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An Integrated Approach To Improve Production Of Starch Tubers

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EXECUTIVE SUMMARY

Production of yam, an essential food in Nigeria and other parts of sub-Saharan Africa, does not meet demand. Problems associated with yam production include transmission of diseases in seed tubers, yields much below the known potential, use of unimproved genetic material and inefficient agronomic methods. Methods, which are applied to other root and tuber species, may be adapted to yam to eliminate disease-causing organisms from propagation material and to clonally propagate select genetic material. Modern methods of cultivation may be adapted to this crop. In this program:

- A germplasm collection of high-yielding yam cultivars was assembled.
- Protocols were developed for clonal propagation of cultivars by tissue culture. One approach, based on culture on semisolid medium was improved. A more efficient system, of propagation in liquid culture, was developed.
- A method for acclimatization of plantlets derived from tissue culture, under greenhouse conditions in both solid substrate and hydroponics was defined.
- Protocols for cultivation and production of seed tubers in solid substrate and hydroponics were developed.
- Physiological aspects of plant growth and tuber development were studied.
- Collaborations were established with institutions in Nigeria and with a university in the Philippines, addressing various aspects of yam propagation, cultivation and improvement.

Three members of the faculty of the collaborating Nigerian institution visited the BIDR for extended periods of time. In addition to participating in the research, each gained an understanding of a western culture that is quite different from their own. Some attended various academic courses offered at the institute.

A tissue culture facility was established at the Nigerian institute, which may be used for continued research on yam and new programs other important crops.

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RESEARCH OBJECTIVES

Background

Yam is an essential food for many millions of people in Nigeria as well as in much of the Southern Hemisphere. Population growth in these regions has generally outstripped increases in production of this important crop, leaving it in critically short supply. Many factors contribute to limited yam production, including insufficient attention to genetic improvement, poor agricultural methods, the spread of disease vectors through the planting material and customs ingrained in the culture (market demand for very large roots) which perpetuate inefficient production.

Although they belong to different botanical families, many parallels exist among the various root and tuber crops, including the problem of proliferation of root- or tuber-borne diseases, appropriate agrotechniques and the need for methods of clonal propagation. The development and implementation of modern methods has changed potato production in developed countries into a highly efficient, high-yielding process. The potential exists to follow the same complex of approaches in order to improve yam cultivation. This, in general terms, was the objective of this program.

Specific Objectives

The specific objectives of the research were to

1. select high-yielding yam cultivars with outstanding characteristics, including long storage life, cooking, organoleptic and nutritional qualities, suitable tuber morphology and above ground habit, and pest and disease resistance.
2. develop an economically viable system for propagation of seed material by improving *in vitro* procedures for plantlet and microtuber production, greenhouse systems for minituber production, and field production of seed tubers.
3. develop and promote the use of improved agrotechnologies, centered on appropriate fertilization and irrigation.
4. conduct a study of field establishment, development and dry matter production in storage roots of unimproved stock and of clean, clonally propagated stock.

METHODS AND RESULTS

GERMPLASM COLLECTION

The order Dioscoreales is thought to have originated in Southeast Asia during the late Triassic or early Jurassic; the ancestral forms of the genus *Dioscorea* having achieved pan tropic, and perhaps worldwide, distribution long before humans appeared on the scene (Ayensu, 1972). It has been suggested that the yams were domesticated independently in Africa, Asia and America, where they are important food crops today (Coursey, 1967). *Dioscorea* species of major economic importance include *D. rotundata* Poir (white yam), *D. alata* L. and *D. esculenta* (Lour.) Burk. in Asia, and *D. trifida* in the Caribbean (Coursey, 1967).

During millennia of vegetative propagation, sexual fertility has, for all intents and purposes, been selected against, so that cultivated yams rarely flower and set seed even less frequently. When flowers occur they are always imperfect, plants are generally dioecious and there is little synchronization of pollen shedding and stigma maturity. Differences have been reported in the regularity and profuseness of flowering as well as in the age at which plants flower, such that there are early maturing (within 200 days after planting (DAP), medium-maturing (240 - 270 DAP) and late-maturing (longer than 270 DAP) types (Okoli et al., 1984). Thus, genetic improvement through conventional breeding has been very difficult and selection is viewed as an important alternative route to improvement.

One of the objectives addressed was cultivar selection. High yielding cultivars with long storage life, good cooking, organoleptic and nutritional qualities, suitable tuber morphology and above ground habit, and pest and disease resistance were sought.

Emphasis was placed on bringing institutions involved in studies of yam into a collaboration on selection. About 11 institutions in 8 African countries have reported yam germplasm collections (Lawrence et. al. 1986). The great majority of the accessions belong to *D. rotundata* and *D. alata*; a smaller portion to *D. bulbifera* and *D. praehensilis*. Nigeria and Cote d'Ivoire hold the largest accessions. The Nigerian germplasm collections are at two

Cote d'Ivoire hold the largest accessions. The Nigerian germplasm collections are at two main centers, the National Root Crops Research Institute (NRCRI), Umuike and the International Institute of Tropical Agriculture (IITA), Ibadan.

The following cultivars have been recommended by the NRCRI on the basis of consumer reaction to storability, cooking time, flavor, eating and pounding qualities, as well as laboratory analyses of protein and amino-acid contents, phenol content, color, starch types and dry matter content:

SPECIES	CULTIVAR	CHARACTERISTICS
D. rotundata	Nwopoko	good eating and pounding quality
	Obiaoturugo	
	Ekpe	good eating quality and for pounded yam flakes
	Abi	roasting
D. alata	Um680	high yield; used in livestock feed
	Ominelu	bread flour

Selections were made from among accessions obtained from NRCRI, IITA and from yam farmers in the rain forest and guinea savanna of Nigeria. Selection was based on physically observable qualities, such that healthy, thick skinned, unbranched tubers were preferred, and rotund tubers with a shape index (S I) <1 were preferred to cylindrical tubers with SI >2. Tubers with these characteristics are likely to be suited to the eventual application of mechanical harvesting.

A collection of Nigerian yam cultivars, representing conventional cultivars and select material obtained from NRCRI, was established in the field at Nnamdi Azikiwe University (NAU). 35 accessions were included. Tissue cultures of these cultivars were established at NAU, forming the nucleus of an *in vitro* gene bank. Six select clones of *D. rotundata*, kindly provided in the form of tissue cultures by the IITA, were also maintained at the NAU *in vitro* gene bank, as well as at the BIDR. A Kenyan acquisition of *Dioscorea* (presumably *bulbifera*) growing at the Tel Aviv Botanic gardens and a widely grown Philippine cultivar of *D. alata* were introduced to tissue culture at the BIDR.

CLONAL PROPAGATION

BACKGROUND

Cultivated varieties of yam rarely flower or set seed and are highly heterogeneous. They are, therefore, propagated vegetatively by means of tuber setts. Good quality, disease-free seed tubers are generally not available in the great quantities needed for extensive agricultural use and what is available is usually priced beyond the means of growers. For these reasons, and in order to facilitate wide-spread use of the better cultivars identified in the selection program, one of the main objectives of this program was to develop an economically viable system for clonal propagation. In the process, *in vitro* procedures for plantlet propagation and microtuber production, as well as greenhouse systems for minituber production were addressed.

Tissue culture techniques have been applied to yam for clonal multiplication (Mantell et al., 1978) and for the production of virus-free plants (Mantell et al., 1980). Indeed, some of the cultivars passed on to us by the IITA (referred to above) were received as *in vitro* plantlets.

Our work began with implementation of tissue culture methods for clonal propagation of yam that had appeared in the scientific literature (Mantell et al. 1978, Mantell et al. 1980. Sengupta, et al., 1984, Mitchell et al., 1995 and 1995a). Propagation rates were slow. Axillary shoot proliferation, leaf expansion and rooting occurred simultaneously. Leaves expanded to several cm. in width, although such size is unnecessary in the stage intended for plantlet multiplication. The entire procedure was very labor-intense. Our objective was to accelerate the propagation rate and to develop a less labor-intensive process.

PHYTOSANITATION

The work plan included a program to develop methods for phytosanitation and testing. The main goal of this was to eliminate tuber-borne vectors of disease, which shorten the storage life of tubers and reduce yield. With the establishment of collaboration with the ITTA, we learned that their yam improvement program included this aspect. Indeed, cultures provided

to us by our ITTA collaborators had been certified virus-free. Rather than repeat their successful efforts, we concentrated on other aspects.

DISINFESTATION AND CULTURE INITIATION

A second aspect of phytosanitation is disinfestation (also known as surface sterilization), the purpose of which is to eliminate microorganisms on the surface of explants, which could develop *in vitro* on the plant tissue or on the culture medium. An efficient method was developed for disinfestation of yam tuber tissue and for initiation of tissue cultures. The plant material used for these trials, the aforementioned Kenyan acquisition of *Dioscorea*, was obtained from the Tel Aviv Botanic gardens. Disinfestation was accomplished by a series of washes of explanted tuber tissue followed by soaking in dilute NaOCl and $HgCl_2$.

***IN VITRO* PROPAGATION**

Multiplication cultures were generated from the Kenyan cultivar as well as from the clones kindly provided by the IITA [identified as TDr131(H1), TDr747(1), TDr205(4), TDr743(4), 87/100264(1) and 87100211A(2)] and from cultures passed on to us by a commercial tissue culture laboratory in Israel, In Vitro S'de Nitsan, which had also originated at the ITTA.

The cultures that had been initiated in the BIDR lab and those from other sources were subcultured to fresh basal nutrient medium composed of the salt formulation of Murashige and Skoog (1962) plus sucrose (30 g/l), glycine (2 mg/l), thiamin, pyridoxine-HCI, calcium pantothenate and nicotinic acid, 1 mg/l each, biotin, 0.01 mg/l, inositol, 100 mg/l and folic acid, 10mg/l. The pH of the medium was adjusted to 5.8 and agar (7 g/l) was added to gel the medium. Medium was added to the vessels prior to autoclaving for 16 min. at 121° C. Tissues were cultured on this semi-solid medium in 25x100 mm borosilicate test tubes containing 10 ml medium or Magenta GA7 polycarbonate vessels containing 60 ml of medium. Nodal cuttings from multiplication cultures were placed upright into jelled medium, one per test tube and six per magenta vessel. Cultures were incubated under a 16/8 hour photoperiod with light provided by cool white fluorescent lights (4000-5000 lux), at 26 +/- 1° C. Cultures were subdivided and transferred to fresh medium every 1-2 months, in order to bring them to a uniform healthy condition and to produce the quantities needed to carry out trials.

acceleration of branching rate / restriction of in vitro leaf extension / microtuber development

Efforts focused on maximizing branching and limiting unnecessary leaf expansion. Studies included changes in the phase of the medium, the manner of contact between tissue and medium and medium composition. Development on shaken liquid was compared to that on stationary jelled medium and on membrane rafts floating above stationery liquid media. Preliminary trials indicated that culture in shaken liquid media and in bubble reactors might be more efficient that in jelled media or on membrane rafts. Also, several concentrations of two gelling agents (agar and gelrite) were tested. Concentrations of kinetin, sucrose, inclusion of activated charcoal and manipulation of gas exchange to the vessel were tested.

explant size and morphology

Microcuttings comprising the apex plus one node were more prolific with respect to shoot and root development than those that consisted of just the apex or only a sub-terminal node. The use of the sub-terminal node as starting material consistently resulted in poorest performance. Cultures derived from explants composed of the shoot apex plus one subterminal node grew taller and more rapidly during 12 weeks of culture than either of the other explant types. They also produced additional nodes more rapidly, produced more internodes and branched more (Fig. 1a-d).

Single-node cuttings 10-20 mm in length with one leaf produced shoots more vigorously than shorter sections lacking a leaf. Tuberization occurred on MS medium supplemented with 3% sucrose and 0.7% agar. After incubation for 5 months, tuberization was greatest in cultures begun from an apex plus one node (60% of the cultures produced microtubers), than in cultures with the apex only (26% of the cultures produced tubers) or one node only (40% of the cultures tuberized). Microtubers generated in cultures started from an apex plus one node attained the greatest individual fresh weight (796 mg), while plantlets from shoot tips gave the lowest fresh weight per microtuber (248 mg) and single node cuttings gave an average fresh weight of 537 mg per microtuber (Table 1). Tuberization was accelerated by use of gelrite

instead of agar, and increased sucrose concentration, as discussed in the section Sucrose and Gelling Agent, below.

sucrose and gelling agent

The gelling agent used in published reports on yam is agar. Trials were conducted to compare the development of the yam cultivars *D. rotunda* and *D. bulbifera* on medium jelled with 0.7% agar vs. 0.24% gelrite, respectively the commonly used concentrations for each. Plantlets of *D. bulbifera* and of the two *D. rotundata* clones tested developed faster, as measured by the production of new nodes, internode length and rooting, in medium jelled with gelrite (Table 2, Table 3). The shoots became thick, leaves dark green, and internodes relatively long (10-15mm) on gelrite medium whereas on agar medium shoots were thin, leaves light green and internodes relatively short (6 - 8 mm).

Sucrose is routinely employed as the carbon source in tissue culture media, at the rate of 3%. Nodal cuttings from cultures of *D. bulbifera* and *D. rotundata* grown on basal MS media were transferred to basal medium supplemented with 0, 3, 6, 12 and 15% sucrose. Cultures grown on medium lacking sucrose survived for only 3 weeks and showed very little growth. Cultures on 15% sucrose also showed very little growth and died after 5 weeks, while those on 12% survived but did not initiate microtubers. Although 3% sucrose supported shoot & root development, microtuber production was observed only after 3 months in *D. bulbifera* and after 5 months in cultivars of *D. rotundata*. 6% sucrose appeared to be the best concentration for production of microtubers, as well as for shoot growth and rooting. Microtuberization was observed within 2 months in *D. bulbifera* and *D. rotundata* on 6% sucrose (Table 3)

While gelrite proved to be better for rapid multiplication of yam cultures, agar was very good for long-term storage, with cultures of both cultivars successfully maintained in agar-media for 6-9 months without replenishment.

activated charcoal

Activated charcoal binds to components of tissue culture media and to tissue exudates, effectively removing them from the active system. Interactive effects of activated charcoal and gelling agent (agar and gelrite) on multiplication and rooting were evaluated. The treatments comprised:

basal medium with agar

basal medium with agar + activated charcoal,1g/l

basal medium with gelrite

basal medium with gelrite + activated charcoal,1g/l

Each treatment was replicated five times with each of the cultivars. TDr 747. TDr 205. TDr 743 and *D. bulbifera*.. Data was collected on the number of new shoots. new nodes. new tips, roots and microtubers each 14 days. Inclusion of activated charcoal in the culture medium consistently resulted in greater plant height, regardless of whether the jelling agent present was agar or gelrite (Fig. 2 and 3c). Similarly, there was a significant increase in the number of new nodes in the presence of activated charcoal in media containing agar or gelrite (Fig. 3a). Activated charcoal enhanced root production in medium containing agar but not in gelrite medium, in which rooting was already significant.

liquid culture

culture on membrane rafts

Culture on liquid medium has been reported to have resulted in faster rates of shoot growth and multiplication than were attained on semisolid medium in a number of plant species (Simmonds and Werr, 1987), including potato (Ziv and Shemesh, 1996), philodendron (Ziv and Ariel, 1991) and nerine (Ziv et al, 1995). It also reduced labor intensive manipulation required for media replenishment and transfer of propagules and facilitated scaling-up of the culture systems (Ammirato and Styer, 1985, Ziv, 1990, Ziv, 1992). However, the use of liquid media in place of agar can be problematic, as it sometimes induces production of vitrified tissues, which are not viable ex-vitro (Hussey, 1986, George, 1993, Ziv, 1990). An

alternative support mechanism for tissue cultures, which is less prone to induce vitrification, is the microporous membrane raft which floats on a liquid medium. Alternatives to the conventional micropropagation of yam on semisolid medium were sought in order to accelerate and reduce the costs of the process.

Plantlets obtained from the IITA were maintained *in vitro* through regular subdivision and reculture every 6 to 8 weeks until a sufficient amount was attained to carry out experiments on liquid culture medium. Propagation was on semisolid modified MS medium described above. Single shoots of clone TDR 743 (4), averaging 20+/-2 mm in length, and weighing 0.45–0.65 g, were cultured in each flask or magenta vessel. Multiplication medium without phytagel (gelrite), supplemented with 0 or 1 mg l^{-1} kin and the pH adjusted to 5.1 (multiplication medium b), was dispensed at the rates of 50 ml per 250 ml capacity Erlenmeyer flask and 150 ml per Osmotec vessel (Vessel No. 700 535) containing floating membrane rafts (Osmotec Cat. No. 600 030). Membrane rafts are characterized by having a 0.3 mm nominal pore size polypropylene membrane, with a buoyant element to support 0-10 g of plant material. These were autoclaved at 121°C for 20 minutes. Flask cultures were rotated on a gyratory shaker at 100 rpm. All cultures were incubated under the conditions described above for stationary cultures.

Cultures on floating membrane rafts developed significantly longer internodes and produced more nodes, shoots and roots per plant than in rotated cultures while those grown in shaken flasks developed significantly longer roots, bigger leaves and a higher percent leaves in the biomass than those on the membrane rafts (Table 4).

Cultivation on membrane rafts floating on liquid medium improved the production of shoots and roots. This may be due to greater aeration at the plant-medium interface on floating membrane rafts than in shaken flasks. Watad et al. (1995) also reported a higher rate of shoot multiplication and explant fresh weight accumulation on liquid medium using interfacial raft technology in *Spathiphyllum* and *Syngonium*. In agar-solidified media, growth rates may be slowed as a result of selective adsorption of growth components by the agar and by plant exudates which diffuse slowly into the agar medium. On the other hand, a serious problem in

liquid cultures is the frequent development of vitrified plants (Ziv, et al. 1995). Culture on interfacial membrane rafts appears to combine the advantages of liquid medium with the relative stress necessary to prevent vitrification (Desamero et al. 1993).

Kinetin

When Kin was added to the medium at a concentration of 1 mg/l, production of shoots, number of nodes and root length increased significantly, but the number of roots decreased by about 36%. The leaf size and percent of leaves in the biomass were comparable in media with or without Kin. Shorter internodes were also observed in cultures grown in kinetin-supplemented media (Table 5).

Kin is known to promote shoot development and inhibit root development (Watad et al. 1995). With kinetin-supplemented media, increased production of shoots with shorter internodes was noted. This effect of kin has also been reported in *Dioscorea bulbifera* (Uduebo, 1971). Addition of up to 2.5 mg l^{-1} kin increased the number of shoots. A further increase in the concentration, to mg l^{-1}, resulted in lower dry weight than in the absence of kin. In *Marsilea drummondii*, Kin treatment caused production of leaves with unusually short petioles or unusually long lobes and leaves that branched repeatedly (Allsopp and Szweykowska, 1960). In *D. alata* shoot cultures, however, kin significantly increased basal and aerial root formation (Mantell and Hugo, 1989).

flurprimidol & liquid medium

Growth retardants, inhibitors of gibberellin biosynthesis, among them flurprimidol, have been used to reduce or prevent abnormal leaf development and enhance the development of compact bud aggregates (Graebe, 1987, Rademacher, 1991, Ziv,1990). We tested the hypothesis that fp might reduce petiole or leaf extension while having a positive effect on axillary branching.

Individual shoots of clone TDR 743, as described above, were placed into 250 ml Erlenmeyer flasks, each containing 30 ml modified multiplication medium, in which kin was replaced with fp (0, 3, 6, and 12 mg/l). The pH of the medium was adjusted to 5.1 prior to autoclaving

for 20 minutes at 121° C. Fp was filter sterilized through a 25 mm diameter, 0.20 micron cellulose acetate membrane into the autoclaved medium after it had cooled to about 35°C. Ten shoots, each about 20 mm long, were inoculated into each flask. Cultures were incubated at 24-26° C and a 16 h photoperiod on a gyrotory shaker at 100 rpm. After 25 days, the cultures were transferred to two sets of fresh medium containing 3 and 6 mg l^{-1} fp.

Untreated plants were taller, had bigger leaves and were less branched than fp-treated plants (Figure 4.). Leaves made-up about 33 % of the fresh biomass of plants in the medium without fp. Fp caused significant inhibition of leaf blade expansion and of petiole elongation, which was greatest at 12 mg/l. While bud cluster development did not occur on the control medium, fp caused production of bud clusters, which was greatest at 3 and 6 mg/l fp. Whereas with 6 mg/l fp bud formation was enhanced but shoots did not develop, buds produced in medium with 3 mg/l fp developed into shoots (table 6).

Following transfer from media containing various fp concentrations to fresh medium containing 3 or 6 mg/l fp, bud clusters proliferated, developed into shoots and/or rooted. Shoot multiplication was greatest, 79 per flask, on material pretreated in 6 mg/l fp and cultured on 3 mg/l fp medium (Table 6). This is about 47%, 52% and 53% higher than when cultures from 3, 0 and 12 mg/l fp pretreatments, respectively, were transferred to medium containing 3 mg/l fp. High amounts of fp in the pretreatment, 6 or 12 mg/l, inhibited subsequent shoot development when on medium containing 6 mg/l fp. However, cultures which came from the same background, when transferred to 3 mg/l, developed shoots with longer roots. Roots were longest on plantlets from the 3 or 6 mg/l fp pretreatment. Fp, thus, enhanced bud multiplication and rooting, while producing compact plants, by limiting leaf development and petiole extension. More but smaller shoots developed in cultures from 12 mg/l fp transferred to 3 mg/l fp than from cultures from 6 mg/l transferred to 3 mg/l fp.

In the present study, leaf growth was notably inhibited by the addition of fp. Bud cluster formation was enhanced at lower fp concentrations (3 and 6 mg l^{-1}) (Fig. 4 and 5) while being totally inhibited at a high concentration (12 mg l^{-1}). The promotion of shoot formation by low

concentrations of fp is consistent with previous reports, which cite increased somatic embryogenesis in *Citrus sinensis* (Spiegel-Roy and Saad, 1986), *in vitro* cormlet formation in gladiolus (Ziv, 1990) and bud proliferation in liquid cultures of philodendron (Ziv and Ariel, 1991). The use of fp in enhancing bud cluster formation and shoot development offers the potential of an efficient micropropagation system for yam.

nitrogen sources - NH_4NO_3 and KNO_5

The Murashige and Skoog nutrient medium (MS) (Murashige and Skoog, 1962), used as the basal medium for yam culture, is characterized by a high nitrogen concentration, contributed by ammonium nitrate and potassium nitrate. This medium has sometimes been reported to be inhibitory to the tuberization process of species of *Dioscorea* (Asahira & Yazawa, 1979, Mantell and Hugo, 1989). The omission of NH_4NO_3 was found to be beneficial to microtuber formation in *D. alata* (Jean and Cappadocia, 1991). However, whether the result was solely due to the absence of NH_4 ions was not confirmed. Alternatively, it could have been due to the reduction of the total nitrogen content or to a high NH_4:NO_3 ratio, as observed in *D. opposita* (Asahira and Yazawa, 1979).

The effects of NH_4NO_3 and KNO_3 were investigated on two clones of *D. rotundata*, which differ in performance *in vitro*. Clone T1.1 is characteristically a good *in vitro* tuber-forming clone while TDR 743 (4) is a poor tuber-former, regardless of the composition of the medium. Longer internodes and leaves were observed in TDR 743 (4) while T1.1 produced significantly more roots. The percent of leaves in the biomass and the growth value (the difference between the final and initial fresh weight (FW) divided by the initial FW (FW_{final}-$FW_{initial}$)/$FW_{initial}$) were similar in both clones.

Explants composed of the shoot tip plus one node were used in trials of N-source effects. The MS basal medium supplemented with 2 mg l^{-1} glycine, 40 g l^{-1} sucrose and Morel vitamins (Morel 1962, as cited by George, 1987), 0.5 mg l^{-1} NAA and 2.4 g l^{-1} phytagel (medium a) was modified as follows:

treatment A - medium a

treatment B - full strength medium a minus NH_4NO_3

treatment C - full strength medium a minus KNO_3 (+5.6 g l^{-1} KH_2PO_4)

treatment D - half strength medium a

treatment E - half strength medium a minus NH_4NO_3

treatment F - half strength medium a minus KNO_3 (+2.8 g l^{-1} KH_2PO_4)

In treatments C and F, devoid of KNO_3, KH_2PO_4 at 5.6 g l^{-1} and 2.8 g l^{-1}, respectively, was added to keep the potassium level constant. The pH of the media was adjusted to 5.8+/-0.1 by NaOH or HCl prior to autoclaving at 121°C for 20 minutes. 60 ml of medium was poured per GA7 vessel (Magenta Co., Chicago) prior to autoclaving. Five explants were placed upright into each vessel. The cultures were incubated at 24-26° C and illuminated by Philips cool white fluorescent lamps at 16 h photoperiod and a light intensity of 60 umol m-2s-1. The number of nodes and roots, length of internodes and roots, number of shoots, % leaves in the biomass and growth value were determined after 3 months.

Cultures in media with both NH_4NO_3 and KNO_3 had the longest internodes. Internode length was reduced in media, whether full- or half-strength, devoid of potassium nitrate (Table 7). In *D. trifida*, an increased concentration of ammonium nitrate in basal media containing MS salts and BAP resulted in decreased internode length (Mitchell et al., 1995). Root formation was improved in half-strength media (Table 7). Similar results were also observed in *D. alata* and *D. bulbifera*, where aerial root formation was reduced when the strength of a low-nitrogen medium was increased (Mantell and Hugo, 1989). Root development was significantly stimulated when ammonium ions were omitted from the MS formulation. However, when KNO_3 was omitted, root formation was poor (Table 7). This parallels the results of other studies, which indicate that omission of KNO_3 from the media had a detrimental effect on the growth of all yam genotypes tested (Jean and Cappadocia, 1991). The plantlets showed poor root and shoot development and early senescence, probably as a result of potassium deficiency. However, in the present study, the level of potassium was maintained at a uniform level by addition of KH_2PO_4 in the various treatments. Therefore, the deleterious effect in this case could not be attributed to potassium deficiency, but, rather perhaps to the high ratio of NO_3:NH_4, as had been observed in *D. opposita* (Asahira & Yazawa, 1979).

ACCLIMATIZATION

Plantlets produced *in vitro* were transferred to hydroponics and to a solid substrate. Three clones of *D. rotundata*, TDr 205 (T_3), TDr 743 (D_4) and TDr &47 (T_1) were used in the acclimatization (hardening off) trials.

Six to eight week old plantlets were placed in 25 l plastic hydroponics troughs containing half-strength Hoagland's solution (Hoagland and Arnon, 1938) supplemented with 2mM NH_4NO_3. Eight plantlets were placed in each container through holes made in the cover, so that only the roots were in the nutrient solution. Plantlets were shaded by a double layer of absorbent paper suspended 3-5 cm above them. One of these was removed after the first week and the other after the second week of hardening. Daily adjustments of the pH were made in order to maintain a pH of 6.0 and the solution was changed weekly. After three weeks in hydroponics, single plantlets were transferred to 45L bottom-perforated plastic buckets filled with a mixture of vermiculite and peat (4:1 v/v). Plantlets were also acclimatized in a form of hydroponics, using Sorbarod boxes. The plantlets were set in cellulose plugs in a liquid nutrient medium. Ventilation ports in the containers were opened in stages in order to reduce relative humidity within the vessels (Fig. 6).

Plantlets were also planted directly from tissue culture to the vermiculite/peat substrate (Fig. 7). The plantlets and surrounding area were sprinkle-irrigated three times daily. Four layers of black shade-netting were suspended 3-8 cm above the plantlets. These layers were removed sequentially at weekly intervals; the last (uppermost) was removed after 4 weeks.

The survival rate in hydroponics was 100%, while about 90% of the seedlings continued to grow well on soil. The plants that were hardened via hydroponics or directly in the vermiculite/peat substrate did not differ with respect to productivity. Plantlets left in hydroponics for more than 6 weeks did not perform well when transplanted into pots, but those left in hydroponics for three months had produced sizeable tubers. Further investigations were, therefore, made in hydroponics culture. These are described below.

GREENHOUSE STUDIES

Plants, produced *in vitro* and acclimatized (hardened off) in the greenhouse in a vermiculite and peat (4:1 v/v) potting mix or in hydroponics, producing large roots at a relatively fast rate.

nitrogen sources

Initial trials of hydroponic nutrient solution composition indicated that nitrate metabolism plays a crucial role in vine growth and minituber formation. The effects of nitrogen sources and concentrations were studied, using *in vitro*-produced plants of *D. rotundata* clones TDr 205 and TDr 743. Three forms of Nitrogen, KNO_3, NH_4NO_3, and NH_4Cl were applied, at concentrations of 2, 1, and 2 mM, respectively. The plants were acclimated and grown for 7 months. Harvests were made at the end of 3, 5 and 7 months. The harvested material was evaluated for effects of N-source and concentration on biomass and tuber production (production of numerous small tubers vs. few large tubers), harvest index, tuber dormancy, and tuber composition.

Increasing concentrations of any of the N sources induced multiple shoot formation and a number of tubers. The number of shoots developed by each plant ranged from 2-12 with over 90% of the plants having more than three shoots and over 80% having 4-6 shoots. In TDr 205, KNO_3-treated plants produced the most shoots but the NH_4NO_3 treated plants had the largest leaf area (Table 8).

All three treatments resulted in 2-5 tubers per plant on about 85% of the plants. Analysis of the harvests showed a strong positive correlation between the number of shoots and the number of tubers. However, there was an inverse relationship between tuber weight and tuber number, i.e., the number of shoots determined the number of tubers but the more tubers formed, the lower the average weight per tuber. Tubers harvested 5 months after planting ranged in weight from 195-376g for plants grown with KNO_3, 396-589g in NH_4NO_3-fed plants, and 191-382g for NH_4Cl-fed plants. A final harvest of tubers produced on plants grown in NH_4NO_3, NH_4NO_3, and NH_4Cl treatments, 7 months after planting, yielded tubers in the size ranges 389-606g; 587-871g; and 532g-699g respectively (Table 9).

NH_4NO_3-fed plants produced the largest tubers at all stages (Table 9). Tubers developed on NH4Cl-treated plants were larger in all the samples than those produced on KNO3 but the differences were not statistically significant.

Analysis of the tubers showed that accumulation of starch and soluble sugars per gram fresh weight was greater in plants starved of nitrogen; but the tuber size was significantly smaller. A study was made of the carbohydrates in tubers at the pre-sprouting stage of clone TDr 743, grown hydroponically and in soil. The results are illustrated in the Figure 8 a-f.

Continuous application of the different nitrogen forms led to appreciable reduction in tuber dormancy. Results indicate that TDr 747 plants treated with NO_3^- were released from dormancy immediately following harvest. NO_3^- reduced the dormancy periods of the other varieties tested, TDr 205 and TDr 737, to 4 weeks and 6 weeks respectively. Tubers treated with NH_4NO_3 and NH_4^+ were also affected, with TDr 747 plants sprouting within one month after harvest.

The effect of nitrate nutrition on tuber dormancy may be due to the lower level of aldehyde oxidase (AO) observed in yam, as well as in barley, ryegrass, maize and tomato plants. Nitrate seems to depress AO, the final enzyme on the biosynthetic path of ABA biosynthesis. Nitrate fertilization during tuber development, by reducing ABA levels, might yield a large number of small tubers with a short dormancy period. This advantage could be exploited to produce seed yams throughout the year with a known short dormancy requirement.

The information on the effects of N fertilization may be used to manipulate the size and number of tubers produced. Thus, the positive correlation between number of shoots and tubers produced with high N fertilization and the negative correlation between number and size of tubers may be utilized to produce large numbers of small seed tubers. On the other hand, the inverse correlation between low N fertilization and tuber size may be used to direct production toward larger tubers whenever edible yams are desired.

Tuber dormancy is one of the major problems in using tissue culture-derived seed tubers. We, therefore, assayed the enzymes that catalyze sprouting and degradation of tubers during storage. The head, middle, and tail portions of tubers were analyzed, with the aim of discovering the relationship among the enzymes and the types of sugar mobilized in any particular part of the yam tuber and the direction of such movement. Nitrate reductase activity was measured in various parts of yam plants grown in soil and in hydroponics. Activity was found to be greatest in the leaves (Fig. 9).

A comparative assay of the influence of the source of N on the induction of nitrate reductase was also carried out. The highest levels of enzyme activity, in both leaves and roots, was found to occur in plants supplied with N in the form of NH_4Cl (Fig. 10).

Because of the low nitrate reductase activity in the tuber, which is the part consumed by humans, it became necessary to ascertain if the nitrate imported into the tuber is accumulated as such and the effect of such an accumulation on the quality of yam tubers. Tubers and other parts of the yam were assessed for nitrate accumulation. The other enzymes assayed were phosphoenol pyruvate carboxylase and glutamine synthetase.

The results showed that there was no direct correlation between nitrate accumulation and the level of nitrate reductase activity (NRA) (Fig. 12 and 13).

The total nitrogen in the various parts of hydroponically grown yams, as affected by the different sources of nitrogen, is shown in Fig. 12. This data offers an estimation of the protein content of the various yam parts.

One of the interesting observations in the hydroponics culture is the formation of multiple shoots, which correlated positively with the production of multiple tubers. Furthermore, there was a correlation of tuber number with tuber fresh weight, as illustrated with results from NH4NO3 (Fig. 13), which is similar to the results from the other treatments. The size of

tubers produced by plants in hydroponics was encouraging. At the final harvest, seven months after planting, tuber yield was, on the average, 580g for TDr 205, 760g for TDr 747, and 820g for TDr 743. Thus, the tubers were reasonably large and, what is more, easier to produce within a very short period that in soil. Among additional advantages of tuber production in hydroponics, the plants did not need staking and weeding was unnecessary.

Tubers produced in the greenhouse form plants propagated *in vitro* were planted in a soil substrate for agronomic studies of harvest index, growth habit and other features of the tubers. The three clones T_1, T_3 and D_4 were used. Each clone was also divided into two groups, (A & B). Group A plants were treated with nitrogen from different sources while plants of group B were treated with only KNO_3.

Results indicate that there were no significant differences between the production of the three varieties; nor did the different N-sources lead to appreciable differences in tuber size when the appropriate pH was maintained for each N-source. For effective induction of tuber bulking - NO_3 should be supplied at pH of 5.5 - 6.0; -NH_4 at pH 7.3 while NH_4NO_3 was best at pH 6.7. The group B plants, which were treated with only -NO_3, produced new tubers, which sprouted simultaneously as they were being formed. This observation was prominent in the variety TDr 747 (T_1 Clone).

Table 1: The influence of size and anatomical origin of the explant on *in vitro* development of nodal shoot cultures of *Dioscorea rotundata* , clone TDr 743, cultured for 12 weeks

Explant size and origin	Shoot length*	New nodes per culture*	Roots per culture*	% plantlets with roots*	% cultures with tuber**	Fresh wt. per tuber
1. Apex	41.3	5	3.3	66	26	248
2. Apex + one node	55.7	7.7	6.3	100	60	796
3. Subterminal node	38.7	4.3	3.7	74	40	537

* Means of six replicates of 30 plantlets
** Mean data from 5 months old cultures.

Table 2: Effects of gelling agent on development of *Dioscorea species in vitro* after 12 weeks

clone	gelling agent	new nodes/ plantlet	internode length (mm)	roots/ culture	% cultures with tuber
D. bulbifera	Agar, O.7%	5.3	8.4	3	60
	Gelrite, O.24%	7.1	10.1	5	50
D.rotundata	Agar, O.7%	4.8	6.5	7.8	0
clone TDr205	Gelrite, O.24%	6.8	11.5	8.3	0
D.rotundata	Agar, O.7%	5.8	8.8	5	0
clone TDr743	Gelrite, O.24%	8	14.5	6	0

Table.3. Responses of nodal cultures of *Dioscorea rotundata* (clone TDr 747), to gelling agents and sucrose on MS medium.

gelling agent	*sucrose conc.*		Shoot Length, mm		New nodes per plantlet		Internode length, mm		Roots per culture		Plantlets with tuber, %	
		duration of culture:	*8 weeks*	*12 weeks*	*8 weeks*	*12 weeks*	*8 weeks*	*12 weeks*	*8 weeks*	*12 weeks*	*8 weeks*	*12 weeks*
agar	3%		55.3	71.5	4	9.8	8.4	10.5	6	9	0	0
gelrite	3%		61.3	75.7	5.7	10.3	8.7	11.3	8	12	0	20
agar	6%		52	73.5	3.5	9.3	9	12	8.5	16.8	50	90
gelrite	6%		55.5	79.3	5	10.5	9.8	12.3	9.8	20.5	60	100
agar	12%		36.8	47.5	1.8	2.8	7.8	10.5	6.3	12	0	0
gelrite	12%		24.3	25.8	0.8	1.3	6.1	7.4	4.3	5	0	0

Each value is a mean of five replicates of 30 plantlets.

Table 4. Plantlet development of *D. rotundata* clone TDR 743 on liquid medium, in shake culture and on membrane rafts.

Treatment	No. of nodes	Internode length	No. of Roots	Root length	Leaf width	Leaf length	No. of shoots	% leaves
shaken flask	4.13 b	1.8 b	7.25 b	14.28 a	3.4 a	2.79 a	1.25 b	55.57 a
membrane raft	5.25 a	3.46 a	16.38 a	7.13 b	1.46 b	2.07 b	2.25 a	33.56 b
cv	5.33	2.39	5.33	7.53	11.31	13.58	23.33	4.93

Table 5. Effects of kinetin on micropropagation of yam.

Kinetin, mg/l	no. of nodes	Internode length	no. of roots	root length	leaf width	leaf length	no. of shoots	% leaves
0	4.38 b	2.99 a	14.38 a	9.14	2.7	2.83	0.75	41.24
1	5.0 a	2.28 b	9.25 b	12.26	2.16	2.04	2.75 a	47.9
cv	8.15	1.82	2.12	8.94	11.56	29.49	32.99	20.04

Table 6. Effects of flurprimidol (fp) on rooting and shoot multiplication of yam in liquid shake cultures.

pretreatment, (mg/l fp)	treatment, (mg/l fp)	no. of roots	root length, mm	no. of shoots	leaves as % of biomass
0	3	27	5.5	38	33
0	6	21	1.2	2	25
3	3	39	11	42	13
3	6	25	8	18	3.5
6	3	49	10	79	11
6	6	0	0	0	0
12	3	30	1.5	37	7.3
12	6	0	0	0	0

Table 7. Effect of NH4NO3 and KNO3 on micropropagation of yam.

medium (treatment)	no. of nodes	internode length, mm	no. of roots	root length, mm	no. of shoots	% leaves	Growth Value
medium a (A)	7.25a	0.75bc	4.88cd	13.03bc	2.88b	0.65a	1.22a
medium a-$NH4NO_3$ (B)	5.0c	0.69c	5.13c	15.53b	2.5bc	0.60abc	0.84b
medium a-KNO_3+5.6 g/l KH_2PO_4 (C)	4.63c	0.43d	4.0d	10.89c	2.13c	0.61ab	0.96b
50% medium a (D)	6.38b	1.13a	8.13a	10.35c	2.38bc	0.56bc	0.83bc
50% medium a-NH_4NO_3 (E)	5.0c	0.90b	8.25a	20.76a	2.0c	0.52c	0.69c
50% medium a-KNO_3 + 2.8 g/l KH_2PO_4 (F)	6.13b	0.34d	6.75b	11.79c	3.63a	0.55bc	0.52d

data followed by the same letter are not significantly different at the 0.05 level of significance (DMRT).

Table 8. N effects on leaf area and numbers of shoots on yam clone TDr 205 growing in hydroponic solution

1a. leaf area

	KNO_3	NH_4NO_3	NH_4Cl
Leaf 1	21.73	37.05	18.72
Leaf 2	11.76	18.24	12.00
Leaf 3	32.20	22.95	20.80
Leaf 4	18.90	23.97	15.97
Leaf 5	11.84	11.04	17.00
Leaf 6	18.50	13.44	8.06
SUM =	93.20	126.69	92.55
AVERAGE	19.16	21.12	15.43

1b. shoots per plant

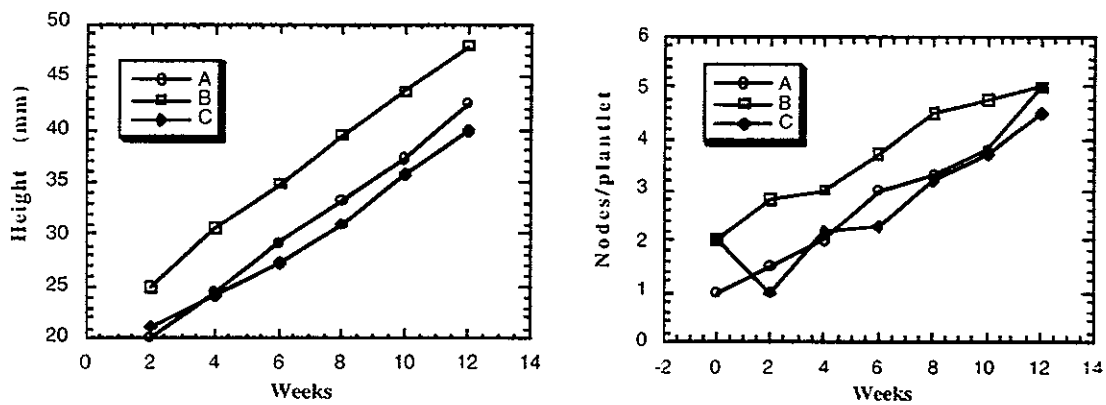
	KNO_3	NH_4NO_3	NH_4Cl
Plant 1	8	7	4
Plant 2	7	6	8
Plant 3	9	9	5
Average	8	7.3	6.3

Table 9. Yam tuber development in hydroponic solution with three N sources

Tuber Wt, grams

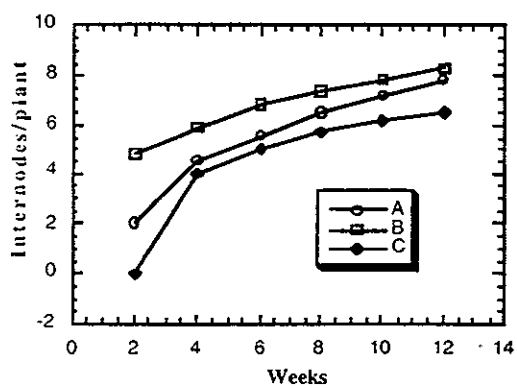
	KNO_3	NH_4NO_3	NH_4Cl
		tuber wt., grams	
3 months:	43.69 ± 12.50	51.57 ± 4.26	45.19 ± 8.08
5 months:	272.11 ± 53.93	488.51 ± 55.99	292.89 ± 55.34
7 months:	510.14 ± 63.86	734.99 ± 82.21	575.80 ± 62.46

Fig. 1. The influence of microcutting composition on rate and morphology of development.

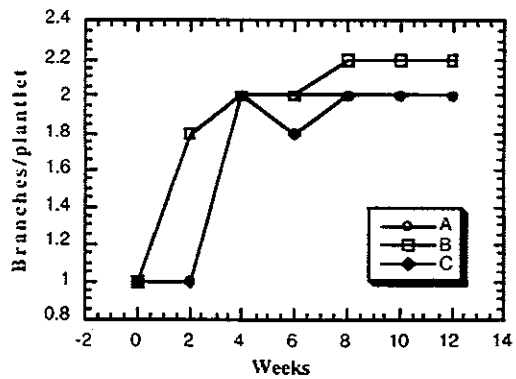


a. influence of explant on plantlet height

b. influence of explant on node proliferation



c. influence of explant on internode length



d. influence of explant on branching

Legend:

Treatment	Explant
A	Apex
B	Apex + 1 node
C	2 nodes

Figure 2. Effects of gelling agent and activated charcoal on plantlet height *in vitro*

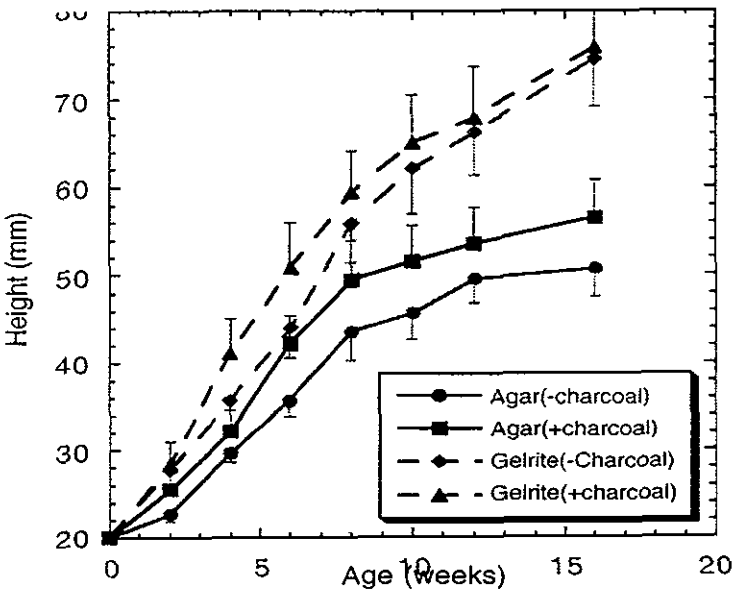
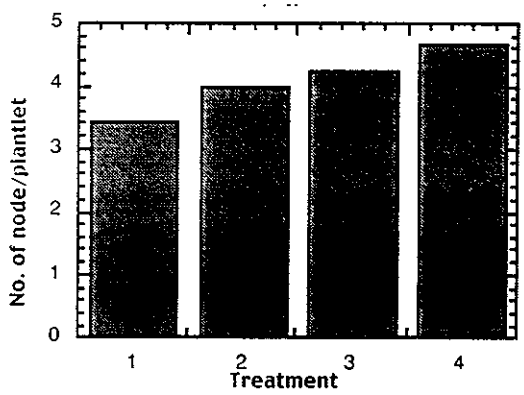
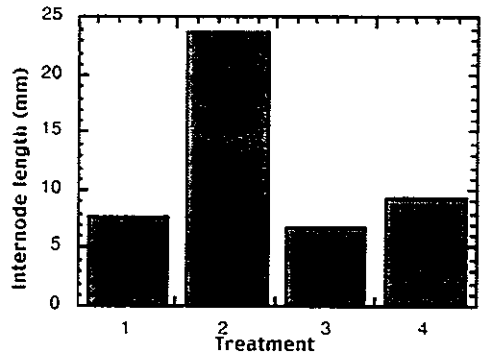


Fig. 3 (a-d). Effects of gelling agent and activated charcoal on node production, internode length, plantlet height and branching.



3a. Number of node/plantlet



3b. Internode length

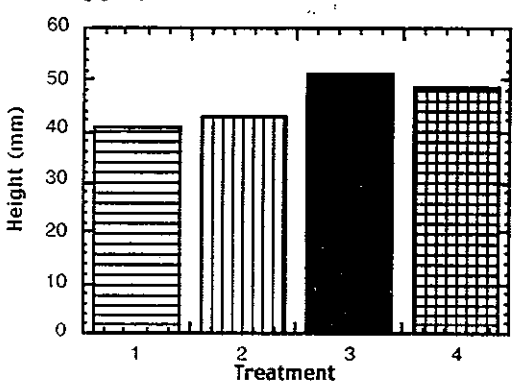


Fig. 3c. plantlet height charcoal.

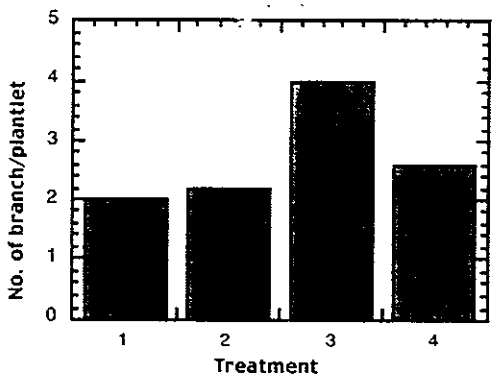


Fig. 3d. branches/plantlet

Legend:

Treatment	Gelling agent	+/-Charcoal
1	Agar	-
2	Agar	+
3	Gelrite	-
4	Gelrite	+

Figure 4. Effects of flurprimidol on *Dioscorea rotundata* plantlet development *in vitro*.

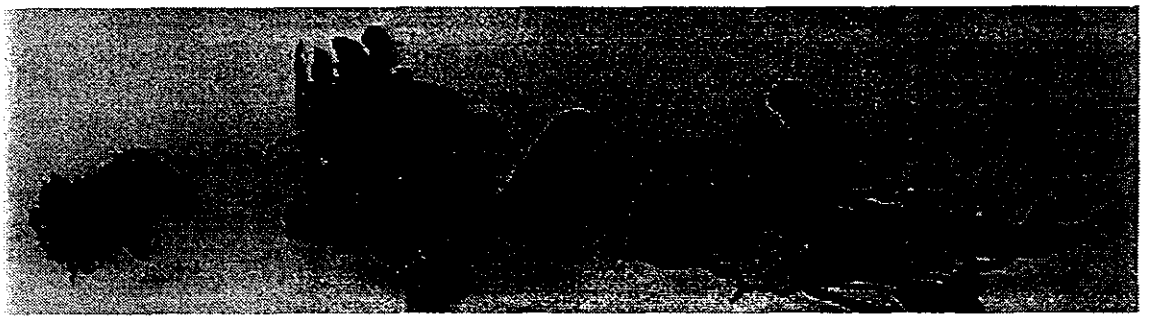
a b

culture in fp for 60 days followed by 55 days on semisolid hormone-free medium

a. 3 mg/l fp

b. 6 mg/l fp

Figure 5. Effects of successive culture on fp-containing liquid medium and culture on fp-containing medium followed by culture on hormone-free medium.



a b c

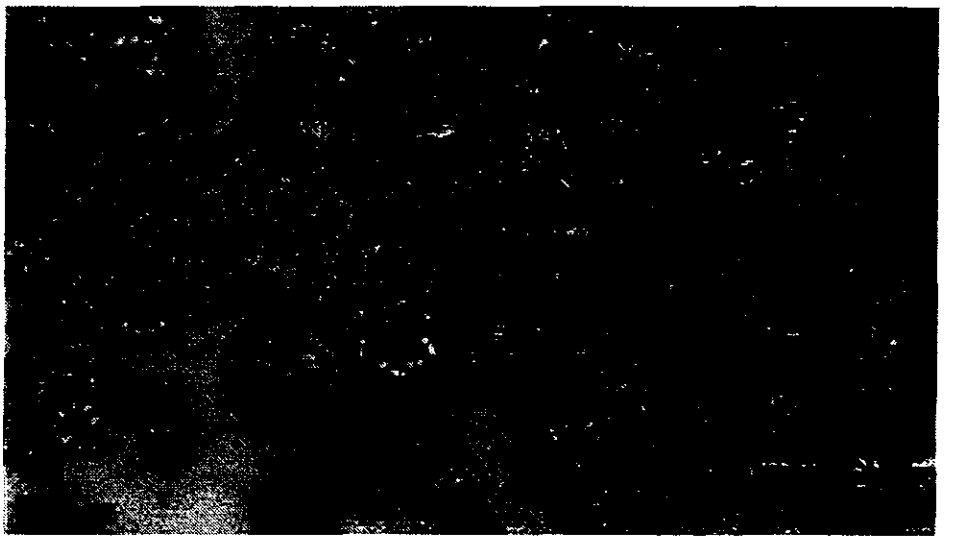
successive culture in fp-containing liquid medium

a. in 3 mg/l fp for 4 30-day periods with subdivision each 60 days

b. in 3 mg/l fp for 2 30 day-periods, followed by 2 30 day-periods without fp. subdivision was carried out once per 60 days

c. fp-free control (fp-free liquid medium) for 4 30-day periods, with subdivision each 60 days

Figure 6. Acclimatization of *Dioscorea rotundata* plantlets, propagated *in vitro* in media containing various concentrations of flurprimidol, in Sorbarod containers with liquid nutrient medium.



a b c d

Acclimatization of subdivided clumps of plantlets for 20 days in hydroponics on cellulose plugs

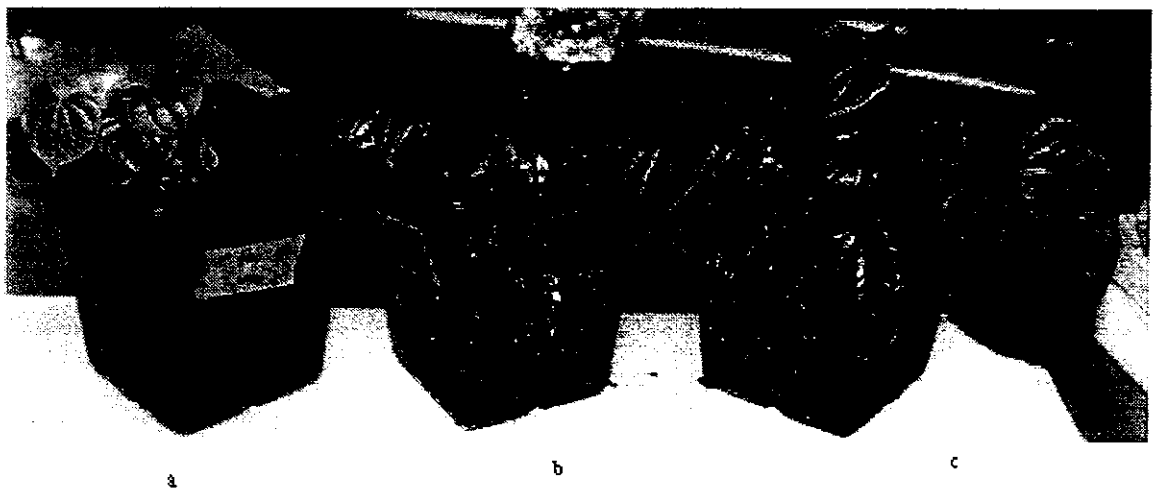
a. following culture in 6 mg/l fp for 120 days (5a)

b. following culture in 6 mg/l fp-containing medium for 60 days and culture in fp-free medium for 60 days (2b)

c. following culture in 3 mg/l fp-containing medium for 60 days and culture in fp-free medium for 60 days (5b)

d. following culture in 3 mg/l fp-containing medium for 2 -30 day-periods, followed by 2 -30 day-periods without fp (4b)

Figure 7. Acclimatization of *Dioscorea rotundata* plantlets, propagated *in vitro* on flurprimidol-containing and fp-free media, on a potting mix composed of vermiculite and peat (4:1 v/v).



Acclimatization on potting mix. Plantlets were held in the lab under cover of saran wrap. The covering was punctured after 7 days and removed after 10 days. The plants were derived from:

a. culture in liquid medium containing 6 mg/l fp for 60 days followed by hormone-free liquid medium for 60 days prior to planting out.

b. culture in liquid medium containing culture in 3 mg/l fp for 60 days followed by hormone-free liquid medium for 60 days prior to planting out.

c. culture in hormone-free liquid medium for 60 days followed by subdivision and reculture on the same medium for an additional 60 days followed by planting out.

Figure 8a-h. Carbohydrate content of pre-sprouting stage tubers of *Dioscorea rotundata* clone TDr 743, grown hydroponically and in soil.

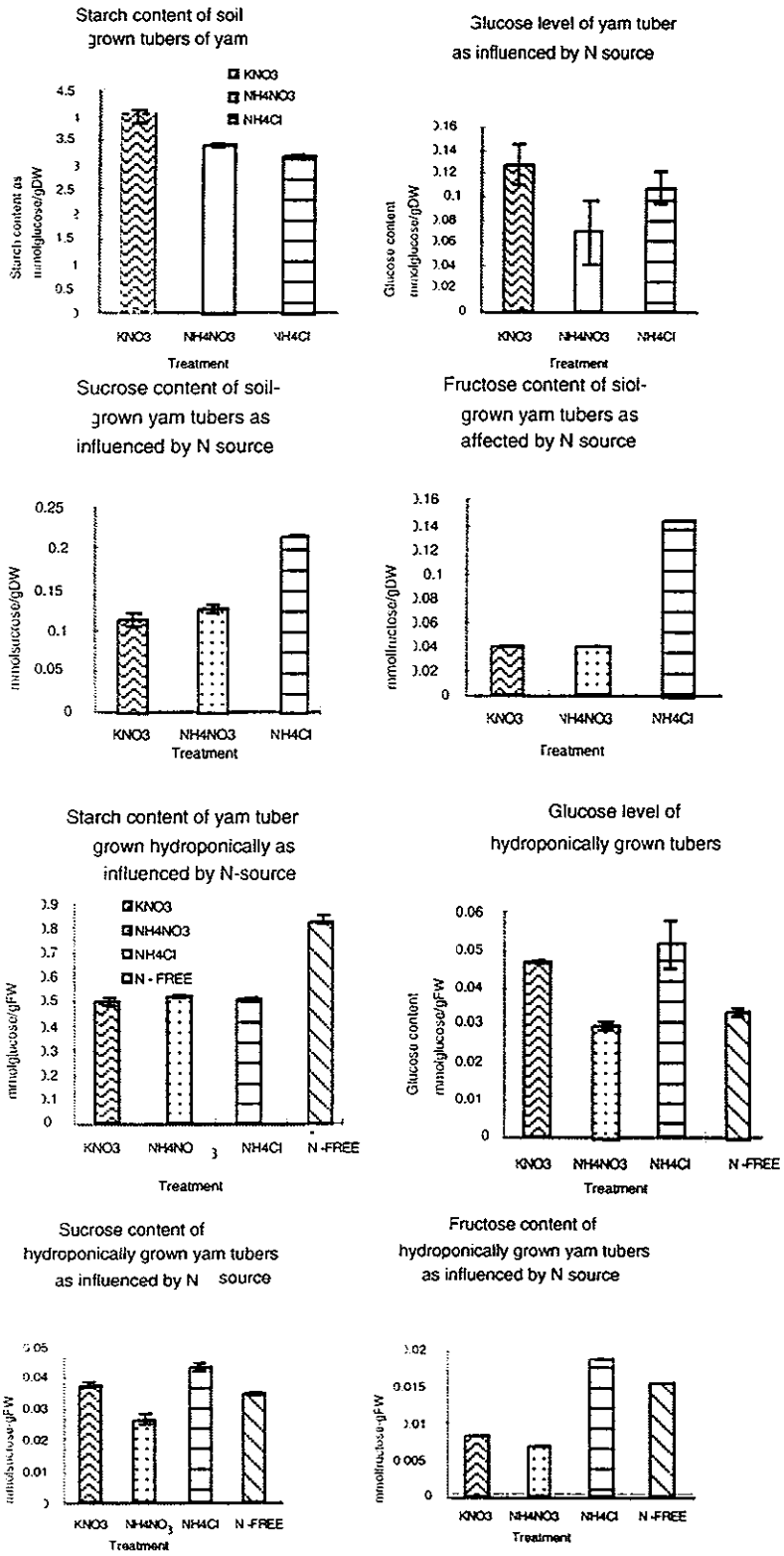


Figure 9. Nitrate reductase activity in various parts of yam plants grown in soil and in hydroponics.

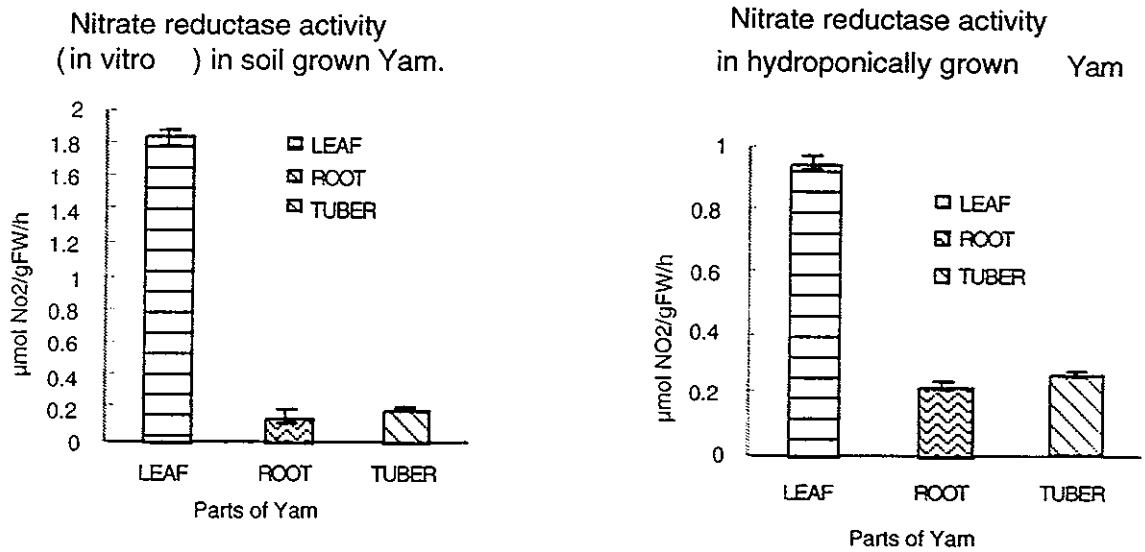


Figure 10. The influence of N source on nitrate reductase activity in leaves and roots of yam.

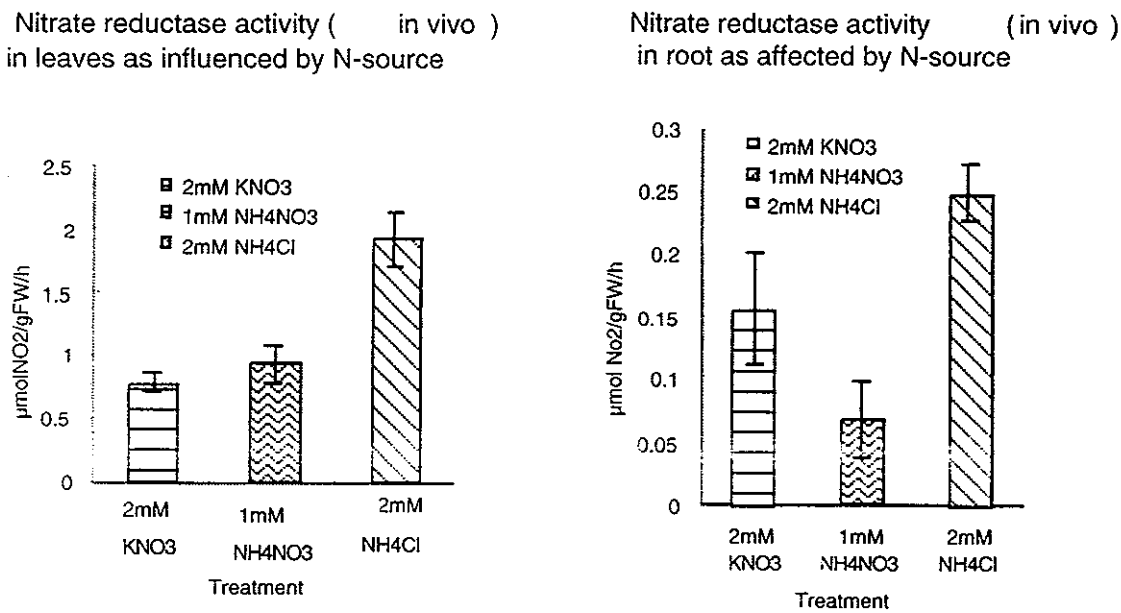


Figure. 11. The influence of N-source on nitrate accumulation in leaves and roots of yam.

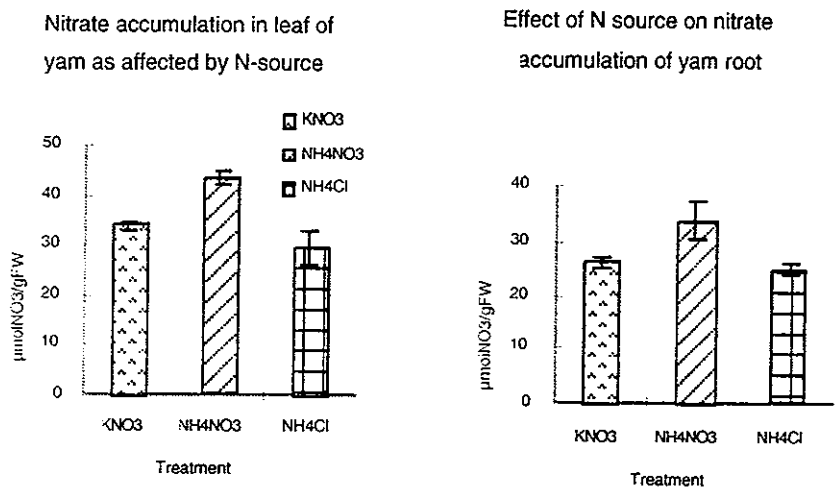


Figure 12. Total N in various plant parts of hydroponically grown yam as affected by the different sources.

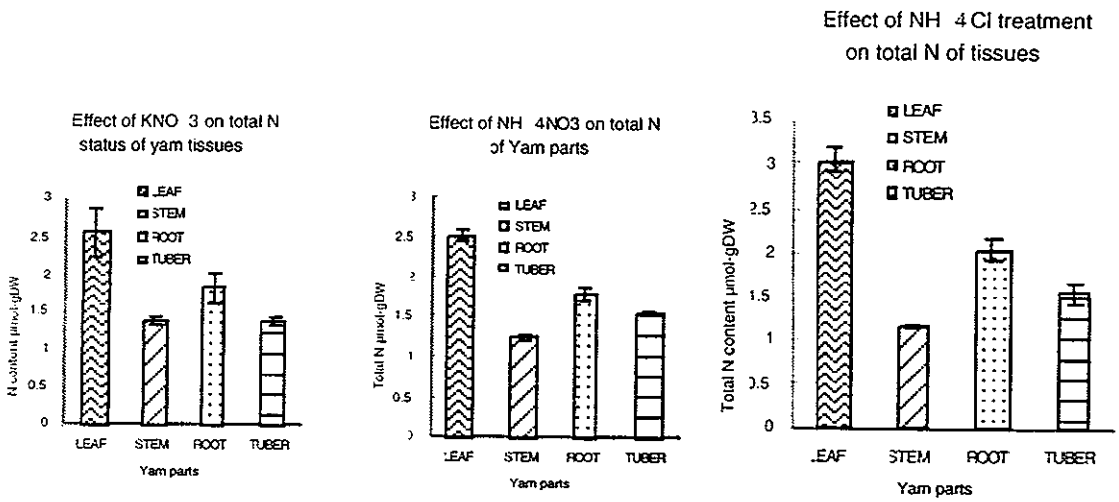
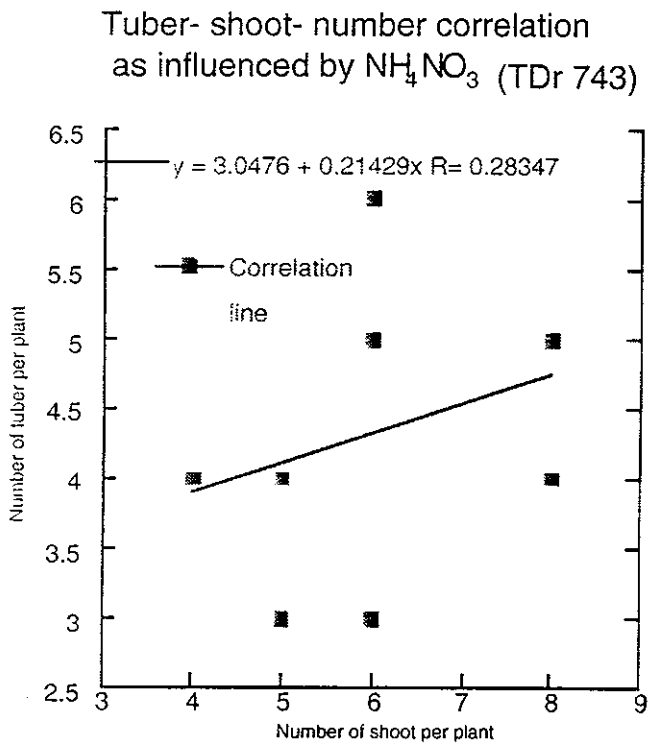
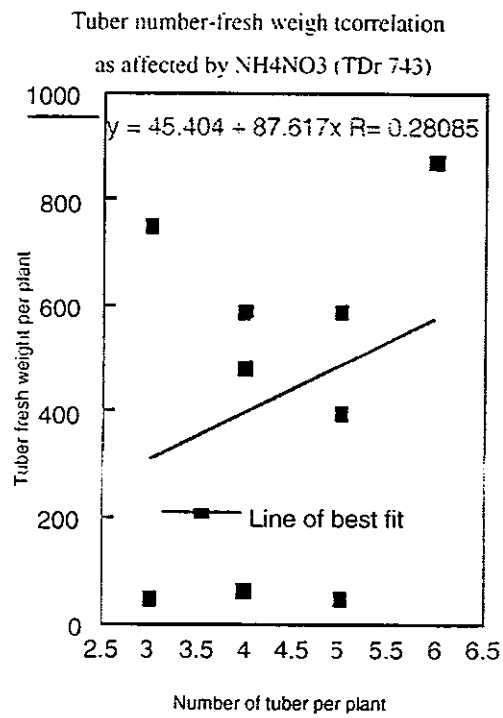


Figure 13. The correlation of numbers of shoots and tubers produced on yam clone TDr 743 in hydroponics.





IMPACT RELEVANCE AND TECHNOLOGY TRANSFER

Yam is a food staple in Nigeria, other parts of sub-Saharan Africa and much of the southern hemisphere. Production has not kept pace with demand, which has increased in parallel with population growth. Research on field plots has revealed the possibility of a four-fold average increase in yam yields over those attained by most growers. The goal of this program was to develop and/or adapt and merge advanced agro- and biotechnological methods, such as are applied in the west to potato and other root crops, in order to improve yam production.

Known methods of *in vitro* clonal propagation were improved, routines for acclimatization of plantlets produced by tissue culture were developed, and plants were grown in a greenhouse, in solid substrate as well as hydroponics, producing tubers. Aspects of the nutrition and physiology of the yam plant and tuber were studied.

Impact on NAU personnel and technology transfer

A member of the NAU faculty, two NAU graduate students and a Ph.D. student from the Philippines took part in the research. All spent various periods of time at the BIDR during the course of this program. Two of the students received training in plant tissue culture; the third studied physiological and biochemical aspects as well as greenhouse aspects, including acclimatization, hydroponics and tuber production. The NAU team left the BIDR with significant hands-on experience and academic background, fully capable of conducting trials and implementing methods developed for yam propagation and seed tuber production. The tissue culture trainees were also capable of conducting in vitro work on other crops that might be of value to their respective countries.

Collaboration with other Nigerian groups

Discussions were held during the course of the program with other research groups in Nigeria, including the International Institute of Tropical Agriculture (IITA) at Ibadan, the National Root Crops Institute (NRCI) at Umudike, the Science Equipment Development Institute (SEDI-E) at Enugu and Lagos State University (LASU) in Lagos. The IITA and the NRCI

collaborated from the onset, with respect to germplasm selection and maintenance. Virus-free clones from the IITA collection were used in our work. The NRCI maintained part of the germplasm bank assembled by Nwana. SEDI-E expressed interest in developing planting and harvesting equipment for yam, for which we hope financial support will be found. LASU. is situated in an area of well-drained, sandy soil, the sort considered ideal for cultivation of root and tuber crops, although yam is traditionally grow in heavier soils. Aspects of yam cultivation in sandy and saline soils may be advanced by researchers there.

Facility Preparation:

A building at NAU was renovated and fully equipped for use as a plant tissue culture laboratory. It was outfitted with standard laboratory furniture and specific equipment and facilities for tissue culture work, including a clean room with laminar flow hood and a controlled environment growth room. By the end of the collaborative program. the NAU team had the facilities as well as the capabilities to undertake large-scale clonal propagation of disease-free yam cultivars by tissue culture and production of seed tubers in soil or by hydroponics.

PROJECT ACTIVITIES/OUTPUTS

Training and Visits of Senior Scientists

Birnbaum visited NAU and collaborating institutions in Nigeria including the IITA. twice in the course of the program and Lips visited once. Nwana visited the BIDR twice during the program (in addition to an extended stay several years earlier. during which this collaboration was initiated).

Three individuals were trained in the program framework. Ms. Virginia Nzegwu. a Ph.D. student at NAU. Mr. Alex Izundu. an NAU M.Sc. student and Ms. Cecilia Villamor. a Ph.D. student at Don Mariano Marcos Memorial State University. in The Philippines (where Yam is also an important crop). Ms. Nzegwu and Mr. Izundu each spent about 18 months at the BIDR and Ms. Villamor was there for two years. Nzegwu and Villamor worked in the tissue culture group. learning *in vitro* techniques and participating in the research: Izundu worked on

the physiological, biochemical and greenhouse aspects, including acclimatization, hydroponics and tuber production. Each gained significant hands-on experience as well as in depth academic background on the subject of his/her specialization and was fully capable of conducting trials and implementing methods developed for yam propagation and seed tuber production following his/her return home.

PUBLICATIONS AND PRESENTATIONS AT INTERNATIONAL CONGRESSES

Anselmo, Belinda A., Elliott H. Birnbaum, Yair M. Heimer and Ali Nejidat. 1998. Random amplified polymorphic DNA (RAPD) in *Dioscorea* germplasm from Nigeria, Kenya and Philippines. Proc. IX Congress on plant Tissue and Cell Culture, Jerusalem, June 14-19, 1998.

Birnbaum, E.H., Virginia Nzegwu, P. Melnikov and I.E. Nwana. 1997. Effects of explant and medium composition on development of white yam, *Dioscorea rotundata Poir* and aerial yam, *D. bulbifera L., in vitro*. Paper presented at the International Conference on Biotechnology for development in Africa: priorities for the early twenty-first Century. Organized by FADIB (Foundation for African Development through International Biotechnology), Enugu, Nigeria February 9-13, 1997.

Villamor, Cecilia and E.H. Birnbaum. 1998. Effects of Flurprimidol on Micropropagation of Yam (*Dioscorea rotundata*). Proc. IX International Congress on Plant Tissue and Cell Culture, Jerusalem, June 14-19, 1998.

PROJECT PRODUCTIVITY

The overall objective of the program was to contribute to the improvement of yam productivity. The specific objectives were to select high-yielding yam cultivars with desirable characteristics, to improve *in vitro* procedures for plantlet and microtuber production and to develop efficient systems for seed tuber production. Additional objectives were to develop agrotechnologies for yam and to conduct study the development of storage roots. All objectives were addressed and largely accomplished. Germplasm collection, selection and field trials were carried out by the Nigerian collaborator together with a network of cooperating scientists at other Nigerian institutions. Virus elimination was accomplished at

the IITA at Ibadan, Nigeria, from which clean cultures were provided for *in vitro* studies in Israel. A known *in vitro* method of yam clonal propagation (on semisolid medium) was improved and a more efficient method, based on culture in liquid medium, was developed. Methods of acclimatization and seed tuber production in both solid medium and in hydroponics were developed. Physiological aspects of plant and tuber nutrition, particularly with respect to nitrogen metabolism, were defined and the associated morphogenesis characterized.

The various results of the program are ready for application to yam propagation and cultivation. If used appropriately, they hold the potential to significantly improve the productivity of this important crop.

FUTURE WORK

Several of the areas addressed in this program should continue to receive attention.

Germplasm selection should be viewed as a long-term objective, to be carried out continuously in Nigeria and other yam-growing areas of the world.

The liquid-based micropropagation system should be defined further and adapted for large scale application. The technology should be transferred to commercial or government-supported laboratories in Nigeria and other yam consuming countries. Similarly, the acclimatization protocols should be made available to propagators in the target countries.

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