LITERATURE REVIEW
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$C_4$ PHOTOSYNTHESIS:

The higher plants are presently understood to exhibit three types of photosynthetic carbon assimilation pathways $C_3$, $C_4$ and CAM metabolism, (Hatch et al., 1971; Black, 1973; Laetsch, 1974; Kluge, 1976). The $C_3$ plants produce 3-phosphoglycerate (PGA) as the first labelled compound as a result of carboxylation, while the $C_4$ plants synthesise the 4 carbon acids as the predominant early photosynthetic products from which $CO_2$ is released through the subsequent decarboxylation and is used for carbon reduction through the calvin cycle. In CAM plants $CO_2$ fixation takes place in darkness resulting in the synthesis of malic acid which is ultimately converted to carbohydrate via the calvin cycle during the following light period with little or no uptake of $CO_2$ directly from the atmosphere during the day (Kluge, 1976; Kluge and Ting, 1978; Osmond, 1978).

Historically speaking Kortchak et al., 1965 have discovered that malic and aspartic acids were labelled predominantly in preference to phosphoglyceric acid when sugarcane leaves were fed with $^{14}CO_2$ for short duration. Hatch and Slack (1966) have confirmed these observations in sugarcane and extended the studies to
a variety of other tropical species of Gramineae and Cyperaceae (Hatch and Slack, 1967; Slack and Hatch, 1967) and in some members of dicotyledonous families Amaranthaceae (Johnson and Hatch, 1968), Chenopodiaceae (Osmond, 1967; Johnson and Hatch, 1968), Portulaccaceae (Hatch and Slack, 1970a) and Buphorbiaceae (Moss et al., 1969). They further presented a detailed analysis of $^{14}$C labelling kinetics of intermediates and enzyme activities in this distinct group of plants. The mechanism which was proposed to account for the unusual pathway of photosynthetic CO$_2$ assimilation has been referred as the C$_4$ photosynthesis (Hatch and Slack, 1970). The C$_4$ photosynthesis is now known to operate in 943 species belonging to 196 genera of 18 different families of angiosperms (Downton, 1975; Raghavendra and Das, 1978).

Photosynthetic pathway in C$_4$ plants involves the initial fixation of CO$_2$ by the phosphoenolpyruvate (PEP) carboxylase into C$_4$ carboxyl of oxaloacetate which is then converted to malate or aspartate. Depending upon the species, one or the other acids is transferred to bundle sheath cells where CO$_2$ is released by the C$_4$ acid decarboxylation and is refixed through the calvin cycle. The C$_3$ compound generated during the decarboxylation is
then returned to mesophyll cells where it serves as precursor for regeneration of PEP (Andrews et al., 1971; Hatch, 1934, 1975b; Black, 1975; Burris and Black, 1976). The conversion of pyruvate into PEP was demonstrated by the discovery of pyruvate phosphate dikinase (Hatch and Slack, 1967, 1968) adenylate kinase and alkaline inorganic phosphatase (Hatch and Slack, 1969c, 1969b).

Downton subdivided the C₄ plants into 'Malate formers' and 'Aspartate formers' depending on the formation of either malate or aspartate as the major C₄ acid. Later the term malate formers and aspartate formers appeared to be misleading (Black, 1975) since all the C₄ plants were known to form both malate and aspartate. Thus, Gutierrez et al. (1974a, 1974c), Hatch et al. (1975); Burris and Black (1976) classified the C₄ plants into three groups such as NADP malic enzyme type (NADP-ME type), phosphoenolpyruvate carboxy kinase type (PEP-CK type) and NAD malic enzyme type (NAD-ME type) based on the decarboxylating enzymes.

Gutierrez et al. (1974a) have compiled an evidence that all the C₄ plants have a number of anatomical, physiological and biochemical characteristics in common.
The structural specialization of leaf which has been termed "Kranz Type" is found in all the plants exhibiting the C\textsubscript{4} photosynthesis belonging to either monocotyledonous or dicotyledonous groups. (Johnson, 1964; Laetsch, 1968, 1969, 1971, 1974; Bisalputra \textit{et al.}, 1969, 1969a,b; Downton and Tregunna, 1968; Downton, 1970; Das and Raghavendra, 1973; Das and Rajendrudu, 1976; Raghavendra and Das, 1976). The existence of a layer of chlorenchymatous cells around the vascular bundles of some plants was demonstrated as early as 1914 by Haberlandt. Hodge \textit{et al.} (1955) noticed the concentrated chloroplasts in the bundle sheath cells of maize. The occurrence of large bundle sheath chloroplasts filled with numerous starch grains and smaller starch-free mesophyll chloroplasts were observed to be common in three tropical subfamilies of graminaceae (Brown, 1955).

The correlation of Kranz anatomy with physiological aspects of photosynthesis was demonstrated by El-Sharkway and Hesketh (1965). They observed that species with high photosynthetic rates which did not leak CO\textsubscript{2} to the environment exhibited 'Kranz Type' leaf anatomy. Subsequently kranz type leaf anatomy was shown to be associated with other photosynthetic

C_4 plants are characterised by a markedly higher rate of photosynthetic carbon assimilation than the C_3 plants. Various workers have demonstrated the carbon fixation rates of 40-80 mg dm^{-2} hr^{-1} in a wide variety of C_4 plants (Hesketh, 1963; Murata and Iwamura, 1963; El-Sharkway et al., 1968). On the other hand, C_3 plants fixed only 16-35 mg CO_2 dm^{-2} hr^{-1} (Rabinowitsch, 1951; Brown et al., 1966). At optimal temperature (35°C) the rate of CO_2 uptake by C_4 plant shows maximum at light intensity of 1000 μE m sec^{-1}. This was apparent in Atriplex (Bjorkman et al., 1970) and other plants (Black et al., 1969; Bjorkman, 1975; Ogawa and Shibata, 1977; Ishi et al., 1977). Net carbon dioxide uptake by C_4 plants reached the maximum at the temperature range of 30-40°C. The photosynthetic capacity of these plants decreased at low temperatures (Miller, 1960; Black, 1971, 1973; Williams, 1974; Bjorkman, 1974).
The effect of oxygen concentration on CO₂ uptake by leaves has been studied by several investigators (Forrester et al., 1966; Bjorkman et al., 1968; Downes and Hesketh, 1968). It was well established that O₂ concentration above 2% inhibited CO₂ fixation in the C₃ plants whereas C₄ plants were relatively insensitive to changes in O₂ concentration (Forrester et al., 1966; Bjorkman et al., 1968). The inhibitory effect of oxygen on photosynthesis was postulated to be due to occurrence of photorespiration in C₃ plants.

The phenomenon of photorespiration was established with the demonstration of increased respiration following illumination (Docker, 1955, 1957). The C₄ plants are now characterised by apparent lack of photorespiration (Forrester et al., 1966b; Black, 1973). However, there is now adequate evidence to suggest that this activity is localised in the bundle sheath cells (Zolitsch, 1966; Goldsworthy and Day, 1970; Troughton, 1971; Laing and Forde, 1971; Chollet and Ogren, 1973; Chen et al., 1974). The lack of any externally detectable efflux of CO₂ in light is due to the efficient internal cycling of photorespired CO₂ (El-Sharkway et al., 1967, 1968; Goldsworthy, 1968; Volk and Jackson, 1972). Photorespiratory ratio (¹⁴CΟ₂ released
light/darkness) obtained for C₄ plants are markedly different from C₃ plants (Zelitsch, 1968, 1973; Kennedy and Laetsch, 1976; Williams and Kennedy, 1977). The C₄ plants are characteristically have light to dark ratio of about one or less using the ¹⁴CO₂ assay of photorespiration.

A few studies are made on the role of dark respiration in relation to growth and yield of crop plants (Thornley, 1970; Penning Devries, 1974; McCree, 1974). Bjorkman, 1968 showed that the oxygen concentration has pronounced effect in light but little on respiration in the dark providing evidence that the two processes are different. Recently Naidu et al. (1980) observed higher rates of dark respiration in C₄ plants when compared to the C₃ plants.

In general, the C₄ plants are characterised by lower (0-5 ppm) CO₂ compensation point (Downton and Tregunna, 1968; Chen et al., 1970; Crookston and Moss, 1970; Black, 1971, 1973; Raghavendra and Das, 1976). Variation on the CO₂ compensation points are often found in the literature. Heinrich and Musgrave (1969) showed that certain maize genotypes grown in tropical environmental possessed CO₂ compensation points as high as 20 ppm, whereas Moss et al. (1977) found that these
varieties grown in temperature environment at 30°C had values never exceeded 5 ppm and these results are yet to be resolved.

The leaf extracts of C₄ species are found to contain higher rates of Chl a to Chl b and higher levels of P₇₀₀ than those of C₃ plants (Black and Mayne, 1970). The total chlorophyll content of C₄ plants was also much higher when compared to C₃ plants. It was thus suggested that the photochemical potentials of C₄ plants was based upon the distribution of chlorophyll between mesophyll and bundle sheath chloroplasts and their potential for photosystem I and II activities (Edwards et al., 1976). Higher chl a/chl b, lower Chl/P₇₀₀ ratio and low Hill reaction actively suggests a relatively more cyclic/non-cyclic electron flow.

Das and Santhakumari (1977) demonstrated that C₄ species were characterised by higher stomatal frequency in upper leaf surface than in the C₃ plants and the same was confirmed recently by Naidu and Das (1981). Transpiration rates of leaves of C₄ plants were lower than those of C₃ species (Hesketh, 1967; Hofstra and Hesketh, 1969; Downes, 1969). A negative correlation between transpiration and photosynthesis within the C₄ dicotyledonous plants was observed by Rathnam et al. (1976). Further, C₄ plants were known to possess
greater water use efficiency than the C\textsubscript{3} species (Black, 1971). During the past several years there has been much emphasis laid the maintenance of water balance in crop plants. Several comprehensive reviews on the physiological responses to water deficits have been published in recent years (Gates, 1964; Henckel, 1964; Crafts, 1968; Slatyer, 1968, 1973a; Laude, 1971; Hsiao, 1973). The water stress effects emphasizing on the physiological processes associated with crop production was reviewed later by Begg and Turner (1976).

**Physiology and Biochemistry under water stress:**

**PHOTOSYNTHETIC RATE:**

The reduction of photosynthesis by water deficits was well documented and has recently been reviewed by several authors (Hsiao, 1973; Boyer and McPherson, 1975; Begg and Turner, 1976; and Turner and Begg, 1978). Boyer (1976) has extensively reviewed the effect of water deficits on photosynthesis. Alexandrov (1964) earlier has stated that the photosynthetic process was also sensitive to high temperature along with water stress. Norcio (1976) found that high temperature stress reduced oxygen evolution from intact leaf sections when stomata are closed. Most of the plant
species exhibit a fairly definite photosynthetic temperature optimum, which can shift in the direction as changes in the ambient temperature (Strain, 1969; Mooney and Harrison, 1970; Lange et al., 1974; Nobel, 1978).

The first observation of the inhibitory effects of water deficits on photosynthesis, appears to have been made by Kreusler (1885). A few years later, Thoday (1910) in experiments designed for other purpose, noticed similar plant responses. Then Iljin (1923), Brilliant (1924), Dasteur (1924, 1928) and Wood (1929) each showed a reduction in photosynthetic activity occurred upon water loss from leaves, and Dasteur (1925) appears to have been the first to have expressed photosynthetic activity as a function of the measured water status of the tissue. His careful determination provided evidence that photosynthesis responded linearly to leaf water content in several species.

The reduction of photosynthetic rate due to increased water stress has been observed in a variety of plant species. Stomatal effects are usually considered to be the first major limitations to CO₂ fixation, although inhibition at the chloroplast level has been
proposed (Hsiao, 1973; Boyer, 1976). Several studies have provided evidence that Boyer, 1981 on some species the observed reduction in net photosynthesis with increasing water stress can be completely attributed to stomatal closure (Troughton and Slatyer, 1969; Boyer, 1970b; Slatyer, 1973b). Decreased photosynthetic rates at low leaf water potentials are often attributed to increased stomatal resistance (Troughton, 1969; Boyer, 1970; Shearman et al., 1972). The non-stomatal effects when present, seem to be operate in association with the stomatal effects (Hsiao, 1973).

PHOTORESPIRATION:

Water deficits have been reported to increase, decrease or have no effect on photorespiration. Photorespiration was observed to be increased with water stress in higher plants (Fisher, 1968; Redshaw and Moidner, 1972). It was speculated that an increase under water stress could be real or could be only apparent, reflecting an elevation of photorespiration, but they did not test the possibility with O₂ free air. Contrary to this, photorespiration was decreased by water stress (Nguyen Duc and Viera dasilva, 1971, 1972) even of glycolate oxidase is not affected. It seems that it is the decarboxylation of glycine in mitochondria that decreases
with desiccation. They stated that the enhanced lipase activity could lead to reduced glycine decarboxylation. This was supported by the finding that inhibition of the decarboxylation of glycine occurs after lipase treatment of mitochondria (Kisaki et al., 1971). Relative sensitivity of photorespiration to drought stress in two species of *Gossypium* were studied (Vieira da Silva, 1974; Dham Thi and Vieira da Silva, 1975b). They concluded that the decrease in photorespiration with drought is much smaller in *Gossypium anomalum* which shows no lipase activity in mitochondria than in *Gossypium hirsutum*, which shown lipase actively with drought.

**DARK RESPIRATION:**

Effect of water stress on dark respiration were reviewed briefly (Crofts, 1967; Slatyer, 1969; Todd, 1972). Early results were often conflicting, showing either no change, an increase or decline in dark respiration with stress (Hsiao, 1970). Recent data demonstrated that dark respiration is generally decreased, more or less proportionately but not very markedly by moderate to severe stress (Flowers and Hanson, 1969; Boyer, 1970; Jarvis and Jarvis, 1970). Contrary to this, Schneider and Childers (1941) Upchurch et al.
(1951); Brix (1962) and Kaul (1966) earlier demonstrated that dark respiration increased in the early phase of desiccation, but then decreased as desiccation became more severe.

The effect of water stress on dark respiration seems to differ with species (Brix, 1962) or according to the degree and length of stress (Greenway and Hiller, 1962). The respiration rates of individual leaves of cotton (*Gossypium hirsutum*, L.) beans (*Phaseolus vulgaris*, C.V.) and Sorghum (*Sorghum vulgare* Pers.) of stressed and non-stressed plants were compared recently by Brown and Thomas (1980). They observed that the respiration rates of water stressed leaves were significantly lower than those of non-stressed plants.

**ENZYMES:**

Studies on enzymes as affected by water stress are limited. Some Russian authors (Mothes, 1956, Review) were among the first to find a link between tissue water content and enzymatic activity as early as 1929. Most of the literature concerning the effect of water stress on enzymatic activity was reviewed by Todd (1972) and showed clearly that hydrolytic enzymes and some oxidases frequently increase in activity with stress. The
activity of RuBP carboxylase was reduced when assays were performed on extracts of desiccated leaves (Huffaker et al., 1970; Jones, 1973; Jhonson et al., 1974; Lee et al., 1974; O'Toole, 1975). PEP carboxylase activity and RuBP carboxylase also decreased but slightly (Huffaker et al., 1970; Shearman et al., 1972). It was shown that the enzyme RuBP carboxylase from spinach, cotton, barley and wheat was sensitive to moderate levels of internal water stress (Plaut, 1971; Jones, 1973; Jhonson et al., 1974; Mayoral et al., 1981).

Plaut (1971) observed an inhibition of CO₂ fixation by isolated chloroplasts at low osmotic potential which was paralleled by decreased Ribulose 1,5-diphosphate carboxylase activity in the spinach chloroplasts but has not decreased the photochemical reduction of NADP. Huffaker et al. (1970) reported that mild water stress to -11 bars did not affect barley seedling RuBP carboxylase activity. However, Plaut and Bravdo (1973) showed an increased mesophyll resistance which might imply either decreased RuBP carboxylase activity or decreased photochemical activity with decreased leaf water potential.

Glycolytic activity was suppressed almost completely in droughted leaves of corn and fodder bean,
while at the same time the rate of oxidation through the pentose phosphate pathway doubled (Abrarov, 1969) phosphomoesterase in cowpea (*Vigna sinensis*) leaves has increased by nearly 50% as a result of drought, whereas phosphatase activity in detached, rapidly desiccated wheat (*Triticum aestivum*) leaves has decreased. Acid inorganic phosphatase activity increased but alkaline inorganic phosphatase activity has decreased during the senescence of turgid leaves. Recently Mishra (1979) observed a decreased in catalase activity and increase in peroxidase activity with time was faster in the water stressed leaves than in the turgid leaves of Rice. Many of the enzymes in the cell exist in more than one form. Limited studies indicated that during desiccation of tissues these isozymes may change at different rates (Stutte and Todd, 1969). Studies on the effect of water stress on the enzymes of C₄ photosynthesis are comparatively few and little is known in this regard.

**PIGMENT COMPOSITION:**

That the light induced chlorophyll formation in etiolated leaves and quite sensitive to mild water stress was first noted by Virgin (1965). This has been further established by later workers in different
plants (Duysen and Freeman, 1974; Alberle et al., 1977; Fisher and Naylor, 1975). Bourque and Naylor (1971) showed that water deficits caused significantly slower accumulation of chlorophyll in greening leaves. They further demonstrated that the lower water deficits induced by low humidities was enough to cause a significant response. The inhibition of the synthesis of chlorophyll is apparently caused by lessened ability to form protochlorophyll (Virgin, 1965). Chlorophyll content of leaves declined only slightly with 2 or 3 days of mild water stress (Huffaker et al., 1970). The decreased nature of chlorophyll concentration by water stress in three species of Quercus was observed by Spyropoulos and Marromattis (1978). The effect of drought condition in the rate of protein synthesis and chlorophyll content was recently investigated in two maize lines by Botha and Botha (1979). They have concluded that protein synthesis and chlorophyll content declined sharply with the progress of drought. The degree of this decrease in chlorophyll content varies with species and environmental factors (Bokhari, 1976; Spyropoulos and Marromattis, 1978). Alberle and Thornberger (1977) observed that the majority of chlorophyll lost in response to water stress occurs in the mesophyll cells with a lesser amount being lost by the bundle sheath cells.
PHOTOCHEMICAL ACTIVITIES:

An early study reported that Hill activity of isolated chloroplasts was unaffected or even increased by water stress of wheat leaves (Todd, 1972). A loss of Hill activity in chloroplasts isolated from leaves of Swiss chard (Beta vulgaris) subjected to stress was first reported by Nir and Poljakoff-Mayber (1970), but only under severe stress. Boyer and Bowon (1970) presented convincing evidence of an inhibition of Hill activity of isolated chloroplasts by mild to moderate stress in Sunflower, and moderate to severe stress in pea. Fry (1970) showed a similar inhibition by severe stress of cotton leaves.

The work of Todd and Basler (1965) suggests that Hill activity in isolated chloroplasts was affected by desiccation, when activity was recalculated on chlorophyll basis. Others have shown that Hill activity (Nir and Poljakoff-Mayber, 1967; Fry, 1970) and cyclic photophosphorylation (Nir and Poljakoff-Mayber, 1967) were inhibited when chloroplasts were isolated from leaves that were previously been severely desiccated. Chloroplasts desiccated in vitro also showed reduced Hill activity (Santarius and Ruber, 1967). Santarius has demonstrated that the levels of NADPH and ATP of
leaves of *Beta vulgaris* have decreased during desiccation.

Chloroplast activities have been evaluated to examine the effect of water stress on the photochemical process. Water stress has been shown to reduce electron transport, photophosphorylation and quantum yield of isolated chloroplasts (Keck and Boyer, 1974; Mohanty and Boyer, 1976). There were also many reports of water deficits and high temperatures affecting the activity of isolated chloroplasts (Santarius, 1967; Boyer and Bowen, 1970; Fry, 1970; Huffaker *et al.*, 1970; Flaut, 1971; Sullivan *et al.*, 1977).

**TRANSLLOCATION:**

Water stress in general causes a reduction in the translocation rate of minerals, carbohydrates and growth regulators. Roberts (1964) has noted a reduction in $^{14}$C photosynthate transport on yellow popular seedlings which was confirmed by many others in different plants (Flaut and Reinhold, 1965; Wardlaw, 1967; Brevedan and Hodges, 1973; Pearson and Rana, 1974). It is generally agreed that drought results in a diminution of the recent photosynthate transported to developing grain. Wardlaw (1967, 1969, 1971) has
shown that the rate of translocation of recently fixed $^{14}$C was reduced in wheat growing under desiccation conditions. Translocation in maize grown in the field under stress conditions showed a similar behaviour (Brevedan and Hodges, 1973). It was shown that during-grain filling of maize, water stress that reduced leaf water potential to $-26$ bars has reduced the vein loading.

The relationship between photosynthesis and translocation rate change as affected by water stress intensity and stage of plant development was evaluated recently in cotton and Sorghum (Sung and Krieg, 1979). They have observed that the photosynthetic rates were reduced as mid day leaf water potentials declined from $-14$ to $-27$ bars in both species. However, Sorghum has maintained higher photosynthesis and translocation rates compared to cotton at comparable leaf water potentials.

NITROGEN METABOLISM:

Several workers have demonstrated an increase in free proline content in plants suffering from water deficits (Kemble and McPherson, 1954; Bernett and Naylor, 1966; Steward et al., 1966; Thompson et al., 1966; Singh et al., 1973a,b). However, the proline
accumulation in water stressed plants is readily reversed if the stress was eliminated by rewatering the plants (Singh, 1973a,c). There was ample evidence that the accumulation of proline is due to denovo synthesis (Barnett and Naylor, 1966; Thompson et al., 1966) rather than the breakdown of proteins. There have been numerous attempts to study the relationship between cellular water deficits and protein synthesis (Hsiao, 1973). Hsiao has provided a clear evidence that even mild water stress can shift the ribosomal profiles in favour of single ribosomes indicating inhibition of protein synthesis. Miller (1938) pointed out that the percentage of protein increases under drought, although the total yield decreases. Evidently the total protein production was inhibited but the total carbohydrate production was inhibited even more.

Studies on water stress and its effects on enzymes of Nitrogen metabolism were somewhat meagre and controversial. Unfavourable temperatures, CO₂ levels and water availability reduce the activity of nitrate reductase enzyme (Beevers and Hagoman, 1969; Morilla et al., 1973) largely because of protein synthesis. Water deficits are known to adversely affect many aspects of growth and nitrogen metabolism.
Few workers have stated that water stress in crop plants has decreased nitrate reductase activity (Maltas and Reuli, 1965; Huffaker et al., 1970; Hsiao, 1973). Contrary to this, an increase in nitrate reduction under mild water stress has been reported in rice seedlings (Mali and Mehta, 1975). Effect of water stress on the enzymes of nitrogen metabolism in two species of Brassica was studied recently by (Gupta and Sheoran, 1979). The two species showed characteristic differences in dry weight, nitrate reductase, aspartate aminotransferase, alanine aminotransferase, glutamate dehydrogenases. A decrease was observed in the activities of all these enzymes and the decrease was more in Brassica juncea than in Brassica campestris.

STOMATAL CHARACTERS:

Several studies have shown that water deficits lead to lowered leaf water potentials and consequently to partial stomatal closure (Brady et al., 1975; Simit and Kramer, 1977; Sivakumar and Shaw, 1978; Turner et al., 1978; Carlson et al., 1979; Wien et al., 1979; Jung and Scott, 1980). Partial stomatal closure in turn, lowers photosynthesis, transpiration, growth and yield (Beardshall et al., 1973). Increased stomatal resistance as a plant response to water stress has
recently been reviewed (Begg and Turner, 1976; Burrows and Milthorpe, 1976). There is overwhelming evidence in the literature that many of the negative effects of water stress on carbon gain are caused by closure of stomata, thus increasing the stomatal resistance (Hsiao, 1973).

Stomatal sensitivity to water stress varies greatly with the species (Gifford and Musgrave, 1973; Henzell et al., 1975) and also between species (Glover, 1959; Beadle et al., 1973; Hsiao, 1973; Turner, 1974). Stomatal movement in stressed plants was regulated primarily by water content of epidermal and guard cells (Stalfelt, 1966), although the leaf water potential at which stomata close differs for each species (Miller et al., 1968; Kanemasu and Tanner, 1969; Boyer, 1970b; Boyer, 1971a; Sanchez and Kramer, 1971).

The relationship between leaf $\psi$ and stomatal diffusive resistance has been quantified for many species (Hsiao, 1973). In most cases there was a threshold value of $\psi$ below which stomatal resistance increases markedly. The value varies between -7 to -16 bars for different species (Hsiao, 1973) and is known to differ with this growth conditions, leaf age, and other factors (Jordan et al., 1975). The relatively
low values of threshold 4 indicates that stomata may not be very sensitive to mild water stress (Hsiao, 1973).

Stomatal response has been reported to be affected by leaf age or stage of plant development (Frank et al., 1973; Hultquist, 1973; Jordan et al., 1973). Leaf resistances of sunflower leaves were shown by Sionit and Kramer (1976) to be higher when stress occurred during flowering than those observed when stress was applied later during seed filling. Morgan (1977) found that for wheat plants at any given value of ψ, the stomata were open more widely during the post-anthesis stage than during the pre-anthesis stages. Hultquist (1973) gave an evidence that Sorghum stomata were no longer responsive to bulk leaf water status after bloom stage of growth. Similar results have been obtained with field grown grain Sorghum (Ackerson and Krieg, 1977).

TRANSPERSION AND WATER USE EFFICIENCY:

Jensen et al. (1971) stated that transpiration decreases logarithmically as the available soil water decreases. The overall stomatal closure and transpiration reduction in response to water
deficiency have been long established. It is well documented that the stomatal closure is the main cause for transpiration decline as water stress develops. The decline in transpiration often parallels the decline in photosynthesis, and this has been interpreted to indicate that stomatal closure limits both processes (Hsiao, 1973). Transpiration generally decreases with decreasing leaf water potential $\psi$ primarily as a result of stomatal resistance (Jhonson et al., 1974; Dube et al., 1975). Jhonson et al. (1974) reported that the transpiration rate of field grown wheat and barley plants become zero at leaf water potentials of -28 bars.

Transpiration ratios of C$_3$ plants were generally about 1.5 mg dry matter per gram of water used while C$_4$ plants have higher water use efficiencies with transpiration ratios of about 3.3 mg (Downes, 1969). Bierhuizen (1976) reported that water use efficiency was independent of irrigation treatment in Sorghum, however a lower supply of water generally increased the water use efficiency of crop of alfalfa. A linear relationship was from early flowering to harvest. Water use efficiency (WUE) generally increased with vegetative stage and was reduced by flowering stage and was reduced by flowering stage and grain filling.
The water use efficiency of Sorghum was earlier studied by Teare et al. (1973). It was stated that the period of greater water use efficiency occurred from boot stage to anthesis. Recently Turk and Hall (1980) have observed a decreased water use efficiency with decreased irrigation levels in coupea.

GROWTH:

Plant water stress affects plant growth by modifying the anatomy, morphology, physiology and biochemistry of plants. Considerable work is available on the effect of drought stress or drought on the growth of the plants (Richards and Woodleigh, 1962; Slatyer, 1967; Hsiao, 1973). In general water stress inhibits growth but the organ of most rapid growth at the time of stress is one which is most affected (Aspinall et al., 1964). Kramer (1963) reported that the plant growth is controlled directly by plant water stress and only indirectly by soil water stress. Water stress has influenced both the rate and period of growth of Sorghum bicolor L. Moench plants (Stout et al., 1978). They stated that water stress has extended the period of leaf and stem growth and inflorescence development was delayed.
The growth of a plant organ depends on cell division followed by the expansion and differentiation of the individual cells. Ample evidence is available that cell expansion is adversely affected by water stress (Boyer, 1970b; Hsiao and Acevado, 1974; Watts, 1974). Cell expansion is apparently one of the physiological processes which is reduced by slight water deficits (Hsiao, 1973). In general, cell enlargement appears to be more sensitive than cell division (Mayer and Boyer, 1972) although Khokham et al. (1972) found an early effect of osmotic solution on cell division.

McCree and Davis (1974) presented an evidence that cell division was at least as important as cell expansion in determining the final leaf area of water-stressed grain sorghum. The exceptional sensitivity of leaf enlargement was first demonstrated by Boyer (1968, 1970a) who showed that the leaf enlargement was reduced to 25% of the controls or even less when the leaf water potentials were decreased to -4 bars in maize, sorghum and soybean. Acevado et al. (1971) have shown a similar response in maize except that the leaf water potentials appear to be little lower, while photosynthesis was unaffected by these leaf water potentials.
Davidson (1969) has stated that a decrease in the availability of water, nitrogen or phosphorous caused an increase in the relative weights of roots. Further in the presence of deficiency of these factors caused a further increase in the root to shoot ratio. In some cases, stress appears to enhance root growth not only relative to shoot growth but also absolutely. Hsiao and Acevado (1974) presented an evidence for this is maize crop and the possible benefit to root crops such as sugarbeet.

It is well established that the effect of water stress on growth and yield depend upon the degree of stress and the developmental stage at which stress occurs (Hsiao, and Acevado, 1974; Sullivan and Eastin, 1974). Previous studies have resulted in conflicting evidence on the sensitivity of cowpeas to water deficits at different growth stages. Hiler et al. (1972) reported that cowpea were less sensitive to stress during the vegetative and pod filling stages than the flowering stages. A 9 to 11 day water stress was imposed during the boot stage and the anthesis through grain formation stage in oat, and observed decreased plant height and the number of florets per panicle.
YIELD AND YIELD COMPONENTS:

Numerous reports in the literature show that water deficits limit the yield and that irrigation increases yield. Crop yields are adversely affected by plant water deficits arising from inadequate soil water (Salter and Goode, 1967; Thompson, 1975). Turk and Hall (1980) recently observed the adverse effect of drought on seed yield of cowpeas. A number of irrigation experiments on the overall effects of water supply in grain yield of sorghum have been reviewed by Ajmad (1975) in the context of different plant populations. The well known ability of sorghum to give relatively high yields under water stress was attributed in past to compensation among the different yield components (Blum, 1973). Nevertheless, if the stress lasts long enough or is severe enough, grain yield may be affected as in other cereal crops and in smaller ways (Lewis et al., 1974).

Generally flowering in cereals is thought to be sensitive to water stress (Salter and Goode, 1967). Nevertheless, studies on the effect of water stress on inflorescence development a key process on the determination of yield, have been done in only a few species, including grain sorghum (Review, Slatyer,

Inuyama et al., 1976 has determined in grain sorghum that the boot stage through bloom was the most critical growth stage for reduced grain yields caused by water deficits. Numerous reports have emphasized the effect of water stress at anthesis or early grain filling on final grain yield (Fasenak and Watson, 1969; Soricino and Gianzo, 1975; Sandhu and Horton, 1977; Sionit and Kramer, 1977). The yield of maize was known to be reduced when water stress develops at various stages of growth. Ruge and Odell (1960) and Sionit and Kramer (1977) found that moisture stress at the vegetative stage reduced yields of soybean. Shaw and Laing (1966) have earlier reported that with soybean, water stress imposed early in growth has reduced pod number. Pod fill has been found to be a sensitive stage in peas (Maures et al., 1968; Muller et al., 1977) and in soybeans (Doss et al., 1974; Sionit and Kramer, 1977), resulting in lower seed weight. Shouse et al. (1981) recently stated that the most sensitive growth
stage to drought in cowpeas are the flowering and pod filling phases with reductions from 35 to 69% depending on the timing and length of drought treatment. They further, observed that the water deficits during the vegetative stage had the least effect on crop yield.

RECOVERY STUDIES:

Compared to the large amount of research which has been conducted on plants exposed to water stress, surprisingly little emphasis has been placed on the recovery of plants from the water stress. Recovery of \( \psi \) after rewatering appeared to be quite rapid if the stress had not been too severe or long lasting (Boyer, 1971; Frank et al., 1973; Sionit and Kramer, 1977). Nulsen and Thurtell (1978) reported that corn plants which were immediately stressed to \(-10\) or \(-11\) bars in growth chamber, recovered to prestressed within 40-50 minutes if allowed to recover in the dark. Plants, when stressed to \(1\) less than \(-11\) bars, required between 95 and 300 minutes. Sanchez-Diaz and Kramer (1973) presented an evidence, that immediately after rewatering turgor potentials of maize and sorghum leaves have increased to values above those obtained before the application of stress. Stomatal opening
also appears to respond very rapidly to the removal of water deficit (Sanchez-Diez and Kramer, 1971; Frank et al., 1973; Bengston et al., 1977). The recovery of photosynthesis after rewatering often parallels the recovery of stomatal conductance (Troughton, 1969; Frank et al., 1973). Krieg (1977) recently reported that the recovery of photosynthetic activity of sorghum after rewatering was dependent on both genotype and stage of development.

The above review on the current status of our knowledge on water relations of crop plants shows that it is mostly limited to sugarcane, maize, sorghum, rice, cotton, cowpea and soybean. It is felt therefore that there is need to extend the studies to C₄ dicotyledonous crops. In this respect a grain crop, Amaranthus hypochondriacus was chosen. The present work was accordingly directed on the following lines to gain fuller understanding of the morphological, physiological, biochemical and agronomical aspects of A. hypochondriacus under variable water regimes imposed at different growth stages. The experiments were designed in two main directions.
I. A detailed characterisation of the $C_4$ photosynthesis in grain amaranth.
   
a. Internal leaf anatomy
   
b. Physiological and biochemical characters.

II. Responses of grain amaranth to different water regimes.
   
a. Growth analysis of plants in the field as well as under pot culture conditions.
   
b. The effect of water stress on morphological, physiological and biochemical parameters.
   
c. The influence of variable water regimes imposed at different growth stages.
   
d. Interaction of water stress and temperature.