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DE L'ADAPTATION A
LA SECHERESSE,.

Evaluation of Membrane Integrity

Electrolyte and Inorganîc Phosphate leakage methods

ISRA - CNRA
BAMBEY SENEGAL
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INTRODUCTION

Alteration of the plant membrane complex has heen considered as a major consequence of drougkt (Iljin. 1957 ; Stocker, 1961).Several workers (Vieira da Silva *et al.*, 1974 ; Pham Thi and Vieira da Silva, 1975) have also shown that cell membranes, in particular those of chloroplasts and mitochondria, are destroyed during drought.This leads to cellular decompartmentation, resulting in solubilisation of enzymes. notably the hydrolases (Vieira da Silva, 1970 ; Todd, 1972 Adjahossou, 1983 ; Jacobsen *et al.*, 1986). Certain products of these hydrolytic enzymes have been observed to inhibit photochemical reactions (Pham Thi and Vieira da Silva, 1976). Lipid and protein composition is also modified during drought (.Pham Thi *et al.* 1982 ; Zuily- Fodil *et al.*, 1990). This modification, which has been observed to be more pronounced in sensitive varieties, may be due to acyl hydrolase and galactolipase activities in the case of lipids and to proteolytic activities in the case of proteins (Pham Thi et *al.*, 1987 ; El-Hafid et *al.*, 1989 ; Zuily-Fodil *et al.*. 1990 ; Roy-Macauley *et al.*, 1993).

These results show that evaluation of membrane integrity in plants under stress conditions is a necessary approach to studying their capacity to tolerate drought. Also, membranes are important living systems involved in cellular compartmentation and exchange with the external environmenr.. Evaluation of the modifications which they are subjected to under stress conditions constitutes a realistic approach to the study of tolerance mechanisms.

Evaluation of membrane integrity by electrolyte and inorganic phosphate leakage

Disorganisation of membranes when tissues are dehydrated is manifested mainly by an increase in their permeability. Under these conditions. electrolytes leak out from cells and its importance could be evaluated by measuring the conductivity of the solution in which they are placed (Sullivan, 1971). Assay of electrolytes leaking out from cells could therefore be used as method in screening for drought tolerance. In this case the integrity of the cell membranes in general is evaluated.

Vieira da Silva (1970) showed that phosphatase acid activity increases in plants subjected to drought. This increase was observed to be higher in varieties sensitive to drought. Adjahossou (1983) working on oil palm showed that drought causes an increase in catalase, phosphatase and invertase acid activities These workers proposed the method of assaying phosphatase acid or inorganic phosphate, a product of its activity, in screening for drought tolerance. In this case, the integrity of chloroplastic membranes in particular is evaluated. In addition this method would complement that of electrolyte leakage.

When membrane integrity of five peanut varieties were evaluated by both electrolyte and inorganic phosphate methods, the results obtained indicated that these varieties presented. for each of these methods the sarne classification (Annerose, 1990). The damage evaluated by inorganic phosphate leakage was lower than that obtained by electrolyte leakage. In addition, the differentiation between varieties seemed to be less precise in the case of inorganic phosphate leakage. Roy-Macauley (1995) evaluated membrane damage in 9 varieties of *Vigna* and grouped them into 3 and 4 groups respectively, using the inorganic phosphate and electrolyte leakage methods. Although the classifications were based on the study of two different methods, a good correlation was observed for certain species. For the rest, the modification went always in the direction of an increase in chloroplastic membrane resistance compared to general membrane resistance.

Normally drought is induced in the laboratory by osmotic shock. However. the varietal difference observed in drought tolerance of the membranes shown by these techniques, does not necessarily reflect the reaction of the plant to dehydration of its tissues under natural conditions. The rate at which stress is applied probably does not allow the plant to express all of its adaptative potentiality. Blum and Ehercon (198 1) and Blum (1984). working on wheat, barley and sorghum. observed an increase in membrane tolerance to dessication of stressed plants. The importance of this phenomenon could thus modifiy the comparison between varieties.

Determination of the osmotic potential of the treatment solution

An osmotic potential which allows significant differentiation of the varieties should be determined. Leaf discs are floated on PEG 600 solution of different osmotic potentials. When membrane damage. evaluated by thc measurement of electrolyte leakage, was plotted against osmotic potential, a sigmotd curve was obtained (Fig. 1). In the case of three varieties of peanut for example (Annerose, 1990), membrane integrity of leaf discs was altered by treatment with PEG solutions with osmotic potentials in the range of -1,35 to -3,5 Mpa.

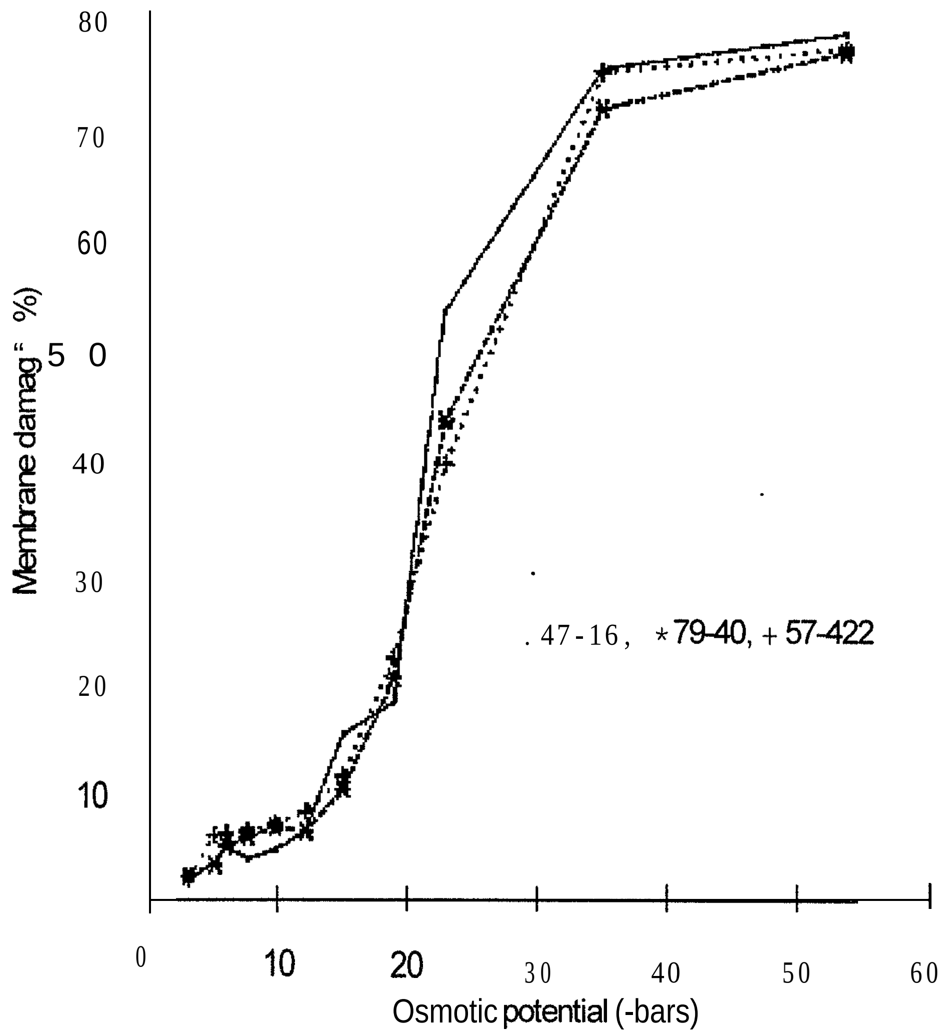

Fig. 1. Effect of induced drought on membrane integrity of 3 peanut varieties. 47-16, 79-40 and 57-422. Membrane integrity, expressed as % membrane damage, was evaluated by conductivity measurement of the water solution in which leaf discs which had heen subjected to induced drought by PEG solutions of differcnt osmotic potentials for 24 hr. were put (Annerose, 1990).

The best differentiation between varieties was obtained for solutions of osmotic potential between -2.0 and -3.2 MPa. Membrane damage was between 32 and 72 %. The theoretical point of inflexion, corresponding to SO % damage, is considered as th.e best point of differentiation between varieties. The osmotic potential of the corresponding solution, which is -2.45 MPa was used in further studies.

Choice of leaf to be used in the study

Annerose (1990) working on peanut observed that membrane damage between varieties varied significantly with the rank of the leaf (Fig. 2). Counting from the stem apex of the plant, leaves of intermediate rank, 3-4 and S-6, are less affected than leaves of rank 1-2 and 7-8. The youngest leaves corresponding to rank 1-2 showed the highest membrane damage. The capacity to maintain membrane integrity depends, therefore, on the physiological age of the leaf.

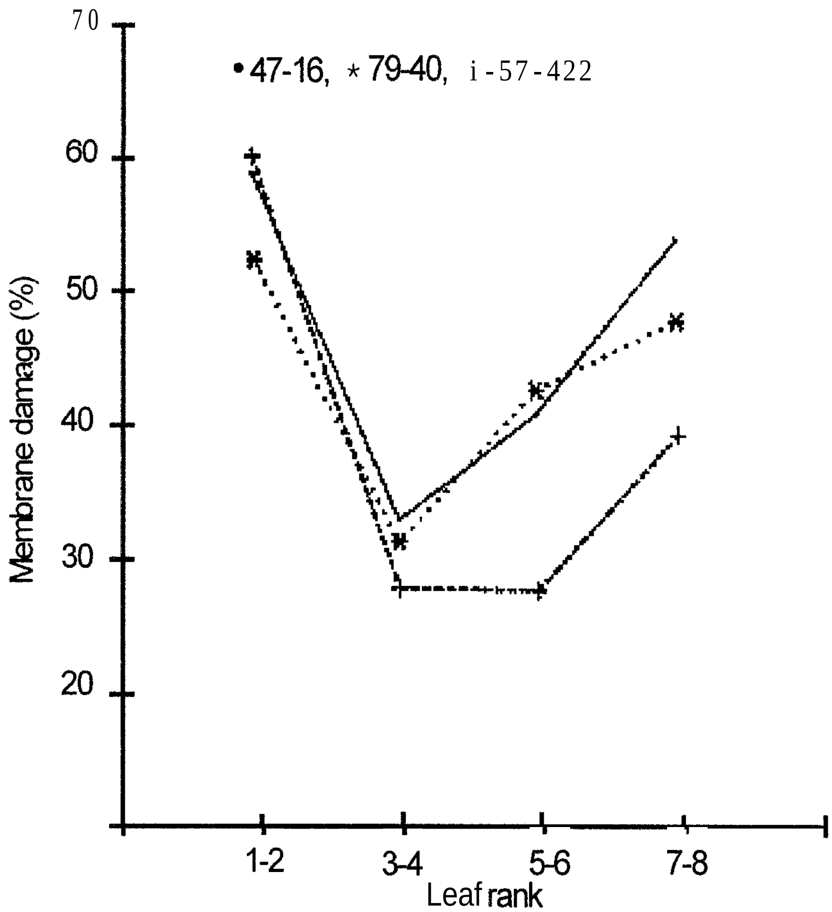

Fig. 2: Membranes integrity of leaves of different physiological age of 3 peanut varieties. Membrane integrity, expressed as % membrane damage, was evaluated by conductivity measurement of the water solution in which leaf discs which had heen subjected to induced drought by PEG solutions of -2.15 MPa for 24 hr, were put. The lowest rank corresponds to the youngest leaves (Annerose, 1990).

In the case of leguminous plants. this corresponds normally to the third leaf counting from the top of thc main branch. Despite the fact that leaves of ranks 1-2, 5-6 and 7-8 might be showing the highest membrane damage, it is preferable to work on leaves of rank 3-4 comprising the youngest most developed leaf which has nor started scnescing. Physiological studies are usually conducted on these leaves.

Effect of the age of the plant

Older plants have a higher capacity of maintaining membrane integrity of their tissues (Annerose, 1990). This author observed that membrane damage decreased from 43,8 % for plants studied 21 days after sowing to 14,2 % for plants studied 71 days after sowing in peanuts. The classification of the varieties was the same but the difference in membrane damage, observed for plants studied 71 days after sowing seemed to be lower than that obtained 21 days after sowing (Table 1).

Table 1. Evaluation of the membrane integrity in 9 varieties of peanut from plants 21 and 71 days after sowing, by the method of electrolyte assay. Leaf discs were treated with PEG solution of -2.45 M Pa for 24 hr. Membrane integrity was expressed as % membrane damage.

Variety of Peanut	21 days after sowing	71 days after sowing
79-40	20.32 ± 2.32 a	6.33 ± 2.71 a
57-422	26.00 ± 5.69 a	3.48 ± 0.81 a
47-16	33.25 ± 1.95 b	8.15 ± 0.99 b
73-30	41.55 ± 5.68 c	18.21 ± 2.68 c
69.101	43.84 ± 1.69 c	15.28 16.74 c
PI-I 174	51.81 ± 5.18 d	15.88 ± 5.74 c
KH 149A	55.13 ± 2.60 d	18.76 ± 4.86c
55-437	56.03 ± 3.20 d	17.30 ± 3.96 c
CHICO	66.48 ± 3.72 e	24.36 ±4.86 d

Values followed by different letters indicate significant differences at P = 5 %.

METHODOLOGY

Preparation of PEG 600 solutions of different osmotic potential

- Weigh x g of the concentrated PEG 600 solution
- Transfer to a 1 L measuring flask and complete with distilled water (Table 2.).
- Verify the osmotic potential ofeach solution by osmometry.

Table 2 Concentration of PEG (g/l) and the corresponding osmotic potential (Lang, 1967).

PEG solution (g/l)	Osmotic potential (-MPa)
50	0.466
75	0.744
100	1.216
12.5	1.476
150	1.913
175	2.256
200	2.838
225	3.360
250	3.902
275	4.908
300	5.901
325	7.091

Sampling of leaf discs

- Cut the leaves to be sampled using a pair of scissors.
- Put them into a plastic bag containing enough distilled water to keep the leaves humid and close the plastic bag.
- Cut out leafdiscs with a 1 cm diameter cork borer and put them in distilled water in Petri dishes.
- Rinse them twice for a period of 15 min
- Wipe the discs rapidly with Whatman paper without syueezing them.

Osmotic shock

- Put 10 discs destined to be treated in 30 ml of PEG solution in a Petri dish.
- Put 10 discs destined to be the control in distilled water also in Petri dishes.
- Leave the Petri dishes on the laboratory work bench for 24 hours with occasional shaking.
- Rinse them three times with distilled water for a period of 5 min.
- Rapidly wipe the discs as already described.
- Put 10 discs in test tubes containing 30 ml of water.
- Put the tubes in a refrigerator for 24 h.
- Shake the test tubes occasionaly.
- Leave the tubes so that the solution returns to room temperature and measure the free conductivity (FC') or free inorganic phosphate (FIP by the method of Ames, 1966) .
- Put the tubes in a boiling water bath for 1 h in order to destroy all the cells.
- Leave the tubes to cool down and put in a refrigerator for 16 hours.
- Leave the tubes so that the solution returns to room temperature and then measure the total conductivity (TC) or total inorganic phosphate (TIP).

Calculation of membrane damage

Absolute integrity (A. 1.) = (1 - CL/CT) x 100.

Relative Integrity (R. I.) = AI treated discs/AI control discs

Membrane damage = 100 - RI.

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DROUGHT RESISTANCE OF FIVE GROUNDNUT (*ARACHZS HYPO*(GENOTYPES DURING GERMINATION AND EARLY SEEDLING STAGE

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Introduction

Rainfed production of groundnut in Senegal has been dwindling in recent years as a (low and irregular pattern of rainfall. Seeds sown after the first rains, more often than an acute water shortage resulting from a dry spell early in the season. This affects ger seedling emergencc, uniform stand developmcnt, growth and final yield. On the other where sowing is deiayed until a more regular rainfall pattem emerges, the crop whose much longer than the rainy season suffers terminal drought. Genotypes whose seeds capable of germinating and establishing under limited water supply would have less ri: experiencing terminal drought. Although studies on the sensitivity of seeds to watcr d well documented (Heikal et al., 1982; Sharma, 1984), Little information is available on resistance during germination and early seedling development of locally grown grounc cultivars in Senegal (Gautreau, 1966; Tourte et al., 1966). The present study, therefor assesses genotypic differences in germination and early seedling survival during droug groundnut genotypcs.

Methods

Seeds of *Arachis hypogaea* L.(genotypes 73-33. Fleur 11, 55-437, 57-422 and GH 1 ! were germinated on filter paper soaked in double distilled water or in polyethylene gly (PEG 600) [illegible] 0.7 MPa [illegible] drought. Incubation temperature was 32 ± 3°C. In order to maintain the same concent thc PEG solutions were changed every three days. The experiment was a completely randomised design, replicated four times. Six days alter incubation. radicle, hypocotyl lengths were measured. Seeds that produced a radicle longer than 2 mm were consider germinated (Heydecker, 1972). Percentage germination and scedling vigour index (SVI = [hypocotyl length + root iength] x germination %), were calculated.

Results

Germination, hypocotyl and root length of all genotypes decrcased with increasing dro (Fig. 1). This resulted in a decrease in the seedling vigour index (Fig. 2). The ability of 55-437, Fleur 11 and GH 119-20 to germinate under severe drought was higher than t| shown by 73-33 and 57-422 (Fig. 3a). The highest seedling vigour index at all drought were shown by GH 119-20, while the least values were shown by 57-422 (Figs 3b and

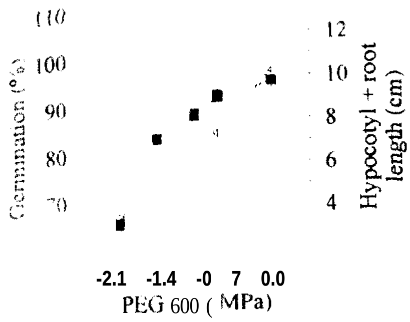


Fig. 1. Effect of PEG 600-induced drought on germination (■) and hypocotyl+root length ( ). Each point represents the mean of the pooled values of all the groundnut genotypes.

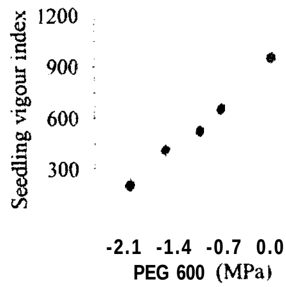


Fig. 2. Effect of PEG 600-induced drought on seedling vigour index. Each point represents the mean of the pooled values of all the genotypes

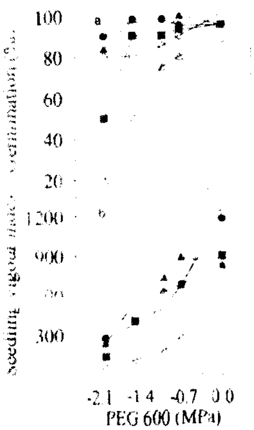
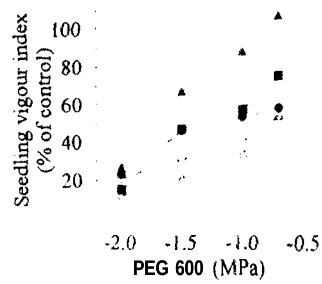


Fig. 2. Genotypic variation in (a) germination, and (b) seedling vigour index in five groundnut cultivars; cultivar 73-33 (■), Fleur 11 ( ), 55-437 (●), 57-422 ( ), and GH 119-20 (A). The SD for germination values varied between 0 and 25, whercas that for SVI values was 59 to 2 17



… lling vigour index expressed as % of control of PEG 600-induced drought in five groundnut cultivars; cultivar 73-33 (■), Fleur 11 ( ), 55-437 (●), 57-422 ( ) and GH 119-20 (A).

Conclusions

Genotypes 55-437 and Fleur 11, in addition to being adaptated to drought based on their short cycle (90 days), resisted drought during germination and seedling establishment. Genotype . il 1 119-20 has a longer cycle (110 days) and is therefore unable to adapt to regions with short and sparse rainfalls. It was, however, able to resist severe moisture stress during germination and at its early seedling stages. These information could therefore be of importance to breeders involved in the improvement of groundnut cultivars.

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Data holding

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1 . Background

CERAAS, Centre d'Etude Régional pour l'Amélioration de l'Adaptation à la Sécheresse (Regional Centre for Studies on the Improvement of the Adaptation of Plants to Drought), is a national laboratory and a regional base-centre of WECARD" for the study of the adaptation of plants to drought It is situated in Thiés (longitude 14° 42', latitude 16° 28'). 70 km east of Dakar, Senegal. The climatic conditions are typical of the semi-arid sudano-sahelian zone. In Thiès, the annual rainfall varies between 400 and 600 mm, and temperature between 19°C and 45°C.

CERAAS found its origin from the expertise developed by a research team in Senegal which started work in 1983, on a new multidisciplinary research programme for improving the adaptation of peanut to drought The aim of the programme was to contribute to stabilising and/or improving peanut production in Senegal. This team consisted of physiologists and breeders of ISRA[3] and CIRAD[4], based at the Plant Physiology Laboratory of CNRA[5] at Bambey, one of the national research centres of ISRA. The programmes were funded by the European Union (DG XII) in the framework of its STD[6] 1 and 2 programmes. Based on the interesting results obtained for agricultural development in the sub-region, in 1987. WECARD and CILSS[7] mandated the research team in Senegal working on this programme, to develop its expertise and to extend it to other crops as well as other NARIs[8] in the region This initiative was further re-enforced with the drought resistance network (R3S[9]), one of WECARD's networks, conferring upon this team the role of organising and conducting research activities at the regional level, on one of its research themes "Physiological mechanisms of plant adaptation to drought and creation of drought resistant varieties" With the association to a regional research network, the laboratory thus assumed a regional dimension and was formally established as CERAAS, within ISRA, in 1989

For a larger number of researchers from the South to benefit from the expertise of CERAAS, a complementary project, prepared by CERAAS with the support of WECARD and CILSS, was jointly financed by the European Union in the framework of its STD3 (DGXII) and Regional Indicative Programme funded by EDF[10] (DG VIII). These funds were destined to improve the research and technical capacity of CERAAS. Furthermore, to facilitate its position as a component of a developing country NARS, open to regional and international co-operation, CERAAS was placed under the tutelage of WECARD, after the signing, on 17 December 1996, of an agreement between ISRA and WECARD

2. Subject and nature of the research

Desertification originating from climatic changes such as drought. is a crucial problem to populations, causing social and economic disaster. Rainfall (water) and productivity feature in the

[2] West and Central African Council for Agricultural Research and Development
[3] Institut Sénégalais de Recherches Agricoles
[4] Centre de Coopération Internationale en Recherche Agronomique pour le Développement
[5] Centre National de Recherche Agronomique
[6] Science and Technology Development

list of critical indicators of desertification. In the sahel, where rainfed agriculture is the order of the day, water is an essential resource for agricultural production. Moreover, its importance increases with the necessity to feed an ever increasing population The development of an integrated water resources management system is therefore an increasing necessity. The integrated approach of water management involves a number of scientific areas, notably the environmental and agricultural sciences, which leads naturally to the development of multidisciplinary research activities

At CERAAS, a multidisciplinary approach conjugating specialists in agronomy, physiology, biochemistry, molecular biology, bioclimatology, crop modelling, biometry and statistics has been developed to work on the research theme "Physiological mechanisms for the adaptation of plants to drought and creation of improved varieties". The programmes on which the research activities are based correspond to national and regional priorities and are grouped under the following three sub themes

1. improving, in an integrated manner, the knowledge on the agronomic performance and physiologic behaviour of crops cultivated locally and/or introduced in semi-arid regions;
2. developing and improving tools that could be used to increase the efficiency of breeding programmes for the creation of crop varieties better adapted to drought;
3. developing and improving tools and methods for forecasting agricultural production and food security

These themes could be characterised as fundamental, strategic and applied as regards tackling the problem of drought and optimising the management of water resources for agricultural production

3. Data holding

Information and data acquired by and/or generated at CERAAS are managed by the information and communication service of CERAAS This service is headed by an information and communication specialist

Existing data archives

The information and data available at CERAAS could be grouped as follows

- methods for investigating the agronomic performance and physiological behaviour of crops cultivated and/or introduced in semi-arid zones;
- information on relevant reproducible physiological and agronomic traits that may be integrated into breeding programmes for creating varieties that are better adapted to drought;
- methods and tools (physiological, biochemical and molecular biological) for screening crop varieties more resistant to drought for the development of plant material resistant to drought ,
- physiological and agronomic methods for determining the water needs of crops, managing water resources and scheduling irrigation;
- methods and tools (crop models and geographic information systems) for predicting yields and identifying zones of potential agricultural calamity; information which could be used by decision makers

Information and data are also available on soil physico-chemical, rainfall and climatic parameters

Data types

The data acquired, elaborated and/or treated are numerical, graphical, textual, photographic, cartographic and tabular In order to facilitate their management, these have been grouped into primary and secondary data

Data quality

Raw data is collected and treated either manually or automatically during research activities conducted in the laboratory, in the experimental station and/or in the farmers field

. in, in addition to the traditional channels provided by the service, receive information and data they require through the internet or by e-mail.

1. Conclusion and perspectives

The operating environment in the information management sector is changing rapidly with the introduction and adoption of new technologies. The information manager at CERAAS needs to be trained to be well equipped for the new "services" environment and technical skills that are required The information manager should also be given the opportunity and means to interact with users to assess the demands to design more appropriate services, to market them and to monitor and assess their impact.

CERAAS possesses a considerable amount of grey literature not available in international systems The grey literature data base has to be developed and strengthened. Moreover, it is necessary to establish a brokerage function to digest and repackage information into more value-added services for users.

In addition, the following actions will contribute to consolidating the information and data management system of CERAAS

- sensitising a large majority of users and decision makers on the necessity to invest in research for a sustainable solution to fight against drought and desertification, through the conduct of modern research programmes;
- improving the valorisation of local results;
- re-enforcing North-South partnership
- re-enforcing the operating environment of the national and sub regional agricultural research systems in Africa (annexe 1)

ANNEXE 1: OUTPUTS, CONCLUSIONS AND ACTION PLAN (DRAFT) RESULTING FROM A MEETING ORGANISED BY THE GLOBAL FORUM FOR AGRICULTURAL RESEARCH (GFAR) IN DAKAR, SENEGAL (26 AND 27TH JULY 1999) ON DEVELOPMENT OF A SUB-SAHARAN AFRICAN AGRICULTURAL INFORMATION STRATEGY).

Outputs and conclusions

The Executive Secretaries and ICT[11] specialists of the three SROs[12] (WECARD, ASARECA[13] and SACCAR[14]) recognised the value of this first consultation to exchange information and compare experiences

The participants reaffirmed that a sub-Saharan Agricultural Information Strategy can only be developed based on the three sub-regional information strategies, and that the information and communications activities of the NARS are the building blocks of the sub-regional information strategies

At the national level, the sub-regional organisations foresee the need of developing and strengthening National Agricultural Information Focal Points (NAIFP), as the information and communication arm of' the NARS These NAIFPs would have a key role to play as: i) the national gateway (within the country and between the country and the regional and international information systems), and ii) the knowledge brokerage agent (acquisition, interpretation, synthesis and dissemination) for the different categories of users The NAIFPs could be hosted by universities or NARIs but should have quality access to communication infrastructure High priority should be given to e-mai! connectivity between NAIFPs and users, especially remote research stations. cstcnsron services and farmers' organisations. without neglecting other types of media (radio/TV, meetings and newsletters, etc.).

The functions envisaged for the SROs are- to gather mformation on mformation systems and networks in member countries; to promote common formats and standards; and to act as the gateway between the national and the global information systems.

With regard to the NARS. the SROs should play the following roles:

- Develop and implement sensitisation programmes for policy-makers and senior managers on the value of IM/IT[15] in order to ensure the allocation of adequate resources to NAIFPs.
- Advise and assist the NARS to develop their own information and communication strategy
- Promote a new role of information and communication professionals (from librarians to "cybrarians") through training curriculum development in the area of library and information services with more ICT-oriented content and agricultural/scientific background.
- Co-ordinate sub-regional programmes (e.g. training. traditiona! and electronic publishing, including newsletters) and to provide advice to NAIFPs on cross-culting issues (e.g connectivity, equipment procurement).
- Mediate, on behalf of the NARS, with international mformation providers over the means and terms of access to STI and with library schools over expertise development.
- Promote the development and maintenance of national databases on research expertise, programmes. activities and facilities and the adoption of management Information systems for improved access and use of these data within and between the sub-regions

The SROs recognised the need for csternal assistance for the establishment of their sub-regional mformation systems and the network of NAIFPs, but considered this need to be temporary They decided to address. in consultation with their NARS members, the basic issue of sustainability as related to recurrent costs and management.

The sub-Saharan Agricult ural Information Strategy will result from the co-ordination of the three sub-regional information strategies that the SROs agreed to develop, and from close interconnection through an electronic network based on interactive Web Pages As the first steps toward information sharing the SROs also agreed to develop a common database with details of their research networks and to establish a regular mechanism of consultation between their respective ICT specialists

Finally, the SROs requested the participating agencies (Africalink/USAID, CAB International, CTA, FAO/WAICENT, ISNAR and the NARS Secretariat of GFAR) and their other partners in the field of ICT to continue to support the development process of their information systems according to their strengths and comparative advantages.

Follow-up actions

The SROs recognised that some of the activities defined above are of a long-term nature and were already addressed in their esisting plans and schedules (i.e ASAREC.4 and the RAIN Project, WECARD and its Plan of Action: and SACCAR and its outline strategy). As a result of this consultation, the SROs agreed to implement the following additional actions within the next six to twelve months:

- Formulation of the sub-regiona! ICM and ICT strategrcs within the framework of their sub-regiona! strategic plans
- Development of their Web Pages
- Establishment of a joint database on research networks
- Initiation of negotiations with scientific information

ASARE CA. WECARD and SACCAR agreed to pursue this Initial dialogue through electronic communications They will formulate their own specific action plan taking into account the issues and options raised and will share them with the other SROs Each SRO will contact the appropriate partners in support for implementation of ils action plan.