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POTENTIAL NON-TUBER MATERIALS FOR YAM PROPAGATION

By

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INTRODUCTION

Boost in industrial development of Nigeria now requires a greater supply of diverse carbohydrate, protein and oil crops as raw materials for industry, feed and food. Grain legumes and cereals have advantage over root and tuber crops because:

1. Grain seeds are small, cheaper and easier to provide planting material for large hectarages; while the tuber crops such as yams are propagated from expensive and bulky whole tuber or sett pieces.

2. Cultivation of grain cereals and legumes is easier to mechanize.

3. Storage of grain cereals and legumes is easier than for yams.

However, yam production and usage have always occupied a pride of place in West Africa where the greater portion, over 75% of the world supply (Coursey, 1967; Onwueme, 1978) is grown. There is need for yam research to improve methods for cultivation, harvest, storage, and tuber use. This is so, particularly the breeding of new varieties that need no stakes, require less labour and planting material (Wilson, 1979), have medium-sized tubers which ease mechanized harvest. One approach in this regard will be to devise and utilize parts of the yam plant other than the tuber as propagules and thus conserve tuber for industrial raw materials, food, or feed. The state of our knowledge of these propagative methods is the theme of this paper.

The following propagative methods are currently being investigated: vine cuttings, cell, tissue, and organ culture, embroyo culture, and true seeds.

VINE CUTTINGS

Since Njoku's (1963) first study of edible yam propagation, after those of Correl et al.,(1955) on wild yams, through vine nodal cuttings, others have followed (Ferguson, 1972; Martin & Gaskins, 1968; Cabanillas & Martin, 1978). Blunden et al., (1966) propagated D. belizensis through vine cuttings, but cuttings of D. deltoides and D. sylvatica failed to develop organs. Blunden et al. (1966) noted an unusual form of multiplication from vine cuttings of D. spiculiflora. The plantlet that originated from the nodal leaf axil tended to multiply itself into several parallel stems that could then be separated and repropagated continously. This rosette type of growth is rare in the

Dioscoreaceae.

Subsequently, Martin & Gaskins (1968) propagated D. spiculiflora through vine cuttings. A cutting usually consisted of a single leafy node excised from the vine in such a way that 2-2.5cm of the vine was left on iether side of the node (Njoku, 1963). Martin & Gaskins (1968) treated the cuttings with fungicides before planting in nurseries of well-drained and aerated medium of vermiculite, peat mass, sand of gravel. Cuttings survived and rooted only when kept moist through periodic, mist spray. Cuttings rooted even in full sunlight if protected against dessication by the mist spray, but, nurseries under shade needed less water spray and often prevented rotting of cuttings due to algal and fungal infection.

Regeneration of new organs began from the axil of the leaf following initiation of new tuberous tissue. Then, roots and shoot buds grew from the tuber tissue. Regenerated cuttings were removed periodically and hardened by being planted in pots or larger beds, before transfer to the field. Propagation was most rapid from nodes bearing fully expanded, young leaves from vigorously growing stems. Such juvenile material was most readily obtained from the vigorous growth flushes occurring early in the growth cycle of plants at the onset of the rainy season. Propagation from cuttings obtained from older plants later in the planting season (as plants approached reproductive stages) became incresingly difficult. Martin & Gaskins (1968) concluded from these studies that the main importance of this method of propagation is for clonal multiplication of superior cultivars and for germplasm preservation, rather than for production of sizeable tubers. Vasanthkumar (1981) also studied propagation of D. floribunda and D. composita by single leaf node cuttings with similar results.

Njoku's (1963) report on the propagation of edible yams through vine cuttings was restricted to the three species of D. alata, D. rotundata and D. dumetorum. He maintained his single-node cuttings in water or potted soil to root. The sequence of regenerative development of the cuttings through callus, root and shoot formation followed the same pattern as described above (Martin & Gaskins, 1968) for sapogenin-bearing yams. Njoku found that the pattern of development of the cuttings was affected by daylength. Their exposure to long daylength promoted vine growth while exposure to short daylength promoted tuber growth. Ferguson (1972) reported that the first new tissue development from yam vine cuttings is a stem tissue and not root. He suggested that harmonal treatments of stem cuttings to stimulate rooting was not necessary.

Okonkwo et al., (1973, 1986) planted cuttings of D. alata, D. rotundata, and D. bulbifera directly in the field, under palm leaf shade and frequent watering. Only D. bulbifera cuttings survived. Further studies on D. bulbifera node cuttings in potted soil in greenhouse and in the open showed that there are three primordia in leaf axils: primary and secondary axillary buds and a bulbil primordium (Fig.1). Of these, the bulbil primordium proliferated in the plated node cutting to form white callus tissue two weeks after planting. By the third week, two stout shoots were produced from the proliferated

tissue (Fig.2). The callus later produced tubers, more roots from the surface of the tuber and laterals from the two primary roots. Shoot differentiation from the proliferated tissue followed 7 to 8 weeks after the cuttings were planted. The primary and secondary axillary buds were dormant as Njoku (1963) also observed. Okonkwo et al. (1973, 1986) showed that the age of the plant affected the regenerative ability of cuttings. Cuttings from young plants (5 weeks or less) regenerated root, tuber and shoot, whereas those from older plants (10 weeks and above) regenerated tubers and roots only, but cutting led to increase in the growth of the resulting organs-roots, tuber and shoots, and therefore more vigorous new plants than those from one-node cutting (Okonkwo et al., 1973,1986; IITA, 1975). Cabanillas and Martin (1978) had good response with D. rotundata, D. esculenta and D. trifida, which confirmed the influence of age in all the satudies. Although the method of propagating yams through vine cuttings has been criticized as not holding out much hope for commercial yam tuber production due to the small size of tubers formed, several workers have continued to experiment on improving tuber yield from vine nodal cutting. When grown under carefully controlled greenhouse conditions, mean tuber size from cuttings reached 420g (IITA, 1975). Vander Zaag and Fox (1981) produced six cultivars of yams from cuttings in the field and obtained large-sized (ware) yams. After five weeks, cuttings that had produced shoots and roots were hardened in potted soil (1:1:2 mixture of vermiculite, perlite and soil). Two weeks later, they were transplanted to he field. The most vigorous cultivar produced tubers at the rate of 46 t/ha, three at 10 t/ha and two failed outright. Akoroda & Okonmah (1982), in a five-year study, employed 4 to 6 node leafy vine cuttings in order to produce "seed tubers" or setts for farmers and researchers, and to maintain germplasm collections in the live form. Estimated field production of 5400 setts/ha (at 0.5 x 0.5 in. spacing) of at least 250 g, or 13248 setts/ha at least 200g were expected for a cultivar of D. alata. Perhaps,additional periodic field dressings of fertilizer on the field-grown cuttings could increase their tuber yield to ware size (Njoku et al.,1984).

CELL, TISSUE AND ORGAN CULTURE

Tissue culture techniques are just being applied for edible yam propagation research, but has been applied to medicinal yams. Defoliated nodal cuttings, excised leaves, vine (shoot) meristem, tuber tissue, callus, cells, and embryos, have been used as source materials to establish plantlets.

To obtain defoliated node cuttings, the lamina was cut off close to the base of the peticle of one-node leafy vine cuttings produced as describe previously. After surface sterilization, the explants were planted on suitable agar nutrient media to stimulate growth of regenerative organs from the axillary meristems at the nodes. One-node vine segments of D. floribunda, a sapogenin-containing yam, could be cultured on basal nutrient medium supplemented with either adenin sulphate and NAA (Lakshmi et al., 1976) to yield vigorously growing plantlets which could be used as source materials for further cultures. In other studies, Fonin & Voloshina (1975) showed that tossue cultures of the steroidal plants D. caucasica and D. nipponica could partially synthesize the

substances which were characteristic of the whole plant. Sinha & Chaturvedi (1979) initiated some cultures of D. floribunda from excised leaves. The leaf explants were devoid of the axillary buds but must contain the pulvinus. They showed that regrowth and development of multiple shoots occurred from the pulvinar tissue. The plantlets obtained were successfully transplanted into soil.

The propagation of edible yam through culture of leafless node cuttings has been studied (Uduebo, 1971; Mapes & Urata, 1970; Okonkwo et al., unpublished).Figure 3 (Okonkwo unpublished) shows that with the right culture medium, roots, tuber and shoots could be regenerated from the axillary complex of the node cutting of D. bulbifera. Basal nutrient medium supplemented with 0.5 mg L^{-1} kinetin stimulated productin of 3 to 4 shoots from the nodal axillary complex (Fig. 3) as Frosyth & Van Staden (1982) also observed in D. bulbifera. Mantell et al., (1978) used the leafless node cutting culture technique to multiply clones of D. alata and D. rotundata. In their studies, regeneration of shoots and roots was high for cuttings taken from plants geown under 16-hour photoperiod. Cutting from plants grown under the natural 12-hour daylength gave erratic regeneration. Plantlets developed from nodal segments after 3-5 weeks, and could be established in steam-sterilized (4:4:3 peat:sand:soil) mixture. However, shoots of plantlets in culture had many branches with numerous leafy nodes and short internodes. Clones were further multiplied by dissecting plantlets in cultures. Shoots were split into 3-5 separate nodal segments, each with one or more leaflets and transfered onto fresh basal medium. After 14 to 20 days, new plantlets developed and these were used to produce further generations of plantlets in a regular 14 to 20 day cycle. By this method, 65,000 plantlets could be obtained from a single node in 6 months. This method is advantageous over the stem cutting techniques (regenerated in soil) on account of a faster multiplication, and only a small area is required. The 16-hour daylength treatment of the mother plants also permits an increase in the number of plantlets produced. This micropropagation method is now being used for the propagation of virus-tested D. alata clones.

Sapogenin-containing yams have also been propagated through meristem culture. Grewal et al., (1977) cultured 0.5mm long apical meristems of D. deltoidea and were able to regenerate many plants. Similarly, Chaturvedi et al., (1977) compared cultured apical meristems and single-node leaf cuttings and found that they had nearly the same rate of growth under one set of nutrient conditions.

Another aspect of propagative research on yam is the use of callus and cell cultures from various organs to achieve totipotency through somatic embryogenesis. Source materials vary; from defoliated vine nodal explants (IITA, 1974), to tuber pieces (IITA, 1973; Ammirato, 1982). Rao (1969) cultured tiny tuber pieces of D. sansibarensis on solid and liquid media and obtained cell proliferation, callus and cell suspension cultures, but not embryoid or plantlet formation from the cells. Similarly, Karanova & Shomina (1977) established liquid suspesion cultures of cells of D. deltoidea and determined the parameters for optimal growth, and achieved five-fold increase in 3 weeks

of cultivation. Grewal & Atal (1976) also regenerated plantlets from callus cultures of D. deltoidea.

SEEDS

Generally, vegetatively propagated plants tend to lose their ability for efficient sexual reproduction (Onwueme, 1978) as is the case with yams. Yams produce flowers and set seeds only rarely (Onwueme, 1978, Okonkwo, 1985) and both activities are required for breeding and improvement of the species through hybridization. Thus, breeders maintain interest in yam flowering and fruiting.

Among edible yams, Dioscorea dumetorum (section Lasiophyton), D. bulbifera (section Opsophyton), and D. trifida (section Macrogynodium) flower and set seed regularly (Onwueme, 1978). The most important food yams, D. rotundata, D. alata, and D cayenensis (all members of the section Enantiophyllum) flower infrequently. if at all. They produce only male flowers, or only female flowers and several cultivars of D. rotundata do not flower at all.

Where flowering occurs, a high percentage of the flowers abort. Even when some flowers remain on the plant, there is a high percentage of ovular abortion resulting in unfilled seeds (Onwueme, 1978; Okonkwo, 1985). In some D. rotundata cultivars, flower abortion can be as high as 94%, with ovule abortions of 70-90% (IITA, 1972). Overall, few filled seeds are found per plant (between 3 and 12) (Sadik & Okereke, 1975a & b).

Propagation of yams through seed has also the problem of seed dormancy. D. rotundata seeds have been shown to be dormant for 3 to 4 months after harvest. Embryos of fruits cellected at tuber harvest have been observed to be partially developed, thus suggesting that seed dormancy may result from a rest period during which the embryo continues its development within the seed (Waitt, 1959; Sadik & Okereke, 1975b; Okezie, 1981). Okezie et al., (1984) showed that D.rotundata seeds contained mostly globular embryos measuring about 685u and 537u in length and breadth respectively at the time of tuber harvest, and grow progressively through heart-shaped and fan-shaped stages to a maximum of about 1115 u and 822 u in length and breadth respectively by the end of seed dormancy. Until the discovery of the dormancy phenomenon in yam seeds, it was assumed that yam seeds were not viable.

Despite problems associated with seed propagation of edible yams, potentials for exploiting this mode of propagation for producing this crop are recognized. There is renewed effort in recent times to better understand the flowering and seed production in edible yams. Hopes are high for an eventual sexual propagation of yam by true seed.

After the discovery of the dormancy limitations in yam seeds, it was found that non-dormant seeds that appeared filled, germinated in 3 weeks with a final germination of 80 - 90% and could be transplanted to the field or larger containers after 8 weeks. This

discovery has raised now hopes for yam breeding studies through hybridization. Yam lines arising from true seed have tended to flower more than those arising from tuber (IITA, 1974, Onwueme, 1978). Propagation from true seed therefore ensures that in addition to increased flowering, there is an increase in the number of fruits and seeds, ensuring continuous supply of source material for further propagation. Increased flowering, which ensures greater fruit set in plants originating from seed is expected to increase after several generations of selection.

Methods for raising large numbers of uniform seedlings from seed have now been standardized (Okoli, 1975; Sadik, 1976; Okezie, 1981). Using such methods, Okezie (1981) has studied the growth pattern of yam seedlings raised from seed and compared them with plants raised from tuber (Okezie et al., 1986). They showed that the growth of plants from seed were superior to those plants from tuber (Njoku et al., 1984; Nwoke et al., 1984). This, in fact, suggested greater yield potentials from seed-raised rather than tuber-raised plants.

Yam propagation by seed eliminates the problems of storage associated with tubers. Apart from the fact that substantial amounts of tubers which would have otherwise been consumed are reused during the yam growing season, both "seed" and ware yams deteriorate in storage as a result of attack by pathogen and pests (Coursey, 1961). Coursey (1965) estimated that over one million tons of yam tuber are lost annually during storage in West Africa. Seeds on the other hand are easier to store, and the problems of handling of tubers between harvest and planting, which could lead to wounding of tubers thereby exposing them to microbial attack is eliminated in seed storage. This seed storage could take place in small compartments. Onwueme (1978) argued that since seeds must be stored for several months before use, there are the attendant risks of spoilage during storage. While spoilage cannot be completely avoided in seed storage, methods have been found for efficient seed storage superior to that attained in tuber storage. At Nsukka, desicator storage preserved seeds for several months without contamination (provided the seeds are properly dried before packaging). In this way, batches of seeds have been stored for 13 years and are still very viable.

While seed-grown yam seedlings grow slowly and yield poorly (Waitt, 1961; Onwueme, 1978) due to the small amount of stored material in the seed (Okonkwo, 1985), it must be recognised that the amount of tuber that could be harvested from seed reaches 500g in one year (Sadik & Okereke, 1975a) and 450g in two years (Waitt, 1959) from seeds that weigh about 0.1 g (Okezie, 1986 unpublished) - a ratio of about 1:5,000. This exceeds yield ratio from tuber-raised yam plants of about 1:58 and 1:62 in 1974 and 1975 for Nwopoko cultivar of D. rotundata (Okoli, 1975). One would then expect that with efficient cultural practices, large (ware) yams could be produced within two or three years, using true seeds as the original planating material.

As most edible yams do not flower or produce only male or female flowers when they do, regular supply of seeds cannot be assured for this exercise. Future possibilities

to induce more flowering and seed production by manipulating environment or applying chemicals exist. Controlled hand pollination especially on application of benzyl-adenine (BA) increased seed set in D. rotundata, D. dumetorum and D. cayenensis (Wilson, 1978). For genotypes that flower regularly, our experience is that their flowering and seed set varies with geographical location. This suggests that there is an interplay between environmental and chemical factors; probably growth regulators, in the flowering and seed production in edible yam in general. With more intensive research, flowering and fruiting of edible yams will be better understood and exploited to ensure flowering and seed production on a regular basis.

EMBRYO CULTURE

The inability of yam seeds to germinate immediately after harvest has been attributed to the fact that they are dormant at this time (Waitt, 1959; Sadik & Okereke, 1975; Okezie, 1981). Okezie (1981) studied D. rotundata embryos in vitro.

Culture of excised embryos provides a level of experimental flexibility not easily attained through the use of seed or seed tubers in the propagation of yams. In vitro embryo culture eliminates the constraints of seed germination (embryo growth and emergence of radicle) caused by the seed coat and seed endosperm (Okezie et al., 1984) and increases the rates of multiplication. It also provides a means of developing long-term storage of germplasms and rapid multiplication of improved cultivars in disease and insect-free form to meet the demands of national progammes world wide (IITA, 1977; Henshaw, 1979, Okezie et al., 1984).

Recently, we induced callus from D. rotundata embryos in the presence of kinetin, benzyl aminopurine and naphthalene acetic acid (NAA) which formed multiple plantlets (up to 9) when transferred to a basal medium devoid of hormonal additives (Figs. 4 & 5).

CONCLUSION AND FUTURE PROSPECTS

From the foregoing discourse, it is evident that useful findings have been obtained so far on yam propagation using various sources of starting material other than whole tuber or tuber setts which are the economically usable parts of the crop. Where the tuber has been used, it has involved extremely small pieces (less than one gram) at start off callus and cell suspension tissue cultures. More intensive studies are therefore needed for deising alternative propagation materials.

Extensive experiments with vine cuttings are necessary; to improve their cultural and management practices to achieve maximum tuber yield from them. It should be possible to assess the effects of various types of fertilizers on the growth and yield of these cuttings in the greenhouse and field studies.

A survey of popular edible yams for genetypes that flower, fruit and set fertile

seeds should be undertaken. For example, the discovery of large populations of flowering and fruiting D. rotundata (cultivar obiaturugo) plants at Uturu, near Okigwe, Imo State, Nigeria, is an asset.

Plant tissue culture techniques should be improve and applied to yam propagation research for germplasm maintenance and micropropagation. Efforts should now be made to promote flowering and fruiting, by attempting parasexual hybridization. It should now be possible to apply yam cell protoplast culture, fusion and hybrid plantlet regenerataion techniques in order to achieve interspecific hybridization techniques in order to achieve interspeifific hyridization to improve the crop. Such techniques, where possible, shorten and ease breeding by removing barriers to sexual hybridization. Inducing pollen mother cells to form haploid plantlets is also fast method for producing homozygous pure lines needed for breeding yams of the future.

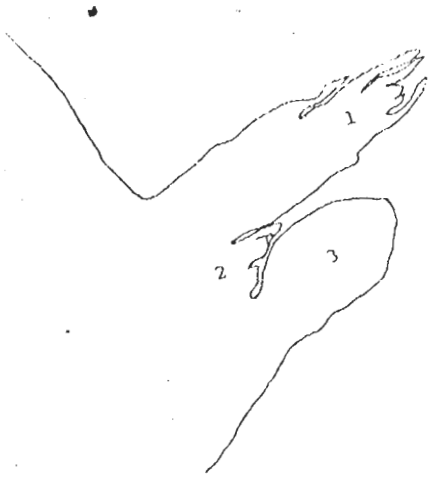

Figure 1

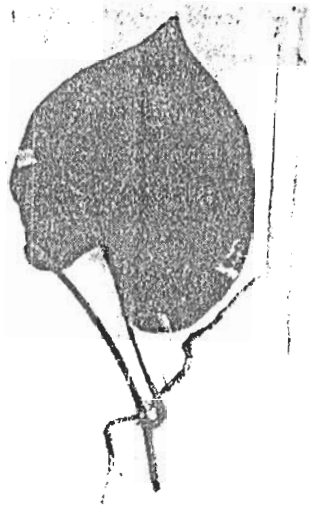

Figure 2

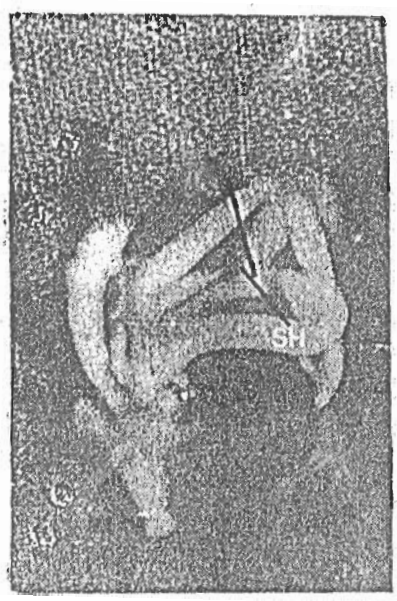

Figure 3

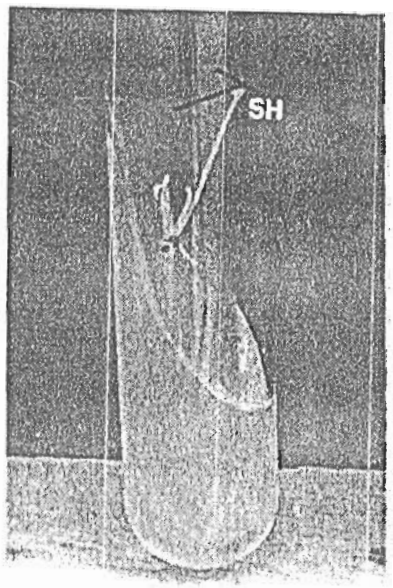

Figure 4

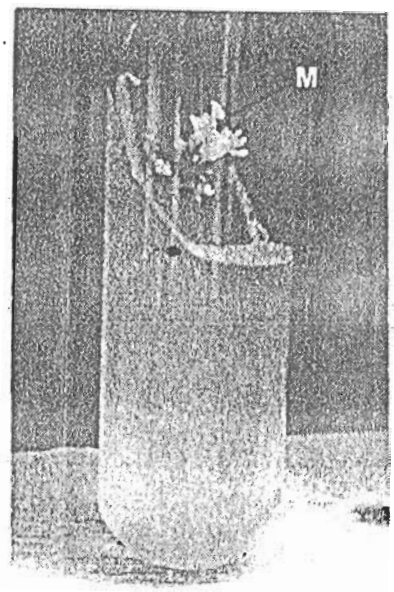

Figure 5

Fig. 1: *Tracing of longitudinal section of a node of Dioscorea bulbifera showing the three axillary primordia: 1= primary bud primordium; 2= secondary bud primordium; 3= bulbil primordium.*

Fig. 2: *Leafy node cutting of D. bulbifera plant observed after 3 weeks growth in potted soil. Note the two stout roots.*

Fig. 3: *Shoots (SH) produced from a D. bulifera leafless nodal explant cultured in a basal medium supplemented with 0.5 mg L^{-1} kinetin.*

Fig. 4: *Shoots (SH) produced in a basal medium devoid of growth regulators, from callus derived from D.rotundata embryo originally cultured in a basal bedium containing 2.0 mg L^{-1} kinetin.*

Fig. 5: *Multiple shoots (M) (up to 11 shoots) produced in a basal medium devoid of growth regulators, from callus derived from D. rotundata embryo originally cultured in a basal medium contining 0.2 mg L^{-1} benzyl aminopurine (BAP).*

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