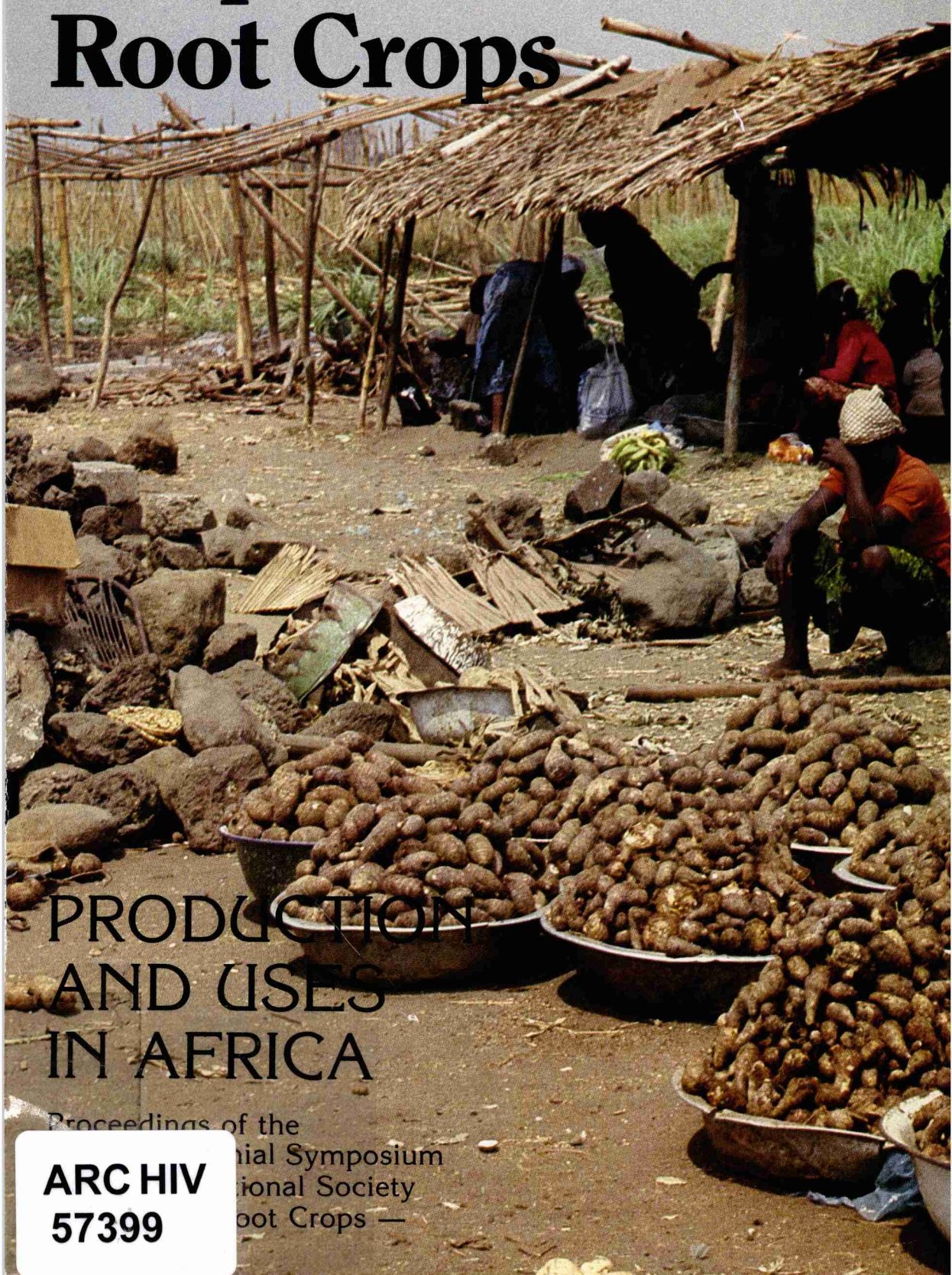


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Tropical Root Crops



PRODUCTION AND USES IN AFRICA

Proceedings of the
International Symposium
International Society
Tropical Root Crops —

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The International Society for Tropical Root Crops — Africa Branch was created in 1978 to stimulate research, production, and utilization of root and tuber crops in Africa and the adjacent islands. The activities include encouragement of training and extension, organization of workshops and symposia, exchange of genetic materials, and facilitation of contacts between personnel working with root and tuber crops. The Society's headquarters are at the International Institute of Tropical Agriculture in Ibadan, Nigeria, but its executive council comprises eminent root and tuber researchers from national programs throughout the continent.

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IDRC-221e

Tropical root crops: production and uses in Africa : Proceedings of the second Triennial Symposium of the International Society for Tropical Root Crops — Africa Branch held in Douala, Cameroon, 14–19 August 1983. Ottawa, Ont., IDRC, 1984. 231 p.: ill.

/Cassava/, /root crops/, /plant production/, /Africa/ — /genetic improvement/, /planting/, /plant diseases/, /pests of plants/, /intercropping/, /fertilizers/, /crop yield/, /sweet potatoes/, /plantains/, /agriproduct processing/, /nutritive value/, /food enrichment/, /feed/, /agricultural research/, /conference report/, /list of participants/.

UDC: 633.68

ISBN: 0-88936-409-5

Microfiche edition available.

Il existe également une édition française de cette publication.

TROPICAL ROOT CROPS: PRODUCTION AND
USES IN AFRICA

ARC 411 V
633.68
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1983

ABSTRACT

A mixture of original research, updates on procedures, literature reviews, and survey reports, this document resulted from the second symposium of the International Society for Tropical Root Crops — Africa Branch, with 77 participants from 16 countries. The focus was cassava, yams, cocoyams, and sweet potatoes, from the perspectives of breeders, agronomists, soil specialists, plant pathologists, entomologists, nutritionists, food technologists, etc. Learning from past successes and failures, many of the researchers directed their efforts toward problems obstructing progress in reaching improved production and use of root crops and attempted to view, realistically, the context in which their results would be applied.

RÉSUMÉ

Résultats de recherches récentes, mises à jour sur les méthodes de recherche, revues de publications et rapports de sondages sont contenus dans ce document issu du Deuxième symposium de la Société internationale pour les plantes-racines tropicales — Direction Afrique, qui a réuni 77 participants de 16 pays. Des communications sur le manioc, le taro, le yam et la patate douce ont été présentées par des phytosélectionneurs, des agronomes, des pédologues, des phytopathologistes, des entomologistes et des spécialistes de la nutrition et des aliments, entre autres. Tirant leçon de leurs succès et de leurs échecs, beaucoup de ces chercheurs ont dirigé leurs efforts vers la solution des problèmes qui entravent l'augmentation de la production et de la consommation des plantes-racines et ont tenté de considérer d'un œil réaliste le contexte qui sera celui de l'application de leurs recherches.

RESUMEN

Una mezcla de investigaciones originales, actualizaciones de procedimientos, reseñas de literatura e informes de encuestas, este documento es el resultado del segundo simposio de la Sociedad Internacional de Raíces Tropicales, Filial Africana, que contó con 77 participantes de 16 países. El simposio se centró en la yuca, el ñame, el cocoñame y las batatas, desde la perspectiva de los fitomejoradores, los agrónomos, los especialistas en suelos, los patólogos vegetales, los entomólogos, los nutricionistas, los tecnólogos alimenticios, etc. A partir de los éxitos y fracasos anteriores, muchos de los investigadores encaminaron sus esfuerzos hacia los problemas que obstaculizan el avance para lograr una producción y un uso mejorados de las raíces y trataron de obtener una visión realista del contexto en que los resultados pueden ser aplicados.

IDRC-221e

TROPICAL ROOT CROPS: **PRODUCTION AND USES IN AFRICA**

EDITORS: E.R. TERRY, E.V. DOKU, O.B. ARENE, AND N.M. MAHUNGU

*PROCEEDINGS OF THE SECOND TRIENNIAL SYMPOSIUM OF THE INTERNATIONAL
SOCIETY FOR TROPICAL ROOT CROPS — AFRICA BRANCH HELD IN DOUALA,
CAMEROON, 14 – 19 AUGUST 1983*

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IN-VITRO CULTURE OF *DIOSCOREA ROTUNDATA* EMBRYOS

C.E.A. OKEZIE, F.I.O. NWOKE, AND S.N.C. OKONKWO¹

Dioscorea rotundata (Obiaoturugo variety) embryos were found to germinate faster in Murashige/Skoog (1962) and Linsmaier/Skoog (1965) media than if left in intact seeds. The ability of the embryos to germinate in the two media increased with time from seed harvest — an indication that recovery of seeds from dormancy is gradual and that the embryos are immature at the time of seed harvest. The latter is further supported by the embryo's gradual increase in size and transformation from globular to fan-shaped during the 3–4 month dormancy. The technique used in this study could enhance breeding programs.

Wilson (1978) reported that the development of methods for producing yam seeds and growing large populations of white yam (*Dioscorea rotundata*) seedlings opens the way for the traditional approach to breeding for crop improvements.

Tissue culture in yam propagation, though still in its infancy, has permitted plantlet formation from yam tuber blocks (IITA 1973), from nodal sections of young yam shoots (IITA 1974), from nodes and shoot meristems of *D. rotundata* (IITA 1977), from single node cuttings of *D. alata* (IITA 1980), and from embryos of *D. rotundata* (Okezie 1981). Perfection of this technique will not only provide the quickest mode of multiplying improved cultivars in a disease-free form (IITA 1977) but will also aid tremendously in generating uniform plantlets that will reveal attributes of the growth characteristics that could be exploited in a breeding program.

This paper reports on the possibility of raising plantlets from *D. rotundata* embryos in vitro on both Murashige/Skoog (1962) and Linsmaier/Skoog (1965) media even without hormonal additives. It also speculates on the potentials of this in-vitro system in yam improvement.

MATERIALS AND METHODS

The embryos were derived from seeds of *D. rotundata* (Obiaoturugo variety) fruits harvested from experimental farms of the National

Root Crops Research Institute, Umudike, in October 1981 at the end of the yam-growing season.

The seeds were surface-sterilized in 1% sodium hypochlorite solution for 10 minutes and rinsed in four changes of sterile, distilled water. The embryos were extracted and inoculated in 20-mL portions of 1% (10 g/L) sterile Difco-Bacto agar slants in two media without hormonal additives, namely modified Murashige and Skoog (1962) basal medium and modified Linsmaier and Skoog (1965) medium. Sucrose (3%, 30 g/L) was used as a carbon source for the growth of the embryos, and the pH of the media was adjusted to between 5.5 and 5.8. Before inoculating with the embryos, we autoclaved the media. Inoculation was done in a sterile environment (ultraviolet radiation for 30 minutes). One embryo was inoculated into each culture tube, and there were 25–30 tubes/treatment. Surface-sterilized intact seeds were also planted in 20-mL portions of 1% Difco-Bacto agar slants to serve as controls. The tubes were kept in a dark incubator at $27 \pm 1^\circ\text{C}$.

In one study, seeds were dewinged and stored in a desiccator for 4 months to ensure complete breakage of dormancy.

In a second study, the fruits were still green on the plant, and germination studies on the embryos were started immediately. The fruits were spread out to dry in the sun and the embryos were extracted monthly for serial germination tests. The tests lasted 4 months. The embryos and seeds were allowed to grow in the media for a total of 50 days.

In the third study, some seeds were soaked in

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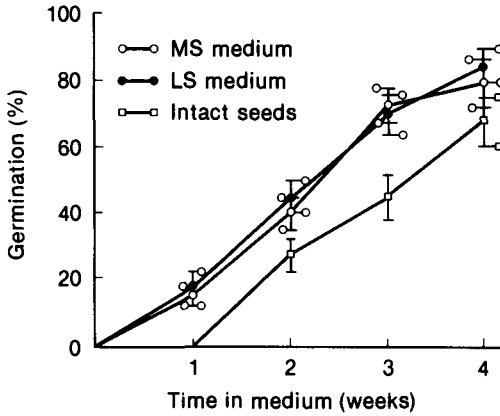


Fig. 1. Germination (%) of *Dioscorea rotundata* seeds compared with embryos in modified Murashige/Skoog and Linsmaier/Skoog media without hormonal additives.

water for 24 h and carefully dissected. The embryos — 40–50 monthly for 6 months — were extracted and mounted on a slide. Length and breadth were measured by means of a calibrated ocular micrometer (Erma Optical Works Limited, Japan). Also, the excised embryos were assessed for their shapes and categorized as globular or fan-shaped and the percentage of each recorded.

RESULTS

Both Murashige/Skoog and Linsmaier/Skoog

media without hormonal additives supported the germination of *D. rotundata* embryos (Fig. 1). Although there was no significant difference in the extent to which the two media induced germination, both supported more germination than did intact seeds (control) within the first 3 weeks of treatment. By the 4th week, only Linsmaier/Skoog medium induced a significantly higher percentage of the embryos than did intact seeds (control).

The number of days to first germination and 50% germination for Linsmaier/Skoog medium, Murashige/Skoog medium, and intact seeds (control), respectively, was 5, 15; 6, 17; and 11, 24, both media inducing germination much faster than intact seeds.

Neither of the two media nor intact seeds (control) could support embryo germination immediately after fruit harvest at the end of the growing season (Table 1). Intact seeds could not even germinate after 1 month from fruit harvest, although both Murashige/Skoog and Linsmaier/Skoog media stimulated germination of some excised embryos. Whereas it took 33 days to notice germination under Linsmaier/Skoog medium, it took 39 days to notice it in Murashige/Skoog medium, with percentage germination also being higher in the former than in the latter. None of the media could support up to 50% germination of embryos within the 1st month. The potential for germination increased with time after harvest.

There was progressive growth of the embryos in length and breadth within the seed after har-

Table 1. Effects of modified Murashige/Skoog (MS) and Linsmaier/Skoog (LS) media without hormonal additives on the germination of developing *D. rotundata* embryos.

Age (months) of embryos from time of first harvest	Treatment given to embryos	Days to first germination	Days to 50% germination	Germination after 4 weeks (%)
0	MS medium	—	—	0
	LS medium	—	—	0
	Intact seeds	—	—	0
1	MS medium	39	—	10.5
	LS medium	33	—	12.5
	Intact seeds	—	—	0
2	MS medium	23	33	40
	LS medium	18	30	45
	Intact seeds	29	39	25
3	MS medium	10	18	70
	LS medium	8	14	70
	Intact seeds	14	24	60
4	MS medium	6	15	80
	LS medium	5	11	85
	Intact seeds	12	20	65

Table 2. Sizes of *D. rotundata* embryos in course of postharvest development.

Time (months) from fruit harvest	Size of embryo		Globular embryos (%)	Fan-shaped embryos (%)
	Length (μm)	Breadth (μm)		
0	685	537	83	17
1	892	624	64	36
2	973	715	52	48
3	1066	787	44	56
4	1090	820	38	62
5	1105	820	32	68
6	1115	822	29	71

vest (Table 2). This increase did not continue beyond the first 6 months from harvest. At harvest, the embryos were mostly globular, but, with time, more than 70% became fan-shaped, reflecting growth of the cotyledon.

DISCUSSION

Both modified Murashige/Skoog and Linsmaier/Skoog media were superior to the natural constituents of the seed endosperm in stimulating the germination of nondormant, viable *D. rotundata* embryos, even in the absence of hormonal additives. Modified Murashige/Skoog medium reduced the germination time by 5 days, and modified Linsmaier/Skoog medium reduced it by 6 days.

In-vitro embryo culture eliminates the constraints of seed germination (embryo growth and emergence of radicle) caused by the seed coat or the endosperm.

Waitt (1959) suggested that seeds contain rudimentary embryos at the time of harvest and that a period of drying permits the embryos to mature and thus ensures germination. In a review of sexual propagation for yam improvement, Sadik (1976) stated that other difficulties in seed germination had masked the 3–4 month dormancy. One of us (Okezie 1981) showed through serial germination of both intact seeds and excised embryos that dormancy in Obiaoturugo variety is completely broken by about 14 weeks after harvest. The present study has shown that intact seeds were not capable of germinating within 1 month of harvest, although modified Murashige/Skoog and Linsmaier/Skoog media were able to induce germination of up to 10.5% and 12.5% of embryos, respectively. This suggests that dormancy can be broken through embryo culture even within the 1st month from harvest. The progressive increase in the ability

of both excised embryos and intact seeds to germinate suggests that recovery from seed dormancy is gradual, not spontaneous. The exact mechanism of recovery from dormancy is not known, but the embryo seems to acquire factors from the endosperm for 3–4 months from harvest.

The in-vitro system described in this paper provides a level of experimental flexibility not easily attained through the use of seed or seed tubers in the propagation of yams. With the efficient development of embryo culture, greatly increased rates of multiplication can be achieved. This technique could also be exploited in generating uniform clonal materials for both initiating a viable breeding program and providing materials for experimental work. Tissue culture is also a means for developing long-term storage of germ plasm and rapid multiplication of improved cultivars in disease- and insect-free form to meet the demands of national programs anywhere in the world (IITA 1977; Henshaw 1979).

Embryo culture could be used to circumvent postfertilization barriers to sexual hybridization. This has been achieved in *Coffea canephora* and *C. dewevri* in modified Heller medium without hormones and *C. arabica* in Linsmaier and Skoog medium (Monaco et al. 1977).

Immature embryos of *D. rotundata*, which would be aborted if allowed to develop in the seed through the 3–4 month dormancy, could be made to germinate in vitro in a properly formulated medium. Some measure of success in this direction has been reported (Okezie 1981) for *D. rotundata* embryos that were cultured 2 weeks from seed harvest. Similar findings have been reported on embryo axis of *Phaseolus vulgaris* (Crocomo 1981) for avoiding seed abortion from interspecific crosses and on coconut embryo (De Guzman 1980) for propagating a non-germinating type of coconut known locally as makepuno. Jagannathan (1980) also used in-

vitro culture for the growth of immature *Carica papaya* embryos, which usually abort if allowed to mature on the pod.

Both in-vitro embryo culture and fertilization have potential to overcome hybridization barriers between species and possibly genera. Tech-

niques are already well established and have been successfully applied in several species including wheat and barley (Nabors 1980) and *C. arabica* (Walyaro and Van der Vorse 1977). This technique could be borrowed for *D. rotundata* and could even permit hybridization with other species.