stress effect on mesophyll factors was estimated.
5. VPD response curves were generated to asses the effect of increased gs on 'A'.
6. Specificity factor of RuBisCO was computed from the initial slopes of CO_2 response curves developed at three low light intensities.

A_{max}, apparent photosynthesis and limitations of 'A' under non stress conditions

Using the experimental approaches mentioned above, A_{max} and apparent photosynthesis (APS) were measured in 35 to 40 days old sunflower and maize plants. Further, to asses the gas exchange traits and to quantify the relative stomatal and mesophyll limitations to 'A', another pot culture experiment was conducted with a few C_3 and C_4 species under well watered conditions. The following inferences can be drawn from these experiments.

1. Chloroplasts have significantly higher capacity for carbon fixation in both C_3 and C_4 plants (Table 1).
2. Substrate availability is an important limitation to photosynthesis. Hence gs is an important component.

3. Under well watered conditions, major limitation on `A' was imposed by stomatal factors (Table 3).

4. Variations in `A' between species could also be due to the inadequate functioning of the mesophyll to utilize the low $CO_2$. A linear relationship between <u>in vivo</u> carboxylation efficiency (dA/dCi) and `A' exists between species. (r = 0.9, Fig. 18).

From these findings it can be concluded that both C_3 and C_4 plants have higher potentials for CO_2 fixation. The stomatal diffusive characteristics and availability of substrate CO_2 are the predominant limitation to achieve the potential `A'. This conclusion gets further support from the fact that the relative stomatal limitations in all the species studied, significantly contributed to the total limitations of `A'.

However, the mesophyll factors, though contributed meagerly to the total limitations, still exerted a degree of control over assimilation rate under non-stress conditions. The fact that the <u>in vivo</u> carboxylation efficiency (dA/dCi) recorded a significant positive correlation with observed `A' (r = 0.9), reiterates the possibilities of improving `A' by enhancing carboxylation efficiency of the leaf.

Since stomatal factors limited `A' to a larger extent, plants were partially defoliated to enhance the stomatal conductances. Both `A' and gs significantly increased within a very short period of time in response to partial defoliation. The CO_2 response curves developed in these plants indicated an increase in both dA/dCi and A_{max}. But in sunflower, `A' did not increase substantially. The increase in `A' by defoliation is attributed to a possible alteration of the mesophyll factors by partial defoliation. But in sunflower, no such conclusions can be arrived.

Quantification of relative limitations of `A' under different abiotic stresses

To assess the gas exchange traits of a few species subjected to abiotic stresses, sunflower, cowpea and sorghum plants were subjected to different degrees of moisture stress, low light stress and nitrogen stress. Based on CO_2 response curves, the relative stomatal and mesophyll limitations were quantified. The following are the salient findings.

1. Under any abiotic stress, both `A' and gs decreased.
2. The limitations imposed by mesophyll factors significantly increased as the stress progressed whereas the limitation imposed by stomata did not change markedly.
3. Mesophyll conductance as measured by dA/dCi showed a significant reduction under all abiotic stresses.
4. It can therefore be concluded that in all abiotic stresses the limitation imposed by gs was marginal and major limitations were from the mesophyll factors.

Although stomatal conductance significantly decreased when plants experienced different abiotic stresses, the limitation offered by stomata either decreased or showed no significant change. But the relative mesophyll limitation significantly increased with the intensity of stress. A corresponding reduction in *in vivo* carboxylation efficiency (dA/dCi) was noticed. The observed assimilation rates under all abiotic stresses had a positive correlation with the reduction in dA/dCi ($r = 0.62$). Thus, it can be concluded that 'A' is limited to a larger extent by the mesophyll factors, especially those associated with carboxylation process under abiotic stresses.

Loop gain analysis

It was noticed that the extent of reduction in gs was more than the extent of reduction in 'A' under moderate stress conditions. The loop gain analysis of plants subjected to moisture stress and low light stress confirmed that the stomata were under a feed back control from the mesophyll factors (Table 11)

Effect of increased gs on 'A' in sunflower subjected to moisture stress

An attempt was made to increase the stomatal conductance by altering the VPD to evaluate the role of stomatal conductance in reducing assimilation rates under moisture stress. The VPD response curves clearly indicated that 'A' did not increase despite a significant increase in gs (Fig. 14). This clearly suggested that the mesophyll metabolism was incapable of utilizing the extra substrate CO_2 provided by enhancing gs. These experiments adequately explain the increased relative mesophyll limitation of 'A' under moisture stress conditions.

CO_2 sensitivity as a measure of mesophyll damage

To further clarify the effect of moisture stress on the mesophyll factors, assimilation rates were measured at saturated substrate concentrations. All the three species studied showed a significant reduction in CO_2 sensitivity under stress (Table 13). Sunflower recorded the maximum reduction in the CO_2 sensitivity indicating a relatively larger damage to the mesophyll.

Assessment of the possible reasons for a high mesophyll limitations in plants experiencing moisture stress

The reduction in `A' under abiotic stress conditions has been attributed largely to an increase in the mesophyll limiting factors. An experiment was conducted to assess a few parameters associated with carboxylation to probe into the reasons for this increased mesophyll limitations of `A' in pot grown sunflower plants subjected to moisture stress.

Gas exchange parameters

A significant reduction in `A' and gs was noticed in all the crop plants when subjected to moisture stress. The CO_2 response curves developed in these plants indicated a reduction in the initial slope depicting a reduction in the in vivo carboxylation efficiency (Table 13).

Photorespiration

Photorespiration was assessed by measuring assimilation rates at ambient and 2 percent oxygen concentration (Table 14). Measurement of PR indicated a contrasting behavior in cowpea and sunflower under moisture stress. Cowpea recorded a significant increase to 31 percent under stress.

Specificity factor of RuBisCO

The *in vivo* specificity factor was estimated following a gas exchange technique originally proposed by Brooks and Farquhar (1985). Cowpea recorded a significant reduction in specificity factor under stress (Table 16). Whereas, sunflower did not show any remarkable change in the specificity factor. It could be possible that the kinetic constants of oxygenation were altered to a larger extent in cowpea in relation to its carboxylase reaction. This assumption gets support from the fact that PR also increased under stress in cowpea.

Total soluble protein

All the three species recorded significant reduction in the total soluble protein contents under stress. Reduction in 'A' under stress correlated well with the reduction in protein content. To assess the efficiency of carboxylation per unit of soluble protein, the ratio of 'A' to soluble protein was computed. The ratio of 'A' to protein did not reveal any significant change under stress. However, there was a large variation in the 'A' per protein ratio between species both under well watered and moisture stress condition.

It can be concluded from these experiments that the reduction in total soluble protein was the primary determinant of `A' under stress. However, the reduction in total soluble protein cannot be solely attributed to the reduction in `A' under stress as the information regarding the stress induces reduction in RuBisCO content or the changes in the ratio of RuBisCO to total soluble protein is not clearly understood. Yet another factor that needs further probing is the aspects associated with the activation state of RuBisCO that determines the total catalytic sites of the enzyme.

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