LEGEND FOR PLATE I.

90 days old field crop of *Amaranthus hypochondriacus* over all view.
PLANT MATERIAL:

Seed material of Grain amaranth, *Amaranthus hypochondriacus* L. were obtained from the National Botanical Research Institute, Lucknow and the plants were grown both in field conditions and pot culture experiments at the University Campus, University of Hyderabad, Hyderabad. The University is geographically situated at 17°19' N latitude and 78°23' E longitude and at an attitude of 542.6 m above the mean sea level. Plants at four growth stages, vegetative, flowering, grain filling and senescence, were used as the source of leaves for subsequent experiments. Fully expanded and healthy leaves were used as the experimental material.

WATER STRESS TREATMENTS:

Field experiments were carried out in the Campus, University of Hyderabad, Hyderabad, during winter and summer seasons of 1979. 4 x 4 m plots were made for this study and the distance between the rows and plants were maintained of 0.5 m (Plate I & II). Later the experiments were carried out in semiglass house chamber as shown in plate III due to problems involved in the maintenance of desired levels of water stress. The glass house was covered with fully transpirant 5 mm
LEGEND FOR PLATE II.

90 days old field crop of *Amaranthus hypochondriacus*

A. Few plants in close up view.
B. Single plant in close up view.
LEGEND FOR PLATE III.

Over all view of semi-glass house, where pot experiments carried out.
glass to prevent rain water while sunlight pass through the sides of the glass house were covered with wire mesh to protect the plants from the attack of rodents.

The experiments were carried out in plastic pots of 30 cm diameter holding 0.5 kg of sand at the bottom and 7.5 kg soil with farm yard (3 parts of red soil + 1 part of farm yard manure) under natural photoperiod. The field capacity of the soil was determined prior to use. The healthy seeds were sown in each pot and 3 plants were allowed to grow after germination. The soil water levels of 100% field capacity (control) and 50% field capacity (mild water stress) and 25% field capacity (severe water stress) were maintained by adding known volume of water at different growth stages: Vegetative, Flowering, Grain filling and Senescence. The experiments were conducted in two seasons: Winter season (Day temperature ranging from 22-32°C) and Summer season (Day temperature ranging from 32-42°C).

**SAMPLING:**

Fully expanded and health leaves were removed from the controlled as well from the water stressed plants, for the purpose of studies on water stress effects at different growth stages: Vegetative,
LEGEND FOR PLATE IV.

View of *A. hypochondriacus* plants grown in glass house.
LEGEND FOR PLATE V.

Water stress effects on *A. hypochondriacus* plants grown in glass house.

A. Vegetative phase

1. 100% field capacity.
2. 50% field capacity.

B. Reproductive phase

3. 25% field capacity.
Flowering, Grain filling and Senescence. The leaves were thoroughly washed first with tap water followed by distilled water and blotted dry. For the measurement of Photosynthetic, leaves were kept in a cold chest maintained at 0.2°C for about half an hour. Fresh leaf material was used for the determination of total chlorophyll content, Hill reaction activity, Cyclic and Non-Cyclic Photophosphorylation NADP Photoreduction.

LEAF ANATOMY:

Fully expanded leaves were harvested washed with distilled water and blotted dry. They were cut into 2.0 cm² bits and fixed in formalin : acetic acid : 50% ethanol mixture (5 : 5 : 90 all v/v). Free hand sections were cut, mounted in glycerine (10% v/v) and examined for the arrangement of chloroplasts on the bundle sheath cells. Photomicrographs were taken with these leaf sections.

LEAF WATER POTENTIAL:

Leaf water potentials were measured with a pressure chamber (model ) (Schneider et al., 1965, Boyer, 1967). Fully illuminated leaf was placed with the petiole in the scale of the pressure chamber
so that the blade could be exposed to pressure but the cut surface of the petiole could be observed from the outside (Boyer and Ghorashy, 1971). Pressure was raised slowly in small steps to minimize heating of the incoming air, and the balancing pressure required to moisten the cut surface of the petiole was determined. 30 to 60 seconds elapsed at the balancing pressure to assure that the pressure inside the chamber was in equilibrium with the water potential of the leaf cells. Each measurement took 8 to 10 min and should have been close to the equilibrium water potential of the leaf measured with a thermocouple psychrometer by the isopiestic technique (Boyer and Knipling, 1965; Boyer and Ghorashy, 1971).

STOMATAL PARAMETERS:

The epidermal peels (both lower side and upper side) were removed from the controlled and water stressed leaves, were plunged in absolute ethanol and were studied in Heath's reagent (Lloyd, 1980). Alternatively imprints were taken with nail polish (Sampson, 1961). Measurements of the width and length of stomatal aperture were made using precalibrated ocular micrometer. An average of 10 measurements were taken and the average pore width and length were calculated from the above readings. The number of stomatal and epidermal cells in the microscopic
field area were noted, the area of which was calculated using a stage micrometer. Stomatal index and stomatal frequency were determined from the above observations.

Diffusive resistance of the stomata was measured with automatic porometer (MK II, DELTA-7 Devices, England). The readings were taken in the humidity range (20–60) during 9 A.M to 1 P.M and 5 P.M. The time taken t seconds was converted into diffusive resistance sec/cm² by the help of the calibration curve supplied by the company with resistance meter. The stomatal conductance was determined by the calculatory i/r were 'r' is stomatal resistance. The plants were grown during winter and summer seasons, with varying temperature. The data of both the seasons were recorded for the comparative study.

PHOTOSYNTHESIS:

\[ ^{14}\text{CO}_2 \] Fixation by Detached Leaves: Fully expanded green leaves from the controlled and water stressed plants were detached, recut under water, and prociliated for 15 min at natural light intensity and temperature. The leaves were fed with \[ ^{14}\text{CO}_2 \] using a technique similar to that described by Berry et al. (1970). A 500ml corning conical flask (containing 0.4 mCi of \[ ^{14}\text{CO}_2 \] with
a cellophane tape covered narrow slit on the top was used as the feed chamber. During the $^{14}\text{CO}_2$ feeding, the tape was removed, a leaf was quickly lowered through the slit with a forceps, withdrawn after 10 min and the slit was immediately closed to prevent the leakage of $^{14}\text{CO}_2$. $^{14}\text{CO}_2$ was obtained by injecting excess of 0.1N HCl into carrier free NaH $^{14}\text{CO}_3$ (specific activity 47.0 mCi/mM, obtained from Radiochemical division, BARC, Trombay, Bombay). The leaves were killed by plunging into boiling ethanol (80% v/v) and turning off the light simultaneously. The leaves were homogenised and the insoluble material was successfully extracted with 80%, 60%, 40% alcohol (all v/v) and then twice with boiling water. The extracts were pooled and were concentrated to a small volume by evaporation. The amount of $^{14}\text{CO}_2$ incorporated was determined by placing 0.1 ml aliquots of the combined extracts on liquid scintillator vials along with liquid scintillation on a liquid scintillation system (model Beckman LS 3133P made in U.S.A). The rate of photosynthesis was expressed as mg CO$_2$ dm$^{-2}$ hr$^{-1}$.

$^{14}\text{CO}_2$ FIXATION BY CHLOROPLASTS:

Isolation of Chloroplasts: The chloroplasts were isolated by differential grinding procedure of Woo et al. (1970) with a slight modification.
The excised leaves were washed with tap water, followed by distilled water and blotted dry. The leaves (about 10.0 g) were cut into 1.0 cm² segments and blended for 10 sec in the isolation medium comprising of 0.3 M sucrose; 0.15 M phosphate buffer pH 7.3; 1 mM EDTA; 1 mM MgCl₂, 1 mM MnCl₂, 5 mM dithiothreitol and 5% (w/v) Polyvinylpyrrolidone.

After homogenisation, the macerate was filtered through layers of cheese cloth. The filtrate was centrifuged at 1500 g for 5 min. The chloroplast pellet was suspended in the medium containing all the components of the grinding medium except that 0.35 M NaCl was used in place of sorbitol.

\[ ^{14}C_{O_2} \text{ FIXATION BY ISOLATED CHLOROPLASTS:} \]

The assay medium (3.0 ml) containing chloroplasts (100 µg chl ml⁻¹) was preilluminated for 5 min. 10 mM NaH\(^{14}\)CO₃ (0.5 uCi/uM) was added to the reaction mixture. Light sources provided an illumination of 35 Klux. Thermal radiations was eliminated by inserting a water filter between the lamp and test tubes. The temperature was maintained at 20°C. To measure the rate of \(^{14}\)CO₂ fixation 0.2 ml samples were removed at 3 min intervals, acidified with an equal volume of 0.1N HCl present in liquid scintillation vials and counted for radioactivity.
CO₂ COMPENSATION POINT:

Compensation points were determined according to the method of Williams and Markley (1973) in a closed system. Infra Red gas analyser model IRGA-20 (Grubb Parsons, New Castle upon Tyne England) calibrated for carbon dioxide was used in a absolute range to measure to changes in CO₂ concentration with detached leaf in a closed system (Plate VII). A 500 ml conical flask was used as the photosynthetic chamber enclosing a detached leaf aside the chamber. The air pathways of the entire system were built with 10 mM glass (corning) and Tygon tubings. The outlet air from the chamber passing through the sample side of the analyser was dried by passing through silica gel with moisture indicator. The air passing out of the analyser was rehumidified and returned to the photosynthetic chamber. The air flow through the system was adjusted to 50 lit/hr. The light intensity and temperature were maintained at 1000 µE m⁻² sec⁻¹ (400-700 nm PAR) and at 30 ± 1°C. In a closed system air was circulated through the leaf chamber, flow meter, drying tower, pump and Infra Red gas analyser. CO₂ content was monitored until equilibrium was reached. This equilibrium values were recorded as the CO₂ compensation concentration. Duplicate measurements were made except when CO₂
compensation differed by more than 2 ppm between duplicate, then five replications were used.

PHOTORESPIRATORY RATIO:

Photorespiratory ratio was estimated according to Das and Rajendrudu (1976). Two fully expanded leaves of equal age and area were chosen and equilibrated in light for 30 min prior to $^{14}$CO$_2$ assimilation in a 500 ml wide mouthed flask. The flask then filtered with a two holed rubber stopper, carrying a thistle funnel and a bent delivery tube. The other end of the delivery tube was dipped in 20% potassium hydroxide. Labelled bicarbonate solution (0.04 mCi, specific activity 53.7 mCi/m mole) was placed into a small vial kept at the bottom of the feeding chamber. $^{14}$CO$_2$ was released by adding 3.0N HCl from a thistle funnel into the vial containing labelled bicarbonate solution. The leaves were allowed to completely assimilate $^{14}$CO$_2$ for 45 min. The two leaves were transferred into two flasks A and B, which were connected on parallel to suction pump. One of the flasks (A) was illuminated by incandescent bulbs at 15,000 lux and the other (B) was maintained in darkness. Suction was applied for about 30 min to trap $^{14}$CO$_2$ released by the leaves into $^{14}$CO$_2$ trapping agent (1.0 N KOH with 0.6% iso-amy1
alcohol). The amount of $^{14}\text{CO}_2$ trapped was measured by taking 0.5 ml into the liquid scintillation vial along with 8.0 ml liquid scintillator and the radioactivity was measured by using liquid scintillator. The ratio $^{14}\text{CO}_2$ released in light/darkness was calculated.

**DARK RESPIRATION:**

The respirometer (model V166 B Braun Melsungen) was used to monitor the dark respiration as the oxygen consumption by excised leaf discs (Naidu et al., 1980). Ten leaf discs (about 3.5 cm$^2$) were floated in 2.0 ml of 50 mM phosphate buffer solution pH 7.2 contained in the center well of the flask. The warburg flask was attached to the manometer and equilibrated in the waterbath of 30 ± 0.5°C. The flasks with the corks open were shielded from light and maintained under constant shaking. CO$_2$ free atmosphere was maintained by absorption of CO$_2$ by 0.2 ml of 20% KOH (w/v) placed a filter paper within the central well. The manometric readings were taken at 30 min intervals after keeping the flask under closed system. A thermobarometer was simultaneously arranged to monitor the changes in the ambient atmospheric during the course of the experiment. The sample manometric readings were corrected to thermobarometer reading to
obtain manometric reading as a result of CO₂ consumption by the leaf samples.

After measurements of respiration the leaf samples were washed repeatedly with distilled water and oven dried for 24 hours at 100°C. The weight of the samples were recorded to the nearest milligram. Finally, the rate of respiration was calculated as ml of oxygen consumed per gram (over dry wt.) of leaf tissue.

TRANSLLOCATION:

Labelling with ¹⁴CO₂ was carried out in the greenhouse at sunlight flux densities of at least 740 ME 2 sec⁻¹ PAR and chamber temperature of 30°C.

¹⁴CO₂ was generated by injecting 4N HCl through a stopper into a small beaker containing 1 ml of 1N Na₂CO₃ containing 50 mCi of NO₂ CO₂ leaves were allowed to assimilate ¹⁴CO₂ for 0.25 hours. The leaf is then removed from the labelling chamber and the plant returned to green house for an additional 5.75 hours. The plant material was later harvested at required time and separated into various morphological components and analysed for the isotope. The percentage of ¹⁴C translocated was calculated with the formula given below:
Total $^{14}C$ recovered in the whole plant

$^{14}C$ in the labelled leaf segment x 10

Total $^{14}C$ recovered in the whole plant

TRANSPERSION:

Transpiration was determined by pot weight method. 30 cm diameter pots with only one plant in each, were used for transpiration determination. Five replications for each treatment were studied. A separate set of pots without plants were also maintained to estimate the surface evaporation and this value was deducted from the total values obtained with each pot to determine net transpiration. Since there were differences in the initial weights of the pots, the transpiration rates were expressed as per cent loss of moisture on the basis of pot weight.

WATER USE EFFICIENCY:

Growth observations were recorded on 10 randomly selected plants and water use efficiency was calculated as grain yield (gms/plant) divided by total seasonal evaporation (mm).
PHOTOSYNTHETIC ENZYMES:

Extraction: Leaf material (3.0 g) was cut into about 1 cm² pieces and ground vigorously in a prechilled mortar with 4 volumes of extraction medium. The extraction medium consisted of 50 mM tris HCl buffer pH 7.8 containing 1 mM EDTA, 2 mM MgCl₂, 5 mM dithiothreitol and 10 mM B-mercaptoethanol. The extract was filtered through 4 layers of muslin cloth and an aliquot was saved at this stage for chlorophyll determination (Arnon, 1949). The extracts were clarified by centrifuging at 20,000 g for 15 min at 0°C using Beckman Spinco Ultra Centrifuge and supernatant was used for enzyme assay.

CARBOXYLATING ENZYMES:

Phosphoenol Pyruvate Carboxylase (PFP): The enzyme was assayed according to Edwards and Gutierrez (1972). The enzyme was assayed estimating ¹⁴CO₂ incorporation into acid stable products. The reaction mixture (2.0 ml) contained 50 mM tris HCl buffer pH 8.0, 2 mM MgCl₂, 5 mM DTT, 1 mM EDTA, 5 mM phenylhydrazine hydrochloride, 1 mM PEP, 3 mM Na H¹⁴CO₃ and enzyme extract. The enzyme mixture was preincubated at 0°C for 10 min with PEP to prevent initial lag in the reaction mixture
was taken and transferred to equal quantity of 20% (w/v). TCA present into liquid scintillator vial and the radioactivity was determined by using liquid scintillator.

**Ribulose, 1,5-biphosphate carboxylase (RUBP):** The enzyme was assayed by following the incorporation of radioactive bicarbonate in a reaction mixture (2.0 ml) containing the same concentration of components used in the grinding medium with 44 mM NaH\(^{14}\)CO\(_3\), 1 mM RUBP and enzyme extract. The reaction was initiated by RUBP. An aliquot of the reaction mixture was transferred to a equal quantity of 20% (w/v) TCA present into liquid scintillator and the radioactivity was determined by using liquid scintillator.

**DECARBOXYLATING ENZYMES:**

**NAD-Malic Enzyme (NAD-ME):** The enzyme was assayed by following NADH\(_2\) formation at 340 nm in reaction mixture (3.0 ml) containing 25 mM tris HCl buffer pH 7.8, 0.25 mM EDTA, 4 mM dithithreitol, 5 mM malate, 5 mM KCl, 4 mM NAD 8 mM ammonium sulphate and the enzyme. Reactions were started by adding Mn\(^{++}\) after the completion of the small and rapid reduction of NAD due to equilibration of malate dehydrogenase reaction (Hatch and Kagawa, 1974).
NADP-Malic Enzyme (NADP-ME): The enzyme was assayed in the decarboxylating direction and the increase in the absorbance at 340 nm was measured (Raghavendra and Das, 1976). The reaction mixture (3.0 ml) contained 25 mM tris-HCl buffer pH 8.0, 0.5 mM BDTA, 2.5 mM malate, 0.25 mM NADP, 5 mM MgCl₂, 2 mM dithiothreitol and the enzyme. Reactions were started by the addition of MgCl₂ so that the blank rate due to NADP malate dehydrogenase could be noted.

PEP-Carboxykinase (PEP-CK) Enzyme: The enzyme was assayed spectrophotometrically by following the disappearance of oxaloacetate followed by the decrease in absorbance at 280 nm. The reaction mixture in a total volume of 1.0 ml contained in micromoles: phosphate buffer pH 7.0, 50; MgCl₂, 2.5; MgCl₂, 2.5; ATP 2.5; OМ⁻¹; and the enzymes extract. The reaction was coupled to endogenous pyruvate kinase which was in excess. The enzymatic activity was expressed after subtracting the non-enzymatic rate of OAA decarboxylase by the addition of the enzyme and ATP from the initial rate resulting from the subsequent addition of the enzyme and ATP (Hatch, 1975).

TRANSAMINATING ENZYMES:

Aspartate Aminotransferase: The enzyme was
assayed by following the absorbance change at 340 nm in a reaction mixtures containing in micromoles; tris HCl pH 8.0, 75; pyridoxial phosphate, 0.1; EDTA, 6; NADH, 0.6; 2-oxoglutarate, 7.5; L-aspartate, 7.5; the enzyme extract and excess malate dehydrogenase in a total volume of 3.0 ml (Andrews, et al., 1971).

Alanine Aminotransferase: This enzyme was assayed in a manner similar to that of the aspartate aminotransferase except that aspartate was replaced by L-alanine, and the malate dehydrogenase by lactate dehydrogenase (Andrews et al., 1971).

NAD-Malate Dehydrogenase: The enzyme was assayed towards malate formation with the oxidation of NADH, in the reaction mixture (3.0 ml) containing 25 mM tris-HCl buffer pH 8.0, 1 mM EDTA, 0.5 mM oxaloacetate, 10 mM B-mercaptoethanol, and 0.2 mM NADH in a final volume of 3.0 ml. The reaction was started by the addition of NADH. The decrease in the absorbance at 340 nm was recorded.

Pyruvate Pi-Dikinase: The enzyme was assayed linking ATP and Pi- dependent conversion of pyruvate into FEP carboxylase and malate dehydrogenase. The reaction mixture (3.0 ml) contained 50 mM tris-HCl buffer, pH 8.0; 3 mM MgCl₂; 2 mM DTT; 2 mM ATP; 2 mM Pi; 2 mM Pyruvate; 1 mM NADH; 3 mM K HCO₃ and the enzyme. The reaction was
started by adding ATP and coupled with endogenous PEP carboxylase and malate dehydrogenase which were in excess. The utilisation of NADH was followed by observing the decrease in absorbance at 340 nm.

PHOTORESPIRATORY ENZYMES:

The enzymes were extracted in 0.1 M phosphate buffer pH 8.0 containing 0.2 mM EDTA and 0.1 M cysteine.

Glycolate Oxidase: The enzyme was assayed spectrophotometrically at 324 nm by measuring the glyoxylate phenyl hydroxyl formation. The assay mixture contained 33 mM phosphate buffer pH 7.8; 2.7 mM EDTA, 0.0083% triton X-100 0.67 mM oxidized glutathione, 0.2 mM FMN, 3.3 mM phenyl hydroxazine chloride, 5 mM potassium glycolate and the enzyme extract. FMN was added before adding the enzyme extract and kept in dark. A standard curve was prepared with glycolate under identical conditions (Fairbend and Beevers, 1972).

Glycerate Dehydrogenase: The enzyme was assayed spectrophotometrically at 340 nm in the reverse direction following the reduction of NAD in the presence of glycerate. The reaction mixture (3.0 ml) contained 33 mM phosphate buffer pH 6.5, 0.8 mM NAD, 20 mM D-glycerate
and enzyme extract. The reaction was initiated by adding glycerate.

Glyoxalate Reductase: The extraction of the enzyme was carried out with 0.1 M phosphate buffer at pH 6.5. The enzyme was spectrophotometrically assayed following the decrease in absorbance at 340 nm consequent of the oxidation of NADH by glyoxylate. The reaction mixture (3.0 ml) contained 33 mM phosphate buffer pH 6.5, 0.3 mM NaOH, 20 mM glyoxylate and enzyme extract. The reaction was initiated by the addition of glyoxylate.

Catalase: The enzyme was assayed spectrophotometrically following the decrease in absorbance at 230 nm. The reaction mixture contained 10 mM phosphate buffer pH 7.0, 2 mM H₂O₂ and the enzyme extract. The enzyme extract was adjusted such that the half time reaction was about 30 sec (Machly and Chance, 1954).

PIGMENT COMPOSITION:

Estimation of Total Chlorophyll: Freshly harvested leaves (1.0 g) were chilled at 0°C in the dark for an hour, ground in 15 ml 80% (v/v) acetone with a pinch of washed sand in a prechilled mortar with a pestle. The homogenate was centrifuged at 6,000 g for 15 minutes.
The supernatant was decanted and the pellet was resuspended in 10 ml of 80% (v/v) acetone and centrifuged at 6,000 g for 10 min. The two supernatants were pooled. The suitability diluted acetone solutions were screened for optimal density at 563 and 645 nm in spectrophotometer. The total chlorophyll was determined according to the formula of Arnon (1949).

Total chlorophyll $\mu g$ ml$^{-1}$ = $(20.8 \times \text{OD 645}) + (8.02 \times \text{OD 663})$

Chlorophyll a, chlorophyll b and chl a/chl b ratio:

The levels of chl a, chl b were determined using the equation:

Chlorophyll a $\mu g$ ml$^{-1}$ = $(12.7 \times \text{OD 663}) - (2.63 \times \text{OD 645})$

Chlorophyll b $\mu g$ ml$^{-1}$ = $(22.9 \times \text{OD 645}) - (4.68 \times \text{OD 663})$

Chl a/chl b ratios were calculated subsequently.

PHOTOCHEMICAL ACTIVITIES:

DCPIP Reduction: The reaction mixture (3.0 ml) contained 0.05 M potassium phosphate buffer pH 7.5, 20 mM 2,6 DCPIP, 1 mM MgCl$_2$, 20 mM NaCl, and chloroplasts (20 $\mu g$ chl ml$^{-1}$). The assay components were illuminated and the reduction of DCPIP was measured by following the decrease in absorbance at 620 nm. The rate of reduction
was calculated with the help of a standard curve prepared with DOPTP in identical conditions.

**Ferricyanide Reduction:** The reaction mixture (3.0 ml) contained 15 mM tris-HCl buffer pH 7.8, 0.5 mM ferricyanide, 1 mM MgCl₂, 20 mM NaCl, and chloroplasts (20 µg chl ml⁻¹), 0.3 ml 20% (w/v), TCA was added after 3 min of illumination. The absorbance of solution was measured at 420 nm after centrifugation to remove precipitate protein. A standard curve was prepared with ferricyanide for calculations.

**NADP Photoreduction:** The rate of NADP reduction with water as electron donor was determined with the reaction mixture (3.0 ml) having 10 mM phosphate buffer at pH 7.8, 20 mM NaCl, 1 mM MgCl₂, 0.5 mM NADP, 5 mM spinach ferredoxin, and chloroplasts (10 µg/ml). Soon after switching off the lights, 0.3 ml of 1 N NaOH was added to the reaction vessels to inhibit the reoxidation of NADPH termed. The NADP reduction was calculated by the increase in absorbance of 340 nm using the extraction coefficient of 6.22 x 10⁶ cm² M⁻¹.

**PHOTOPHOSPHORYLATION:**

The rate of photophosphorylation by chloroplasts was estimated according to Whatley and Arnon (1963).
The chloroplasts were isolated at 0°C in 0.2 M tris-HCl buffer at pH 8.0, containing 0.35 M NaCl, and were suspended in 0.02 M tris-HCl buffer pH 8.0, containing 0.035 M NaCl to give a final concentration of about 0.5 mg chlorophyll ml⁻¹. The basic reaction mixture for the measurement of photophosphorylation contained the following (in micromoles ml⁻¹), tris-HCl buffer at pH 8.3, 50 MgCl₂ 2; ADP, 1; Pi + ³²P, 3.0; (5 x 10⁵ cpm), PMS (cyclic), 0.02 or ferricyanide (non-cyclic), 0.2 and chloroplasts equivalent to 5 µg chlorophyll.

The reaction mixtures were illuminated at 28°C and 200 Wm⁻². The reactions were terminated by turning off the light and adding 0.3 ml of 20 per cent (w/v) TCA. After centrifugation, the supernatant was assayed for its ATP³²P content.

To measure the "organic phosphate" formed, 1.0 ml aliquot of the supernatant fluid was mixed with 1.0 ml of the magnesia mixture. A drop of 0.2 per cent (w/v) phenolphthalein was added to check unit the pH remained alkaline. It was found desirable to add small amount of inorganic phosphate to the stock magnesia mixture to insure the presence of 'seeds' of magnesium ammonium phosphate for starting the precipitation. The precipitate contained unesterified inorganic phosphate. The
mixture was allowed to stand for an hour at room temperature and then the precipitate was washed twice with a 1:10 dilution of the magnesia mixture. The filtrate contained the radioactive ATP. Radioactive phosphorous was estimated in a 0.1 ml aliquot of the filtrate by evaporating to dryness over the planchets. The counting was done using a GM counter. The ATP formed was calculated by comparing the radioactivity in the filtrate with the total radioactive phosphate present in 0.1 ml aliquot of the original supernatant, which was similarly evaporated but not precipitated with magnesia mixture.

ENZYMES OF NITRATE ASSIMILATION:

Nitrate Reductase (NR): The enzyme was extracted in 20 mM potassium phosphate buffer pH 8.8 containing 1 mM EDTA, 10 mM cysteine, 3% (w/v) BSA and 0.1% (w/v) polyvinylpyrrolidone, and was assayed with NADH as the electron donor (Hageman and Huckleby, 1971). The assay mixture in a final volume of 2.0 ml contained in micromoles, phosphate buffer pH 7.5, 50; potassium nitrate, 20; NADH, 0.4; and the enzyme extract. The reaction was initiated by the addition of the enzyme. After incubation at 30°C for 15 min, the reaction was terminated by the addition of 1.0 ml of 1% (w/v) sulphanilamide in
3.0 M HCl, and 1.0 ml of 0.02% (w/v) N-1 naphthol ethylene diamine dichloride (together called as diazo-coupling reagents). The colour was allowed to develop for 30 min at 30°C prior to reading at 540 nm.

Nitrite Reductase: The enzyme was extracted in 10 mM tris-HCl buffer pH 8.0 containing 1 mM EDTA and 10 mM dithiothreitol and was assayed with dithionite reduced methyl viologen as reductant (Losada and Paez, 1971). The reaction in a final volume 2.0 ml contained in micromoles; tris HCl, pH 8.0, 150; sodium nitrite, 4; methyl viologen, 1.5; dithionite, 4.5; and the enzyme extract. After incubation for 10 min at 30°C the reaction was stopped by vigorous shaking until the dithionite was completely oxidised and the dye becomes colourless. To an aliquot of 0.2 ml, 1.0 ml each of the diazo-coupling reagents were added and the volume was made up to 30 ml prior to reading the colour intensity at 540 nm.

Standard curve was prepared by treating varying nanomolar quantities of NaNO₂ with diazo coupling reagents (Rinal Volume 3.0 ml) and was utilized in the calculations of nitrate in both the above mentioned assays.

Glutamine Synthetase (GS): Glutamine synthetase was extracted in the medium containing 0.05 M tris-HCl
buffer pH 7.9, 1.5% (v/v) of 1 M MgSO₄ and B-mercapto-
ethanol to a final concentration of 7 mM. The standard
reaction mixture (2.0 ml) consisted 0.10 M tris-HCl
pH 7.9 buffer containing 20 mM MgSO₄, 1 mM EDTA, 80 mM
L-glutamate, 6 mM NH₂OH, and 8 mM ATP (final pH was
adjusted to 7.8). The assay components were preincu-
bated for 2 min at 35°C and the reaction was initiated
by the addition of the enzyme. After incubation for
6 min at 35°C, the reaction was stopped with 2.0 ml of
a solution containing 0.37 M FeCl₃, 0.67 N HCl and 0.2 M
TCA. The contents were centrifuged and the amount of
r-glutamyl hydroxymate formed was determined colorimetri-
cally (540 nm) by comparison with a standard curve pre-
pared using authentic r-glutamyl hydroxymate in the
presence of all assay compounds except ATP (O’Neal and

Glutamate Synthase: The enzyme was extracted in
50 mM tris-HCl buffer pH 7.5 consisting of 1 mM EDTA,
5 mM B-mercaptoethanol, 1% (w/v) BSA and 0.4 M sucrose.
The reaction mixture (3.0 ml) contained, 50 mM tris-HCl
buffer pH 7.5, 1 mM EDTA, 15 μmoles glutamine, 250 μmoles
NADH and 0.1 ml enzyme extract. The decrease in absor-
bance was read at 340 nm (Beever and Storeg, 1976).
GROWTH PARAMETERS:

Leaf area was measured by placing the leaves on a graph sheet. The area was calculated from the graph sheet by predetermining the weight of specific area of the graph sheet.

Plant height measurements have been taken from the plants grown in controlled and water stressed plants. Both the shoot height and root heights were recorded during four growth stages: vegetative, flowering, grain filling and senescence by using meter scale.

For determination of fresh weight of the shoot part and root part, the plants were removed, washed with tap water first and then with distilled water blotted gently and weighed in an analytical balance. Dry weights were obtained by keeping the shoot part and root part in an oven for 48 hr at 80°C till a constant weight was obtained. And shoot/root ratio was obtained from the above. Estimations were made for the controlled and water stressed plants during vegetative, flowering, grain filling and senescence stages.

GRAIN YIELD:

After maturation, the Amaranthus plants from all the five replicates were brought to the laboratory and after threshing, the grain yield per plant was recorded and presented here as gms/plant. The collected grain later utilised for taking observations such as 1000 seed weight and measurement of seed size.
LEGEND FOR PLATE IV.

View of *A. hypochondriacus* plants grown in glass house.