Centre d'Etude Régional pour
l'Amélioration de l'Adaptation à la
Sécheresse

CERAAS

TRAINING REPORT UN THE PHYSIOLOGY OF DROUGHT TOLERANCE IN GROUNDNUT : Physiological techniques

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INFORMATION ABOUT C.E.R.A.A.S.

Centre d'Etude Régional pour l'Amélioration de l'Adaptation à la Sécheresse (C.E.R.A.A.S.) is a national laboratory of Institut Sénégalais de Recherches Agricoles (I.S.R.A.) based at Bambey. It is the basic component of a scientific network which contributed to generate a scientific community shared by European, African and South American scientists working together with a goal to stabilize yield production of annual crops grown in the semi-arid zones. C.E.R.A.A.S. was created in 1983 from a new ISRA programme based on plant adaptation to drought

OBJECTIVES

The objective of C.E.R.A.A.S. is to improve crop production in semi-arid zones through creation of cultivars well adapted to rainfall conditions without more investment from farmers. The programme is based on a multidisciplinary approach involving physiology, selection, agroclimatology and agronomy.

CLIMATIC CONDITIONS

Longitude : 17 ° W, Latitude : 14 ° N

Climatic condition	: semi arid (sudaneese sahelian)
Raining season	: end of june till end of september
Total rainfall	: 350 - 400 mm
Temperature	: between 35°C and 40 °C
Dry hot season	: march to june.

I - INTRODUCTION

Drought tolerance has been defined in relation to the level of stress that kills 50 % of the cells. The level of drought stress has been determined by the water content or water potential of plants, or the relative humidity of air equilibrium with plants (Levitt, 1972). Drought tolerance at different stages of development may also depend on previous and subsequent environmental conditions.

Climate plays an important role in determining which crop species and cultivars can be grown in an area and also determines the resultant yields. Successful crop production requires efficient use of the climatic resources of solar radiation, carbon dioxide and water. The phenology and rate of development of a crop depends upon climatic factors such as temperature, day length and water supply

Peanut (*Arachis hypogea* L.) genotypes have been identified that differ in their adaptation to drought (Gautreau and al, 1980). The genotypes best adapted to dry zones of Senegal had the lowest leaf water potential. Also, observations suggest that Spanish types are more tolerant to drought than Virginia types under severe water stress and high evaporation demand conditions.

C E.R.A.A.S. gives breeders and physioiogists working in the semi-arid countries access to infrastructures to study the physiology of their vegetal materials and conduct programmes for genetic improvement of adaptation to drought.

The objective of this training was to familiarize me with the techniques used in plant physiology and how ta use both the field and laboratory equipments, and aise to reenforce the existing cooperation between Botswana and Sénégal in the field of groundnut research.

II - DIFFERENT TECHNICS USED FOR LEAF WATER POTENTIAL AND GAS EXCHANGE MEASUREMENTS

It is important to know the amount of water available in the soil and its relation with plant water status and gas exchange (e.g. transpiration). In a groundnut trial, conducted under fieid conditions, three treatments were compared: well watered, stressed and rainfed. Moisture tubes (270 cm long) were installed in each plot, and measurements were made once a week at every 10 cm until maximum depth, using a moisture gauge type 4300 Troxler.

With the data collected, the result are plotted, moisture by depth. The intersection of two successive curves gives the maximum depth where water is used up by the plants The difference between the two readings is due to water uptake by the plants if there is no rain or irrigation between the measurement dates

The well watered treatment gives the maximum evapotranspiration (ETR), stressed shows how drought can influence evapotranspiration. Since water content of the soil is known, it is interesting to know how water content can influence peanwt transpiration by measuring the stomatal resistance and conductance (because the stomata are responsible for gas exchange) with a porometer.

II - 1- Stomatal resistance and conductance

Porometer allows the study of gas diffusion through pores particularly through leaf stomata. Since plant transpiration is mainly controlled by the openrng and closing of stomata, the use of porometer is fundamental to many areas of plant research. Stomatal aperture controls the water loss from plant leaves and the uptake of CO_2 for photosynthesis. It is an important indioator of the physiological condition to environmental factors.

Measuring was done with a delta-T automatic porometer (MK ii Cambridge England). The porometer has :

- Pump - it pumps air through the crystal
- Crystals - dry the air from the pump

Leaf chamber : - measures humidity of the air in the chamber, also that of the leaf which will be reflected by increase due to transpiration. - also records the temperature of both the cup (leaf chamber) and the leaf.

The porometer is calibrated before and after each measurement according to standard procedures (Kanemasu, Thurtell and Tanner, 1969). For calibration, an acrylic plate which had II, 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 water, with a strip of adhesive paper on one side, was sealed over the lower side of the acrylic plate, 4 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 value.

Measurement were taken on the upper (Rsl) and lower (Rs2) sides of the third leaf of the main stem, since there is variation in stomata number. With the data collected, total resistance of the leaf was calculated with the equation :

$$\frac{1}{Rs1} + \frac{1}{Rs2} = \frac{1}{Rs} \Rightarrow Rs = \text{Resistance (Inverse } 1/Rs \text{ is conductance)}$$

If the air humidity in the cup is equal to 5 % of the first humidity, the pump will stop, also if there is less transpiration the porometer will not stop reading

If the resistance is higher (more than 1) it shows that the peanut plant is transpiring less If the conductance is equal ta zero (0) the stomata are fully closed

The interest here is to see at which moisture level transpiration is influenced

II - 2 Leaf water potential measurements

Water potentiaî was measured with a Peltier cooled thermocouple psychometer microvcrltmeter. Before using the psychrometer is calibrated with salt solutions of known concentration (four or six salt solutions). The linear regression (y = b + ax) derived from the individual curves is used to calculate the water potentsal of each sample.

When sampling the third leaf from the main stem was collected. The leaf was rolled and quickly placed in the sample compartment of the psychrometer an d left to stand undisturbed (for equilibration) on a laboratory bench for 3 hours after which the psychrometer microvoltmeter was used to read the microvolt produced by the sample. Then the water potential was calculated.

The principle of the psychrometry is based on the measurement of the depression of the air humidiy in the leaf chamber. The thermocouple measures the temperature in the chamber, also the potential difference (DDP) in the extremis (+ or -) of the thermocouple. Since the DDP and temperature are known. the calibration curve of the psychrometer is used to convert the DDP to water potential

It was observed that well watered peanut had high water potential (- 6 to - 12 bars). Stressed plants have a reduced water potential (-6 to - 50 bars).

II - 3 Relative water content

In order to know the water status of the plant, water potential and relative water content (RWC) are measured. The sample (leaf discs) were collected from the third leaf of the main stem with a punch or pasty cutter.

When collecting the sample the leaf discs were punched, quickly placed in a small battle (whose initial weight was determined) and quickly replacing the stopper, In the laboratory the fresh weight (FW) of the leaf discs was determined, and distilled water was added to discs so that they should absorb maximum possible water for 3 hours, after which they was carefully blotted between folds of filter papes, Then quickly the blotted leaf discs placed between a fold of parafilm paper whose weight was known or zeroed, and the turgescent weight (TW) was then determined. The leaf discs were dried at 85°C in the oven for 48 h and dry weight (DW) was determined. Then relative water content (%) was calculated from

$$RWC = \frac{FW-DW}{TW-DW} \times 100$$

* FW - DW = quantity of water in the leaf (leaf disc) from the field.
* TW - DW = maximum water the leaf (leaf disc) can absorb.

- When there is enough moisture, RWC can vary from 88 % to 99%
- When plants are stressed, RWC can fall to 30 %.
- Below 30 % RWC, the crop will die.

NB : For all the test, i.e. stomatal resistance and conductance, water potential and relative water content, samples were collected from one trial, closer to the moisture tube, so that correlation should be made with soil moisture content.

III - PROTOPLASMIC RESISTANCE TO HEAT AND DESSICATION

This test was used to determine the temperature at which 50 % damage was done to the protoplasm. Leaf samples (third true leaf) were collected from three cotton varieties grown under uniform field conditions. Samples collected were immediately placed inside a plastic bag (moistened with distilled water to reduce excess water loss). The interest was not on the variety but the species, and -amples of the 3 varieties were bulked together.

From each leaf, 10 leaf discs were punched with a puncher or pasty cutter, then floated in distilled water for about 30 minutes in petridishes, then blotted between folds of paper towelling and placed in test tubes. The test tubes with discs were placed in a water heater with temperatures ranging from 35, 40, 45, 50, 55, 60, 65, and 70°C and the other four test tubes were kept at room temperature to serve as the control with 30 ml of distilled water added. All test tubes were closed with parafilm paper.

Another set of leaf discs were placed in different concentrations of polythethyleneglycol 600 (PEG 600) as follows 50, 100, 150, 200, 250, 300, 350 and 400g/l and four controls with distilled water.

It shoulcl be remembered that, for every treatment, there were two replicates with four leaf discs from each variety which makes twelve leaf discs in each tube.

The leaf discs for the temperature treatment were subjected to various emperatures as indicated for 1 hour then 30 ml of distilled water was added in each tube and tubes were left for 24 hours at 5 °C in a refrigerator. The solution was left to cool down at room temperature for about 20 minutes and free conductivity (FC) was measured with a micro computer conductometer (consort K 220). The same solution together with the control was boiled at 100°C for 1 hour and cooled down to room temperature. It was left in the refrigerator for 24 hours and total conductivity (TC) was measured.

Leaf discs whiçh were left for 24 hours in the PEG solution were rinsed several times with distilled water, blotted between paper towelling and 30 ml of distilled water were added in the test tubes and left for 24 hours at 5 °C in the refrigerator. FC of the solution was determined. The same solution with leaf discs and the control were boiled at 100 °C for 1 hour, cooled down to room temperature, placed in the refrigerator for 24 hours and TC was measured.

Using the values obtained from for free oonductivity and total conductivrty. the following parameters were calculated.

Percentage Absolute integrity : $PIa = [1 - \frac{FC}{TC}] \times 100$

Percentage Relative integrity : $PIr = [\frac{PIa\ treatment}{PIa\ control}] \times 100$

Percentage of Relative damage : PD = 100 - PIr.

IV ROOT PARAMETERS STUDIES USING RHIZOTRONS

Oil palm plants were grown in tubes consisting of cylindrical PVC tubes measuring 100 cm by 15.5 cm. Each tube had one side flattened out by cutting off and a flat transparent PVC material (100 cm by 13 cm) was used to seal tne cut surface This transparent material (PVC) allowed visual observation and measurement of roots development. The bottom part of the tube was sealed with a flat PVC material with 5 holes (each 10 mm in diameter) to drain off excess water.

There was a layer of gravel at the bottom, then filled with different top soils Seeds were sown 2 cm from the transparent flat PVC. Each tube was encaseo in an easily removable black polythene casing, to reduce excessive heat to the roots Tubes were placed in an inclined position.

The following parameters were measured, roots length, density and numbers of roots within each horizon (each tube was marked out in 20 cm horizons, giving a total of 5 horizons/100 cm tube) and roots measurements were taken from the main root and two other long roots Part of the twenty tubes under study were stressed at 78 days after sowing by stopping irrigation.

The put-pose of this stwdy was to see how oil palm roots will behave in different soils, and also how it respond to water stress.

V UTILIZATION OF CEPTOMETER TO DETERMINE % SOIL COVER AND LAI

The ceptometer consist of a long light-sensitive probe with 80 photodiode cells) sensors. Reading of the average light level over the whole probe length, or of a single photodiode at the tip of the probe may be selected A short probe version s also avaiiable.

A ceptometer can be used to estimate canopy structure parameters such as leaf area index, ground cover, gap fraction and leaf angle distribution, In our case, it was used to estimate leaf area index of a groundnut crop. Calibration is derived from comparison with a standard PAR Quantum Sensor in a natural day light conditions. When the probe is not under the plants or in operation it reads 100 %.

When collecting data, the light sensitive probe is placed between two plants to intercept tight rays through the plant canopy. Readings can be taken five (5) times or more, then the average is determined and stored in the memory.

Samples were collected from a groundnut trial with moisture tubes installed in order to determine correlation between soil moisture content and plant development At the time of measurement and within the same plots, two plants groundnut) were collected for area determination with a leaf area meter.

If the data collected with a ceptometer correlate with that of leaf area meter, it will be always easy to calculate leaf area for the plants and leaf area index from the percent soil covered. The advantage of a ceptometer is that crops are not destroyed whereas with leaf area meter crop samples are collected to the laboratory

VI - RESULTS AND OBSERVATIONS

Stomatal conductance : water stress sharply decreased stomatal conductance (from the second day of stress) of stressed and rainfed plots. The decrease went on until the stomata were fully closed from the ninth day for both rainfed and stressed plots The well watered plots had high conductance ranging from 0,3 to 0,8 cm/s.

Water potential there was no difference between the treatments from the first day up to the ninth day. From the tenth day of stress, there was uniform decrease in water potential for rainfed and stressed plots (-15 to -62 bars). Well watered plots maintained a high water potential though it was erratic.

Relative water content during the first three days of stress, RWC was not highly reduced, but as water deficit progressed, there was a great decrease in RWC for both stressed and rainfed plots (around 23 %). Generally well watered plots maintained high RWC between 85 - 91 % throughout.

Protoplasmic resistance : with the data collected, it was observed that for cotton, the 50 % relative damage done to the protopfasm was obtained at 43°C It was low as compared to that of groundnut, which was 53°C, reported by Annerose D. and Cornaire B., 1991.

Rhizotron although the data were not statistically analysed, with visual observations from the values obtained during measurements thera was no difference in roots tength and density in all the treatments.

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Table 1 : Stomatal conductance of groundnut at 13 : 00 (cm/s).

DAS	Well Watered	Rainfed	Stressed	CV
1	0.506 a	0.320 a	0.314 a	9.84
2	0.409 a	0.113 a	0.120 a	66.32
3	0.505 a	0.094 a	0.083 a	36.74
4	0.678 a	0.109 b	0.096 b	27.63
8	0.304 a	0.089 b	0.084 b	21 01
9	0.404 a	0.000 b	0.000 b	15.14
11	0.440 a	0.000 b	0.000 b	29.98
12	0.522 a	0.000 b	0.000 b	17.36
13	0.519 a	0.000 b	0.000 b	25.45
15	0.461 a	0.000 b	0.000 b	14.50
25	0.580 a	0.000 b	0.000 b	26.20
31	0.391 a	0.000 b	0.000 b	10.49

DAS Days After Stress

CV : Coefficient of Variation (%)

Values in the same line with different letters are significantly different at 1%