CENTRE D'ETUDE REGIONAL
POUR L'AMELIORATION DE L'ADAPTATION
A LA SECHERESSE

CERAAS

I.S.K.A. - C.N.R.A.
BP 53 Bambey Sénégal
Tél 73-60-W

# ADAPTATION OF COWPEAS TO DROUGHT : PHYSIOLOGICAL ASPECTS

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Dr. MARCEL C. NWALOZIE

JULY 1991

FINAL REPORT SUBMITTED TO CENTRE D'ETUDE REGIONAL POUR L'AMELIORATION DE L'ADAPTATION A LA SECHERESSE (CERAAS), BAMBEY, SENEGAL, AT THE END OF MY RESEARCH FELLOWSHIP.

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## I).INTRODUCTION:

Drought is one of the most complex problems facing agricultural food production in arid and semi arid regions. The slow progress made in developing cultivars that are adapted to drought is as a result of this complexity. Thus drought has persistently taken its tolls on crop yield. This reduction in yield is becoming more alarming in view of the continued decline in the availabity of irrigation water. The increased tempo of studies in this regard may, however lead to the elucidation of the physiological mechanisms of drought resistance which could act as sound bases for genetic engineering and selection.

*Vigna unguiculata(L)* Walp.,also commonly called cowpea,is a very important food legume,and is established by seed.A number of workers (Hall and Dancette,1978;Turk. Hall and Asbell, 1980) have demonstrated that cowpeas can considerably withstand water stress.Some cultivars can, however, resist drought better than others. Understanding the basis of this resistance is needed for selecting and breeding for drier regions.

The studies on drought adaptation of cowpea reported here consist of two separate trials. The first was designed to study the development of cowpea roots under intense drought, where as the other investigated the effect of pre-sowing hydration-dehydration drought hardening technique on water stress resistance in cowpeas. The later tria1 was based on prelimnary reports from my laboratory in Nigeria which hinged on certain scientific observations. There are reports in the literature that when seeds of some species are imbibed in water and then dehydrated (by air-drying) to near their original water content they subsequently germinate more rapidly than untreated seeds, and this leads to enhanced growth of the seedlings and greater resistance to water stress (Henckel, 1964; Enu-Kwesi, Nwalozie and Anyanwu, 1986). The experiments carried out in the present studies were designed to rigorously test the suitability of this technique with cowpeas in actual drying conditions. A successful technique such as this can be usefu] in a tested cultivar to protect it against unpredicted drought(or a dry spell).

## II). MATERIALS & METHODS:

### 1)EEDS:

Two varieties, B89-504 (from the Senegalese Agricultural Research Institute, ISRA ) and 17'84 S2246-4 (from IITA, Nigeria) were used. For ease of description, B89-504 will be referred to as V1, while IT84S2246-4 will be tagged V2.

### 2) TEMPERATURE AND HUMIDITY OF GLASSHOUSE:

The temp was 35°C in the day and 19°C in the night. Humidity was 40% and 72% day and night respectively.

### 3')RHIZOTRONS:

The tubes consisted of cylindrical PVC tubes measuring 100 cm by 15.5 cm. Each tube had one side of itflattened out by cutting off!, and a flat transparent PVC material measuring 13 cm by 100 cm was sealed to the cut surface (this transparent PVC permited visual observation and measurement of root development). The bottom side of the tube was also sealed with a flat opaque PVC material with 5 drainage holes (each 10 mm in diam) to drain off excess water.

The bottom of each tubes was layered with grave1 and thereafter filled with top soil that was sieved through a 2 mm mesh. (The physical and chemical properties of the soil was known). Three grams (3 g) of finely ground N P K (6:20: 10) were applied 10 cm below soil surface before the soil was irrigated and seeds sown. The soil in each tube was watered to field capacity before 4 seeds were sown. The seeds were treated with Granox fungicide.

The tubes were placed in the glasshouse in 2 variety by 2 hydric levels by 4 replications (a randomized complete block design, 3 factor factorial). Each tube tube was encased in an easily removable black polythene casing fastened to the neck of the tube with a rubber band (this was to prevent the penetration of light to the roots). Each tube was inclined at an angle of 47".

At the completion of germination (6 days after sowing, DAS) the seedlings were irrigated 2 times daily with 100 ml of water during each irrigation. Eigth DAS the plants were thinned down to one seedling tube. Water stress was applied 10 DAS: for V1H1 and V2H1 plants irrigation was discontinued throughout the duration of the experimcnt (i.e for the remaining 26 days -this expt lasted for 36 days); for V1H0 and V2H0 plants irrigation was continued as already stated, uninterrupted.

Ferric chlorosis was observed on the seedling leaves 11 DAS. This chlorosis was induced by the accumulation of calcium carbonate from the irrigation water. This was corrected according to the procedure of Blandel (1968). The method consisted the foliar application of 4% ferric sulphate in solution with Guardar Adjuvant (obtained from SnWn Associates, 3610 Garden Brook, Dallas Texas 75234). The Guardar soln only acted to ensure that the ferric sulphate was not washed away, and so ferric sulphate could be applied alone. Ferric sulphate was applied again 25 DAS, and distilled water was used to replace tap water for irrigation.

Root growth and development were monitored 2 times weekly from the commencement of stress to the termination of the experiment. The following parameters were mcasured; a) root length, b) number of roots within each horizon (each tube was marked out in 20 cm horizons, giving a total of 5 horizons/100 cm tube), and c) root density within each horizon.

This experiment was terminated when it was observed that the first roots had reached the bottom of the tube, and this was at 36 DAS. At termination the following measurements were made; d) soil moisture content of each horizon, e) total root length, f) total root dry wt, g) root volume, h)leaf area, i)leaf dry wt, j) stem dry wt, and k) leaf water potential (the water potential of the plants at the termination of the experiment was as follows; V1H0 = -13.7, V1H1 = -27.0, V2H0 = -18.5, V2H1 = -31.2 ). The procedure used for the measurements in (d) to (k) were as described for pot trials.

4) POT TRIALS:

4.1 Seed treatments:

A batch of V1 seeds were imbibed in distilled water for 3 h (the imbibition time of cowpea seeds. The imbibition time is the period required by a seed to imbibe water to full capacity, and is determined by imbibing seeds and weighing them at regular intervals. Extrapolation from a curve ofthe data gives the imbibition time). The imbibed seeds were decanted and gradually air-dried to half the moisture content of the fully imbibed seeds (Henckel,1964; Enu-Kwesi et al, 1986). This constituted the hardening treatment, T1. The other batch of seeds were not imbibed and this constituted the non-hardened, T2. These treatments were repeated for V2, thus giving the following seed treatment combinations; V1T1, V1T2, V2T1, V2T2. The seeds were treated with Granox fungicide as described earlier. Six seeds were sown in each pot.

4.2 Culture and growth of plants :

The seeds were sown 1.5-2.0 cm below the soil level in cylindrical PVC pots (25 cm diam. by 40 cm height) containing 28 kg of top soil with similar composition as described in section 2.3 of this report. The pots were arranged in a 2 by 2 by 3 strip split plot factorial design, giving the following combinations V1T1H0, V1T1H1, V1T1H2, V1T2H0, V1T2H1, V1T2H2, V2T1H0, V2T1H1, V2T1H2, V2T2H0, V2T2H1, V2T2H2 (12 treatments/ replication);H0 = regular irrigation, H1 = -25 bars of water stress, H2 = -40 bars water stress. This was replicated 5 times (i.e 2 by 2 by 3 by 5 = 60 pots). The pots were raised about 4 cm above the floor of the glasshouse with welded steel bars. Another set of similar pots numbering a total of 24 containing seedlings af similar treatments (2 by 2 by 3 by 2 =24) were also arranged in a strip randomized fashion around the main experimental pots (These served as borders, and data were not collected from the borders).

The pots were irrigated to field capacity with tap water (at field capacity excess water would drain off through 10 mm drainage holes punched at the bottom of the pots) before seeds were sown, and no additional irrigation was done until 2 days after 80% seediing emergence (i.e 6-7 DAS ). At this stage, the pots were irrigated with 400 mls of tap water once every other day. When the plants were older and required more water (i.e at 20-25 DAS) they were irrigated with 600 ml of water once every other day. At the on-set of flowering (39-40 DAS) the plants were irrigated with 500 ml of water ‘2 times every day.

At 7 DAS each pot was given 500 g of organic fettilizer, whose characteristlcs were known. Additional fertilizer, N P K (6:20: 10) was applied in the proportion of 3 g/pot. The number of plants per pot was thinned down to 2 on 21 DAS. Ferric chlorosis was also corrected by 17 DAS as previously described in section 2.3. This solution was sprayed again by 29 DAS, and the plants were subsequently irrigated with distilled water.

4.3 Water stress:

Prelirninary results from my laboratory In Nigeria indicate that cowpea is more sensitive to water stress at the flowering stage of the life cycle. Water stress was therefore applied to Hl & H2 plants at 37 DAS, the on-set of flower buds. HO plants recieved regular irrigation as already described, while irrigation for H1 & H2 plants was suspended, and their water potential determined at regular inter-vals. Water stress was terminated for H1 plants when their leave water potential registered -25 bars at 47 DAS at the termination of each stress level irrigation of the hitherto stressed plants was done as in the regularly watered plants. Similarly, water stress was terminated for H2 plants at 61 DAS when they had reached a water potential of about -40 bars. (Tnteresting enough, V 1T1 H2 plants never reached -40 bars, but the stress had to be terminated since the life cycle of the plants was almost ended, and other measurements were to be taken. The varieties used in these studies completed their life cycles at 65-67 DAS).

In addition to monitoring leaf water potential, soil water content was determined gravimetrically shortly before the termination of each stress level. The gravimetric method involved determining water content of soil on a mass basis (Kg ha-l). The mass of the sample was determined when it was collected from the pots and after oven drying to a constant wt at 105°C for 12-24h. The mass wetness (w) was determined from the relationship:

$$w = ( (Mw + Ms) - Ms )/ Ms$$

where Mw = the mass of water in the sample, and
Ms = the mass of the solid particles.

The mass wetness (Kg ha-l) is thus equivalent to the quantity (wet mass-dry mass)/dry mass (Mulla, 1987). This can also be related to percentage moisture by;

( ( wet mass - dry mass)/dry mass ) * 100%.

(All dry weight measurements of in these expts were taken from a Mettler balance - model AE100, precision balance).

4.4 Water potential measurements:

Water potential was measured with Peltier cooled thermocouple (or chamber (CC30, WESCOR)) psychrometers connected to a PR-55 psychrometer microvoltmeter readout device (Wescor, Utah U.S.A). The psychrometers were initialy calibrated with different concentrations of NaCl as previously described (Lang, 1967). The linear regression derived from the individual curves were used to calculate the water potential of each sample.

Sampling involved cutting out a small rectangular piece of leaf tissue (3rd leaf from the apex), avoiding the lager veins. (Three swift cuts were made to obtain each rectangular piece of leaf tissue). The excised tissue was quickly placed in the sample compartment of the psychrometer and left to stand undisturbed(for equilibration) on a laboratory bench for 4 h after which the psychrometer microvoltmeter was used to read the microvolt produced by the sample. Water potential was calculated as earlier described.

Water potential was measured at the inseption of stress (38 DAS) and at 2 - 3 days intervals, to the termination of the expt (64 DAS).

4.5 Leaf RWC:

Leaf relative water content (RWC) was similarly monitored from the begining of stress to the termination of the experiment. Individual treatments were sampled by cutting a section of the 3rd leaf, and swiftly placing it in a small glass vial (Pyrex grade), whose initial weight was determined, and quickly replacing the stopper. The fresh weight (FW) of the leaf tissue was determined, and the leaf was then floated on distilled water for 2 h, after which it was carefully blotted, between folds of paper towelling, avoiding pressing out much sap from the tissue. The blotted leaf tissue was quickly placed between a fold of parafin paper, whose wt was known or zeroed, and the Turgescent weight (TW) determined. The tissue was thereafter dried at between 100-105 °C for 12-24 h, and the dry weight (DW) determined. RWC (%) was calculated from:

$$(FW-DW)/(TW-DW) * 100.$$

4.6 Stomatal resistance/condutance:

Thesc were measured with a Delta-T automatic porometer (MK II Cambridge England). Leaf stomatal resistance and conductance were measured on both the adaxial and abaxial surfaces of the 3rd leaf from the apex of the main stem. Measurements were taken from the central part of the centre leaflet. The porometer was calibrated before and after each measurement according to standard procedures (Kanemasu, Thurtell,and Tanner, 1969). For calibration, an acrylic plate which had 11, 6, 4, and 3 holes respectively drilled at different levels was used. These were used to simulate stomatal resistance, the number of holes and thickness of the plate correspond to determined diffusion resistance. A piece of Whatman N° 1 filter paper which was saturated with distilled H20, with a strip of adhesive paper on one side, was sealed over the lower side of tht: acrylic plate. A calibration curve was developed for each set of measurements, using the average of before and after measurement calibration readings from the perforated plate with already calculated standard resistance values.

Correction factors (CF) obtained by multiplying the porometric readings by standard calculated values ; 1.0, 0.94, and 0.88 (where 1.0 was used for temps between 0-25°C, 0.94 for 26-35°C, and 0.88 for 36-45°C ). Such CF values were extrapolated from the calibration curves to obtain the unit resistance for the adaxial or abaxial surfaces of the leaf.

Conductance of the leaf was calculated from:

$$(1/ADr) + (1/ABr)$$

where ADr= unit resistance of the adaxial surface

ABr= unit resistance of the abaxial surface.

Total leaf resistance (TLr) was calculated from:

(1 /conductance).

4.7 Protoulasmic resistance: This was done at 50 DAS:

4.7.1 Determination of the temp at which 50% damage was done to the protoplasm; Leaf samples of V1T2H0 and V2T2H0 (control of the unhardened plants) were collected from the 3rd leaf as usual. These leaf samples were collected in plastic bags whose inside was moistened with distilled H2O to reducc excess H20 loss.

Ten leaf discs were punched with a 10 mm cork borer from the leaves of VI, rinsed in distilled H20, blotted between folds of paper towellings, and placed in a test tube. The test tube with the discs was then placed in a water bath at 40°C. Series of 10 leave discs were similarly placed at 45, 47.5, 50, 52.5, 55, 57.5, and 60°C, and a batch was placed at room temp (26°C) to serve as the control. These were repeated for V2 samples.

In another set up, series of 10 leaf discs batches from V1 were placed in di fferent concentrations of polyethene glycol 600 (PEG 600 ) viz; 0, 20, 25, 30, 35,and 40 g/l These were also repeated for V2 samples.

The leaf discs in the temp controlled water bath were treated to the various temps for 30 mins, after which 30ml of distilled water was added,and they were then left to stand for 24 h at 5°C in a refrigerator. The temp of the solution was then allowed to equilibrate at room temp for about 15 mins, and conductivity of the solns, free conductivity (FC), measured with a microcomputer conductometer (Consort model K220). These same discs in the solutions were there after boiled at 100°C for 1 h, placed on a bench for 20 mins to cool to room temp, and then retumed to the refrigerator at 5°C for another 24h. Total conductivity (TC) was then measured for each tube.

Meanwhile, the discs in the PEG solns were left to stand for 24 h, after which they were rinsed 2 times in distilled H20, placed in 30 ml of distilled water, and left to stand at 5°C for 24 h. The FC of the solns were measured. These discs were then boiled at 100°C, placed at 5°C for 24 h, and the TC measured.

The results were plotted, then the temperature or PEG 600 solution at which 50% damage was done to the protoplasm was used in the actual determination of solute Ieakage from the membranes. The results of the temperature trials are shown in, and this indicates that the average temp at which 50% damage was done to the protoplasm of these two cowpea varieties was 49.2°C. This temp was used as the working temp for actual determinations.

4.7.2 Actual determinations of protoplasmic resistance; One leaflet (the centre leaflet) were collected from each of 12 treatments in 5 replications. These leaves were placed in polythene bags as described earlier. Leaf discs (10 discs) were punched from each of 5 leaves (a total of 50 discs from each treatment). The discs from each treatment were floated on distilled H20 for 2 h with 2 changes of the float H20 within the floating time (the discs were thoroughly rinsed during each water change).

Four test tubes were prepared for each treatment (a total of 48 tubes for all 12 treatments). The first tube of each treatment constituted the sample control, into which 30 ml of distilled H2O were added (no H20 was added at this stage to the other 3 tubes for the specific sample treatments). These 3 tubes from each treatment were treated to hot temp at 49.2°C (as previously determined) in a water bath for 30 mins. (Note that the specific sample control tubes from each treatment were not treated to hot temperature). The tubes were then removed from the water bath, placed on a bench at room temp for about 15 mins after which 30 ml of distilled H20 were added to each tube. AH the tubes, including the sample control tubes, were then kept at 5°C for 24 h.

The tubes were allowed to equilibrate at room temp thereafter, and the FC was measured for each soln. The tubes were then boiled at 100°C in a water bath for 1 h, and placed at 5°C for 24 h. TC was then determined for each tube.

% of absolute damage(AD) to the protoplasm was calculated from

(FC/TC)*100;

% of absolute integrity(AI) of the protoplasm was calculated from

(1- (FC/TC) * 100 or from 100-(AD);

% of relative integrity (RI) was calculated from

( (1-(FC/TC) of st) /(1-(FC/TC) of SC) ) *100

where st = sample treatment; SC = sample control.

% of relative damage was calculated from

(1- ( (100-(FC/TC) of st) / ((100-(FC/TC) of SC) ) * 100

4.8 Osmotic regulation:

Leaf samples of all treatments were collected severally in moistened polythene bags as usual. Eleven leaf discs were punched from each leaf sample of each treatment with a 10 mm cork borer. The discs were rinsed and subsequently floated in distilled H2O in a Petri dish for 2 h to obtain full turgidity.

Eleven test tubes, each containing a solution of NaCl in the following concns; 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.2, 1.4, and 1.7 molal concns were prepared. These concns corresponded to the following values in bars; -12.72, -16.93, -21.15, -25.39, -29.67, -33.98, -38.32, -42.70, -51.60, -60.68, and -74.60 all at 5°C respectively. A concave-shaped wire mesh was suspended at 4-5 cm above each soln in the tubes. A leaf disc from the first treatment (V1T1H0) was taken from the Petri dish in which it was floated, blotted between folds of paper towelling, placed between folds of parafin paper (to reduce H20 foss) and quickly weighed (wet wt or turgid wt) on a Mettler AE 100 balance. The disc was immediately placed on the suspended wire mesh of the tube containing the first concentration, i.e 0.3m NaCl. The tube was immediately wrapped close with a piece of parafin paper. The other discs of the same sample treatment were similarly treated and placed in the other concentrations respectively. This was repeated for each of the 12 treatments, thus giving a total of 132 tubes. The tubes were left to stand in a refrigerator at 5°C for 48 h after which the fresh wt (FW) of each disc was determined. The discs were then placed in an oven at 1 00-105 °C for 16 h, and the dry wt (DW) measured. Relative water content (RWC) of the discs was calculated as previously described.

4.9 Mcasurements at final harvest:

The following measurements were made at final harvest;

a) Leaf area was measured with a portable leaf area meter, LI-COR brand (model LI-3000).

b) Leaf dry wt

c) Stern dry wt

d) Root dry wt

e) Dry wt of root nodules

f) Number of flower buds not yet opened

g) Number of flowers opened

h) Fruit number and dry wt.

4.10 Statistical treatment:

All data so collected were statistically analyscd according MSTAT (1985) package, using a factorial analysis of vatiance.

## III) RESULTS & CONCLUSTONS:

### 1) RHIZOTRONS:

The elongation growth of the first root (i.e the primary root) was significantly ($P < 0.01$) increased by the stress treatment throughout the period of the experiment. The differences in growth between the varieties was not significant. The second and third roots of the same plants measured during this period were not significantly increased during stress. There were, however, differences in root length between measurements.

The number of roots within the first horizon (O-2Ocm) was higher ($P < 0.01$) in the regularly irrigated plants than in the stressed ones irrespective of varieties. However,as the tubes graduated into deeper horizons (e.g 60-80cm,and 80-100cm) more proliferation of roots was observed ($P < 0.01$) in the stressed plants. This was especially so in V1.

Similarly, water stress increased ($P < 0.01$) the density of roots only in the deeper horizons of the tubes (40-60cm, 60-80cm, and 80-100cm). There was also an important difference between the varieties in there rooting characteristics, with V1 producing a higher density than V2.

The fact that these cowpea varieties showed a higher proliferation of there roots in the first horizon of the tubes which were regularly irrigated was a normal reaction of plants grown under adequate soil moisture conditions. These plants did not need to dessipate useful energy in the growth of deep root systems in search of water, since there was adequate moisture in the shallow zone of the first horizon. Under soil moisture stress, however, the development of large numbers of roots in the deeper horizons was necessary to sustain the water demands of the actively growing plants. The higher ability of V1 plants under stress to produce more roots under these conditions appears to contributed to its higher leaf water potential (-27.0 bars ) over that of V2 (-3 1.2 bars), since large number of roots provided more surface area for water absorption.

At harvest, it was observed that water stress significantly lowered the total number of leaves per plant irrespective of the varieties. The total length of roots and leaf area were significantly affected. Water stress, however significantly decreased the dry wt of leaves and stems in both varieties alike. It was also observed that both root dry wt and volume mass were not significantly affected by the stress.

The cummulative behavior observed in root development at final harvest was a strange one. The non significant difference in length, dry wt, and volume mass of roots at this stage probably points to the fact that the roots were merely re-aligned in their distributions during stress rather than any significant increases in their dry wt and total area (or volume). In that case it was most likely that the stressed plants did not expend extra metabolic energy to grow deep and higher proliferating roots. The stressed plants, however, lost in the above soil biomass, since dry wt of leaves and stems were decreased by water stress.

The soil moisture content of the stressed tubes was significantly lower than the non stressed ones. There was also a high level of significance in the moisture content of the different horizons; deeper horizons contained more water than shallow ones.

2) POT TRIALS:

RWC; During the early stages of the stress treatment (i.e before the plants reached -25bars), leaf RWC was not significantly reduced by the stress in both varieties irrespective of the hardening treatment. As water stress progressed, however, (i.e between -25 and -40bars) RWC of V1 plants was significantly higher than that in VI plants. Generally, there was a higher RWC in regularly irrigated plants than the stressed ones. The hardening treatment did not enhance the RWC in the stressed plants irrespective of the variety.

Leaf water potential; During early soil moisture stress the leaves of these cowpea varieties significantly had lower water potential than the stressed ones. V1 had higher water potential than V2. The hardening treatment did not confer a better ability to retain more water to the varieties during stress. There were important differences in the water potential of the plants between measurements; as stress progressed water poten tial was decreased .

At higher moisture, soil moisture stress a highly significant difference was observed in the leaf water potential of the plants. Firstly, there was a difference between the control and the stressed plants. Secondly, V1 had higher potential than V2. Lastly, there were differences in water potential between measurements. Although the hardening treatment did not produce any differences, its interaction with the varieties significantly enhanced the water potentials. No V1 plant ever reached -40bars, the original intention af the experiment; but the stress could not be continued for reasons explained earlier in materials and methods. (It would be recalled that such V1 plants that never reached -40bars were grown in the same conditions as V2 that reached -40bars much earlier).

The higher water content (RWC & water potential) of V1 over V2 was probably a result of the higher ability of V1 to quickly establish and re-align their root systems to meet the water requirements of the plant under stress as reported in the rhizotron expts. (section 3.1 of this report). Prompt and aggressive development of roots are important factors considered in drought adaptation of crop plants. The rhizotron experiments. confirm that V1 were able to promptly grow deeper and higher density roots, without expending additional energy.

Protoolasmic resistance; The percentage relative damage done to the protoplasm of stressed plants was significant irrespective of the varieties and the hardening treatment.. There was, however, a high inter-t-action between varieties and the hardening treatment as much as between the hardening treatment and stress.

On the other hand, percentage relative integrity was significantly different between V1 and V2 grown under soil moisture stress. V1 retained higher protoplasmic integrity than V2 under stress. The interractions between stress and varieties on one hand, and the hardcning treatment and stress on the hand were important.

Therc are reports in the litterature which relate protoplasmic integrity to the moisture status of a plant.When films of moisture surrounding the protoplasm were removed by the drought the protoplasm lost its integrity and was damaged, leading to leakage of protoplasts. Depending on the extent of drying, the protoplasm may or never recover on restoration of adequate moisture.

Stomatal resistance and conductance; Water stress significantly lowered both stomatal resistance and conductance irrespective of the variety or the hardening treatment.

At high moisture stress the stomata behave sluggishly in relation to gas exchange. Generally, under water stress stomates close, and impede the diffusion of both CO2 and water vapour. This may also reduce photosynthates (except in crassulean acid metabolism plants, which have a mechanism for the storage of CO2 for photosynthesis).

Stomatal closure apart from the beneficial reduction of water loss in the stressed plants, in addition reduced the total dry mass (see the subsection on agronomie results) of such plants since enough photosynthates were not availabe for incorporation into the carbon skeleton of the plants.

Osmotic adjustments(regulation); Because of the large number of samples involved in this expt. (a total of 132 samples) and the painstaking nature of the procedure, it was difficult to replicate the trials, and so statistical analysis was not done.

The general pattem of response of these varieties to osmotic adjustment was, however similar to earlier results reported here. The results indicate that the ability of the plants to adjust to osmotic pressure was increased with stress. That is, the higher the soil moisture stress, the better the adjustment to osmotic pressure.

There are similar reports in the literature which indicate that the response of plants to stress is improved when they have been exposed to sublethal doses of a similar stress. In a similar vein, when these cowpea varieties were exposed to soil moisture stress, and then to osmotic stress, their percentage RWC was found to be increased over that of the controls. The control plants having never experienced any drying conditions, could not maintain an equally high RWC (compared with the stressed plants) when exposed to the same osmotic pressure.

Agronomic results; Water stress significantly reduced the number of fruits, flower buds, flowers, leaf area, dry wt of nodules, dry wt of leaves, dry wt of stem, dry wt roots, and dry wt of fruits. The hardening treatment did not influence these parameters under stress.

Both varieties did not produce fruits at high water stress level (i.e -40bars), whereas the number of fruits in V2 was more than that in V1 at -25bars, although there was no significant difference in there weights. In a similar observation, the numbers of flower buds and opened flowers considered separately were significantly higher in V2 than in V1 under stress (i.e at -25bars) and the controls. The interractions between the varieties and stress were also highly significant.

The total leaf area of V1 plants was significantly higher than that of V2 under stress and regular irrigation. Again, the hardening treatment did not enhance the surface growth of leaves of both varieties under any of the soil moisture regimes. The dry matter of leaves was reduced by moisture stress irrespective of variety or hardening.

The dry weights of roots and root nodules, determined separately, were also significantly reduced by the stress treatment in both varieties. The reduction in stem dry wt was similarly without regard to variety or treatment.

Photosynthates constitute the primary products in green plants. One of the important effects drought has been reported to be a reduction in photosynthesis through stomatal closure. The exchange of gases needed in the configuration of organic matter in the plants was impeded as adduced from their stomatal behaviour as previously reported above. Consequently dry matter was reduced.

Soil rnoisture content; There were significant differences between the soil moisture content of stressed and unstressed soils. The soil moisture of the pots at recovery were similar.

## REFERENCES

ENU-KWESI,L., NWALOZIE,M.C.and ANYANWU,D.I. 1986. Effects of presowing hydration-dehydration on germination, vegetative growth and fruit yield of Abelmoschus esculentus grown under two soil moisture regimes. Trop. Agric. (Trinidad) 63(2): 181-184.

HALL,A.E. and DANCETTE,C. 1978. Analysis of fallow farming systems in semi arid Africa using a mode1 to simulate the hydrologic budget. Agron. J. 70:816-823.

HENCKEL,P.A. 1964. Physiology of plants under drought. Ann. Rev. of Plant Physiology 15:363-386

KANEMASU,E.T., THURTELL,G. W. and TANNER,C.B. 1969. Design, calibration and field use of a stomatal diffusion porometer. Plant Physiol. 44:881-885.

LANG,A.R.G. 1967. Osmotic coefficients and water potentials of sodium chloride solutions from 0 to 40°C. Aust. J. Chem.20:2017-2023.

MULLA,D.J. 1987. Measurement and characterization of soil water relationships. In Soil, Crop and Water Management in the Sudan-Sahelian Zone. A publication of the International Crops Research Institute for the Semi Arid Tropics (ICRISAT) pp75-83.

TURK,K.J., HALL,A.E. and ASBELL,C.W. 1980. Drought adaptation of cowpeas. 1. Influence of drought on seed yield. Agron. J. 72:413-420.