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

Electrolyte leakage and fatty acid profile as indices of membrane integrity in groundnut (*Arachis hypogaea* L.) subjected to water stress

TECHNICAL REPORT

Marrh 1 to May 27, 1999

Dr. Emmanuel Akubugwo
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TABLE DES MATIERES

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SUMMAKY

Membrane integrity of the leaves of three groundnut cultivars (Fleur1 1, GC-85 and 57-422) subjected to varying levels of water stress was assessed using electrolyte leakage and changes in fatty acid profile as indices. The effect of the treatments on leaf dry matter was also studied. To date, studies on the leaf membrane electrolyte leakage as well as dry matter have heen completed while studies on the fatty acid is still in progress. Data obtained from the completed studies have been statistically analysed. Results indicate that the absolute integrity of the leaf membranes were perturbed by the treatments. Membrane damage was evident and increased in magnitude in the different cultivars with increase in the severity of the applied drought. Significant variety and water regime effects were observed in the electrolyte leakage. In the dry matter however, there were no variety effect, rather strong water regime effects were observed. In all the data analysed so fat-, there were no block effects.

INTRODUCTION

Groundnut is an important food and cash crop in many tropical and sub-tropical countries including Nigeria and Senegal. Like other crops, drought is a major limiting factor in its growth and production. Classical breeding techniques have been employed by breeders to select drought resistant cultivar to counter the adverse effect of the incessant water deficit problems prevalent in these regions. These techniques, though effective are time consutning There is therefore, need to develop rapid and effective methods of screening of plants for drought resistance. It is generally accepted that biochemical methods offer a good alternative. It is known that when plants are subjected to water deficit, they respond through alterations in their biochemical make-up. These changes when properly characterised are veritable tools for screening of plants for drought tolerance. Such changes of interest have been abserved in the membranes.

The fluidity of membranes (which is essential for it to function under variable conditions) is governed by the chemical composition of the membrane. Drought destroys cell membranes especrally those of chloroplasts and mitochondria (Vieira da Silver et al., 1974; Pham Thi and Vieira da Silva, 1975). When the integrity of the membrane is compromised by dehydration (as occurs in drought), electrolytes leak out of the cell. This leakage can be assessed by measuring the conductivity of the solution in which they are kept (Sullivan,1971). There exists a direct relationship between the ability of membranes to maintain fluidity and the nature of their lipid composition. It is therefore expedient that the lipid content and fatty acid composition of the leaves of plants be properly characterised especially under water stress conditions. This is so because of the critical role cell membrane stability plays in drought tolerance.

Protoplasmic tolerance involve mechanisms which include osmoregulation, control of enzymatic activities as well as control of re-arrangements in membrane structures (Losch, 1986; Turner and Jones, 1980). Reports (Ferrari-lliou et *al.*, 1984 ; Pham Thi et *al.*, 1985 ; Quartacci and Navari-lzzo, 1992) indicate that many annual plants respond to drought stress by changing the polar lipid composition of their leaf cell membranes (Repellin et *al.*,1997), reported similar changes in the leaf membrane of young coconut palms subjected to water stress. These changes present in the form of reduction in the polar lipid content especially the glycolipids. Another feature of these modifications is decreases in the poly unsaturated fatty acid content. This effect is more pronounced in the monogalactoctosyl diacylglycerol (MGDG) fraction, than in the other polar lipid types. These modifications are products of the inter play of three events which simultaneously occur in the plant during water deficit, Viz.: 1/.Increased lipolytic activities (El-Hafid et *al.*,1989). 2/ Inhibition of lipid synthesis (Pham Thi ef *a/.*, 1985,1987), and 3/ Enhanced production of scavenging free radicals (Ferrai-lliou *et al.*, *1992).*

Available reports indicate a good correlation between the extent of modification of the polar lipids composition of the leaf membranes and the abilities of the different cultivars within a species to tolerate and adapt to water deficit (Pham Thi et a/. ,1990). Leaf membrane polar lipid characteristics is considered a good index for the evaluation of protoplasmic tolerance to drought stress within a plant species (Hubac et *al.*, 1989 ; Monteiro de Paula et a/. ,1990 ; Pham Thi *et* a/.,1990 ; Chen and Liu, 1991) and could be used by breeders as a screening test for drought tolerance. The present investigation aims at correlating membrane integrrty as assessed by electrolyte leakage with a parallel assessment using changes in the fatty acid profile in three peanut cultivars subjected to varying levels of water stress in the green house.

MATERIALS AND METHODS

Seed collection

Certified seeds of three peanut cultivars viz. -57-422, Fleur-l 1, and GC8-35 were obtained from CNRA/ISRA in Bambey Senegal. They were chosen because agronomically they are known to have good drought resistance capacity.

Growth Conditions

Sandy soil was used as the potting medium. The soil characteristics were as previously described by Annerose (1990). The soil (6.3kg) were packed in plastic pots of volume 3.51 (diam =15cm, ht. =20cm) with drainage holes at the bottom. The seeds were treated with the fungicrde Granox (Captafol 10%, Benomyl 10% and Carbofuran 20%) to prevent fungal attack After irrigation to field capacity, three seeds were sown per pot at a soil depth of 3 cm. Ten days after planting, the plants were thinned down to one plant per pot to obtain plants of uniform growth vigour. The soil was fertilised with N.P.K. (8-18-27) at the rate of 150kg/ha to remove nutrient deficiency as a limiting factor. The plants were kept in the green house at CERAAS, Thiès, Senegal, (longitude 14°42' ,latitude 16'57').

Soil Moisture Treatment

Four water regimes were chosen after a preliminary experiment. They are-

1 .Field capacity (full irrigation).

2.Low water stress (-1.25 to -1.54 MPa).

3.Medium water stress (-2.31 to -2.52 MPa).

4.Severe water stress (s-3.15 MPa).

Experimental Design and Statistical Analysis

Block randomised design with three replicates and four watering regime was employed. A total of 144 pots in three experimental blocks separated by inter block distances of 70cm were used. Within a block, there were twelve (12) plots. One plot is made up of four pots and constitute an experimental unit.

Measurements

All measurements were made on the third leaf counting from the stem apex during the vegetative/early flowering stages of the plant. Eleven hours after the choice of a particular stress level from the preliminary experiment was made, studies on the following parameters were performed.

Physiological Measurements

1. ***Leaf waterpotential (ψ, MPa)***

This was determined in the green house using hydraulic pressure (Campbell JIG) in the apicai part of the leaf. Measurements were taken at a fixed hour in the morning to avoid variations in water potential associated with time of the day.

2. ***Leaf dry matter determination***

Fresh leaves from the plants were weighed and kept in the oven at 80" C for 48h, re-weighed and the difference in weight noted.

3. ***Membrane electrolyte leakage***

Membrane integrity was assessed at the various water potentials using electrolyte leakage method of Ames,(1983).

Biochemical Analysis

1. ***Extraction of membrane lipids***

The Rarvested second developed leaves were first fixed in boiling water for 2-5 minutes. Total lipids were extracted in chloroform: methanol : water (1:1:1,v/v/v) according to the method of Allen and Good, (1971). The lipid extracts were dried under a stream of nitrogen and stored in ethanol : toluene mixture(1:4 v/v) in the refrigerator till when required for analysis.

2. ***Separation of the lipids into various classes by Thin Layer chromatography (TLC)***

This was performed according to the procedure described by Lepage, (1967)

3. ***Saponification and transmethylation of Lipids***

Both the total lipid and the various lipid classes obtained after TLC was effected by acid catalysed transesterification of the saponified lipid.

4. ***Analysis of the methylesters of the fatty acids by GLC***

The Varian GLC system equipped with flame ionisation detector (FID) was used with C- 17 as the internal standard while Helium was the carrier gas. The flow rate was 2ml/min while column temperature was maintained at 200°C.

RESULTS

The effect of water stress on the absolute integrity of the leaf membranes of the studied groundnut cultivars are shown in Table 1 a. In each cultivar, the effect was most pronounced in the severe stress. GC5-35 was the most resistant while Fleur-l 1 was the least resrstant. This picture is shown clearer by the percent membrane damage in Table 1 b.

The nature of the interactions of the water stress on the electrolyte leakage in the different cultivars is shown in Table 1 c. There are significant ($P<0.05$) differences between the amount of electrolyte leakage in the control (Field capacity) and the values obtained in the various levels of applied stress. However, the difference of the effects of low and moderate stress were not significant ($P>0.05$). This Table also shows that amongst the varieties, the only significant ($P<0.05$) interaction occurred in Fleur-l 1 relative to GC8-35 only.

Table Ia. Absolute membrane integrity in three groundnut (*Arachis* hypogaea L) cultivars subjected to different levels of water stress.

	Percentage membrane integrity Fleur- 11	GC8-35	57-422
Field capacity	88.17a	87.02a	88.17a
Low stress	74.07b	83.55b	76.70b
Medium stress	51.21b	77.57b	75.04b
Severe stress	42.15c	66.78c	58.31c

In each column, means followed by the same letters are not significantly (P<0.05). different according to Duncan's multiple range test.

Table 1 b. Percentage membrane damage associated with water stress levers in three groundnut *(A.* hypogaea L.) cultivars.

	Percent membrane damage Fleur- 11	GC8-35	57-422
Low stress	12.71	3.55	8.63
Medium stress	16.78	10.48	9.78
Severe stress	38.85	22.88	30.56

Table Ic. Nature of interactions (variety effects) within various water regimes observed in the membrane integrity studies using electrolyte leakage method.

	Field capacity	Low stress	Moderate	Severe stress
Fleur- 11	88.17 a	74.07a	51.21a	42.15a
GC8-35	87.02 a	83.55b	77.57b	66.78b
57-422	88.17a	76.70ab	75.04ab	58.31ab

In each column. means followed by the same letters are not significantly (P<0.05). different according to Duncan's multiple range test.

The effect of the water stress on biomass accumulation in the groundnut leaves is shown in Table 2. Biomass (dry matter) calculated on wet weight weight basis increased in the leaves with increased intensity of water deficit. GC8-35 had the highest dry matter. However. the dry matter content of 57-422 was slightly grater than that of Fleur-II. There are significant ($P<0.05$) differences between the dry matter content of the control (Field capacity) and those of the various levels of applied stress. However, the difference of the effects of low and moderate stress were not significant ($P>0.05$). The interactions observed among the varieties were very complex.

Table 2. Leaf dry matter changes associated with water stress levels in three peanut (A. *hypogea* L.) cultivars.

	Leaf dry matter (g/100g wet weiaht).		
	Fleur- 11	GC8-35	57-422
Field capacity	17.54	24.55	30.67
Low stress	38.71	33.14	35.09
Moderate stress	46.27	54.3 1	51.25
Severe stress	61.01	66.48	59.36

DISCUSSION

Our projections in this study are 1. Application of stress will cause membrane damage whose severity will increase with increased level of water deficit. 2. The membrane damage will vary amongst the different cultivars depending on their drought resistance capacities and 3. The pattern of the observed changes in 1 and 2 above will be the same both in the electrolyte leakage and in the fatty acid profile studies.

The results obtained showed that absolute integrity of the leaf membrane was compromised by the application of water stress. The intensity of this compromise was heightened by the severity of the stress.(Table la.). This result is in deed amplified by the percentage membrane damage (Table 1 b.). Various workers have shown that water stress results in membrane damage (Vieira da Silver et *al.*,1974; Pham Thi and Vieira da Silva, 1975).It is also accepted that measurement of the conductivity of the medium in which the damaged membranes are kept will give a true picture of the extent of the damage (Sullivan,1971).Our results are consistent with these earlier observations and also with our projection. Based on the result so far analysed, the water deficit tolerance capacity of the three cultivars is in the order Fleur-I 1> 57-422> GC8-35. If the result from the fatty acid profile studies (which is in progress) corroborates this finding, our projections in this study would have been fully actualised.

PROBLEMS

The major problem we had in prosecuting this project was that of adapting the new equipment which CERAAS recently acquired to the local environment. A number of the methodologies also needed to be modified. Much success have been achieved in this regard. With the level of commitment shown by the staff on the ground, these issues will soon be effectively completely resolved.

PERSPECTIVES

Based an the facilities available in CERAAS for lipid research, I have the following perspectives-

1. That the centre be used to train Regional expert in the use of lipid techniques in drought research.
2. That special training workshop on lipid study techniques be mounted soon for the staff on the ground ta enable them make effective use of the ultra modern equipment in CERAAS.

ON-GOINC EXPERIMENT

During my stay at CERAAS, I also participated in an on going experiment titled "Root growrh of cowpea genotypes in hydroponics, aeroponic and under different soil moisture conditions in the green house". Data is currently being generated from the study.

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