In-vitro Performance of Some Selected Improved and Farmers' Varieties of Yam (*Dioscorea* spp)

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Abstract

Yams are cultivated worldwide especially in West Africa and South Eastern Asia. Approximately 95% of the world's annual production comes from West Africa. Ghana is the leading exporter of yams in the world even though it is the third producer behind Nigeria and Cote d'Ivoire. Apart from the export market there is also domestic use for the crop as well. Seedyam production in Ghana is a major constraint in yam production in that it is estimated to take 40-50% of production cost making yam accessibility difficult. Tissue culture micro propagation is one of the growing agricultural techniques that helps to increase seedyam stocks for breeders and commercial seed companies. However, different yam varieties in Ghana differ in physiological characteristics when grown under the same conditions. The objective of the study was to evaluate growth performances of two improved (CRI-Pona, Mankrong Pona) and four landraces (Dente, Pona, Labariko, Matches) yam varieties in-vitro. Yam Minisetts obtained from CSIR-CRI breeding program were established in screen house for in-vitro manipulations. Actively growing shoots were initiated in-vitro on Murashige and Skoog's(MS) medium supplemented with BAP, NAA, Kinetin and AdSO4, adjusted pH 5.7± 0.1 under 5000 lux for 8 weeks. Mankrong Pona gave the highest growth (80%) on all the medium used followed by Matches, (79%), Labariko (75%), Dente (73%), CRI-Pona, (70%) and Pona (50%) being the lowest. The results obtained indicate that the improved varieties have a greater performance than the landraces.

Key words: Establishment, Initiation, Kinetin, Multiplication, Murashige and Skoog.

Compartment in vitro de certaines variétés d'ignames améliorées et de certaines variétiés espèces d'ignames d'agriculteurs *(Dioscorea spp)*

Résumé

Ignames sont cultivées dans le monde entier, en particulier en Afrique de l'Ouest et en Asie du Sud-Est. Environ 95% de la production annuelle mondiale provient d'Afrique de l'Ouest. Le Ghana est le premier exportateur d'ignames dans le monde bien qu'il soit le troisième producteur apres le Nigeria et la Côte d'Ivoire. En dehors du marché d'exportation, il existe également une utilisation domestique pour la culture. La production de semence au Ghana est une contrainte majeure dans la production d'igname dans la mesure où elle est estimée à 40-50% du coût de production, ce qui rend difficile l'accès à l'igname. La micropropagation des cultures tissulaires est l'une des techniques agricoles en pleine croissance qui

contribuent à augmenter les stocks de semence pour les sélectionneurs et les entreprises semencières commerciales. Cependant, différentes variétés d'igname au Ghana diffèrent dans les caractéristiques physiologiques lorsqu'elles sont cultivées dans les mêmes conditions. L'objectif de l'étude était d'évaluer les comportments de croissance de deux variétés améliorées (CRI-Pona, Mankrong Pona) et quatre varietés locales (Dente, Pona, Labariko, Matches) d'igname in vitro. La production d'igname par mini-fragmentation obtenus à partir du programme de selection CSIR-CRI ont été mis en place dans des serres pour des manipulations in vitro. Des pousses en croissance active ont été initiées in vitro sur du milieu de Murashige et Skoog (MS) additionné de BAP, NAA, Kinetin et AdSO4, ajusté à pH 5,7 ± 0,1 sous 5000 lux pendant 8 semaines. Mankrong Pona a réalisé la plus forte croissance (80%) sur l'ensemble des supports suivis par Matches, (79%), Labariko (75%), Dente (73%), CRI-Pona (70%) et Pona (50%) étant le plus bas. Les résultats obtenus indiquent que les variétés améliorées ont une meilleure comportement que les variétés locales.

Mots-clés: Établissement, Initiation, Kinetin, Multiplication, Murashige et Skoog.

Introduction

Yams are one of the most important sources of carbohydrate in Sub-Sahara Africa. However, yams belong to a group of crops labeled "orphaned crops", which have not received much research attention for improvement in a long time (Otoo, 2007). Even though yam has been cultivated in Ghana from time immemorial, until May 2005, there had not been any formal release of any yam variety. There are many yam varieties as named by various ethnic groups (Asemota *et al.*, 1996; Dansi *et al.,* 1999; Otoo, 2001).

West Africa countries involved in yam production includes Nigeria, Cote d'Ivoire, Ghana, Togo and Benin. Globally, Ghana is the leading yam exporter even though it is the third producer behind Nigeria and Cote d'Ivoire having exported 21,000 metric tons of yams annually. Over the last decade, increasing global demands for yam indicates that there is a potential for higher production and export (MiDA, 2009).

Yam is vegetatively propagated using the edible tuber sectioned into minisetts. Conventionally, this method of propagation is slow and not adequate for rapid multiplication. Bacteria, viral and nematode infections are transmitted through the seed tubers or minisetts to the new generation of plants. This reduces yield and therefore affects production and costs. To counter this effect, propagation of yam through tissue culture is one of the in-vitro techniques which has in recent years become major agricultural importance in the area of plant propagation, disease elimination, plant improvement and production of secondary metabolites in-vitro. There is therefore the need to use this technique for rapid multiplication of seedyam (Quain *et al,* 2015).

The objective of the study was to evaluate growth performance of two improved yam varieties (CRI-Pona, Mankrong Pona) and four landraces (Dente, Pona, Labariko, Matches) *in-vitro,* during initiation and rapid multiplication. The response of these varieties on two different media is also investigated and reported here.

Materials and Methods

The study was carried out at the laboratory of

Tissue Culture, Plant Biotechnology Unit at CSIR - Crops Research Institute, Fumesua, Kumasi.

Plant Material and Surface Sterilization

All genotypes used in the experiments were obtained from the Yam Breeding Programme at CSIR - Crops Research Institute, Kumasi as minisetts. During initiation in-vitro mother plants were obtained from stock plants which had been established in the screen-house.

The excised nodes were thoroughly washed under running tap water for 10-15 minutes and then soaked in 70% ethanol for 5 minutes and rinsed with distilled water, followed by disinfecting with 20% and 10% NaOCl which had 2 drops of tween20 added for 20 and 10 minutes respectively. The excised nodes were washed with distilled water and trimmed at the edges at each level of disinfection, finally rinsed with sterile distilled water and trimmed again. This was done to get rid of the micro-organisms that might be present in the node. Nodal cuttings were then labelled appropriately and cultured on appropriate media and labelled accordingly. Also meristems of approximately 1x1 mm were excised from the shoot tips using a dissecting microscope.

Initiation medium for yam

At initiation experiment using explant from greenhouse, inoculation was done using one node per test tube with diameter of 25 mm × 150 mm height. Nodal explants were removed from sterile distilled water onto sterile petri dishes. Explants were inoculated by means of forceps onto a complete Murashige and Skoog (MS) medium (Murashige and Skoog 1962) with vitamins (Caisson brand), 30 g/l sucrose, 80 mg/l Adenine sulphate (AdSO4) and 20 mg/l L-cysteine, 5 µM Benzylaminopurine (BAP), 0.5 µM Naphathalene acetic acid (NAA) (Plates A & D). The rim of the test tubes was flamed each time to ensure sterility and covered with plastic closure. The pH was adjusted to 5.7± 0.1 and was sterilized at temperature 121 ºC and 15 psi for 10-15 min. All the cultures were incubated at a temperature of 27 ± 2 ºC with a photoperiod of 16 hours light and 5000lux (Quain, *et al.,* 2002).

In-vitro Rapid Multiplication

Explants initiated developed shoots by the 8th week in culture (Plates A - D). The developed shoots were excised and divided into two and subcultured onto a fresh initiation medium (labeled as medium 1) as above and multiplication medium with composition; Complete MS medium with vitamins, supplemented with 2.5 µM Kinetin, 80 mg/L AdSO4, 20 mg/L L-cysteine, 3 % Sucrose (labeled as medium 2). The pH was adjusted to 5.7± 0.1 and sterilized at temperature 12 ºC and 15 psi for 15 min. All the cultures were incubated at a temperature of 26 ± 2 ºC with a photoperiod of 16 hours light and 8 hours darkness (Quain, *et al.,* 2015).

Data collection and Statistical Analysis

Yam genotypes used in the experiment include improved yam (Mankrong Pona, CRI-Pona) and landraces (Dente, Pona, Labariko, Matches). Data taken included: Number of shoots / culture, Number of leaves/culture, Height of shoot (cm) after eight weeks of *in-vitro* culture during initiation and multiplication. One nodal explant per test tube, making 34 explants as total sample size. Survival of the cultures was assessed on the basis of criteria as suggested by Reed (1992) as dead and brown shoots were considered as un-survived while those with vigorous growth and having healthy leaves were considered as survived.

$$\text{Percentage performance} = \frac{\text{Shoots Culture}}{\text{Number of Shoots Proliferated}} \times 100$$

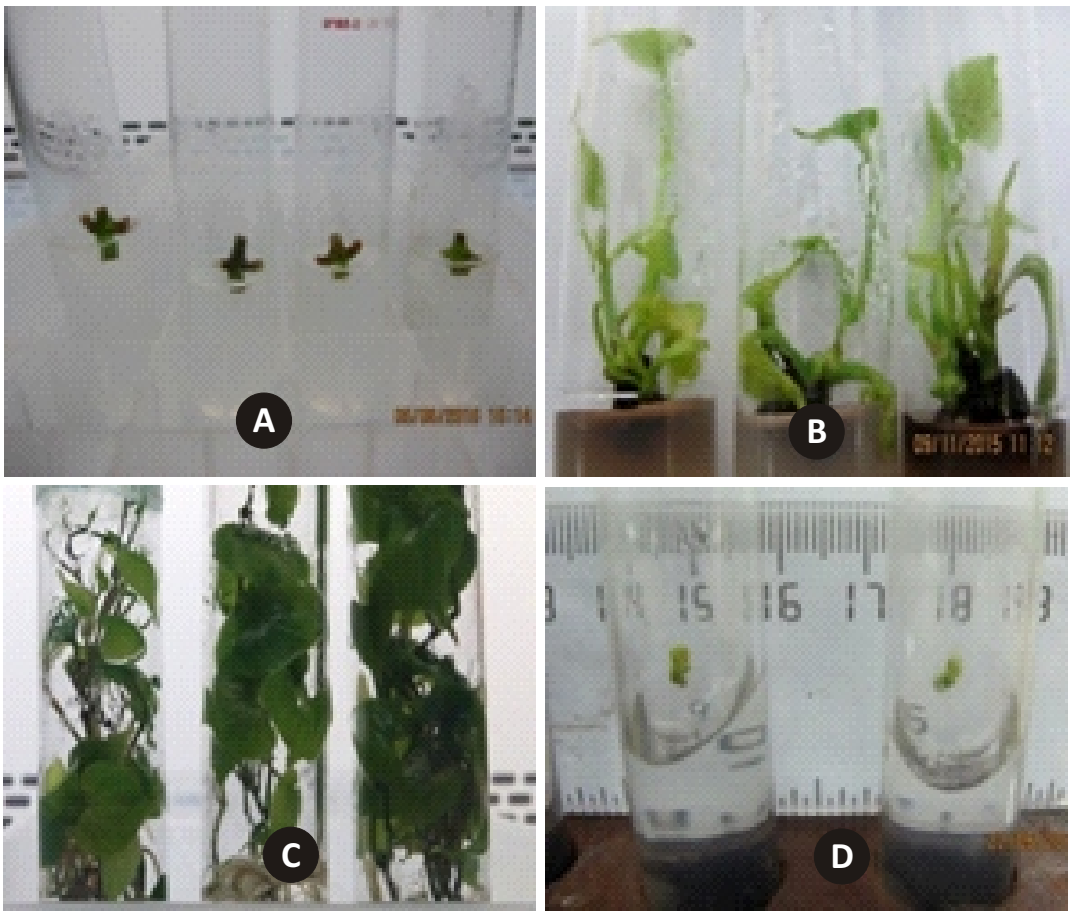
The number of leaves, number of shoots and shoots heights (cm) after eight weeks and standard error were calculated using Windows Excel 10.1.

Results

At eight weeks after initiation, well-developed shoots were obtained in most cultures although some were not successful and died or were lost to contamination, however, no contamination was observed in cultures initiated from shoot tip meristems. The frequency of shoot proliferation was used to measure the percentage success. The highest survival percentage was observed in Mankrong Pona (80%), followed by Matches (79%), Labariko (75%), Dente (73%), CRI-Pona (70) and Pona (50%) being the lowest.

Shoot Development

Following initiation, and sub-culturing unto media 1 and 2, Mankrong Pona variety gave the highest number of shoots (2.13) at initiation, 3.63 on medium 1 and 4.71 on



Plates A - D showing initiated yam explant cultured in vitro (A), plantlets proliferation after eight weeks (B), actively growing yam cultures (C) and cultured yam meristem at eight weeks (D)

medium 2 at multiplication. Matches variety had a higher number of shoot (4.19), than Labariko (3.82) and CRI Pona (3.86) on medium 2 during multiplication. However, Pona performed poorly in shoot development 0.43, 0.83 and 0.17 respectively at initiation, and on medium 1 and medium 2 during multiplication (Fig. 1).

Leaf Development

Dente variety had the highest number of leaves (16.73) on medium 1 and 17.45 on medium 2 during multiplication. Matches variety gave a higher number of leaves of (9.06), (9.08) and 9.15 at initiation, on medium 1and medium 2 respectively during multiplication. Mankrong Pona gave (9.36) and (9.45) on medium 1 and medium 2 during multiplication, however, at initiation it had a lower number of leaves (4.16). CRI-Pona gave (9.45) and (9.36) in media 1 and 2 respectively (Fig 2) during multiplication.

Shoot Height

Dente recorded the highest figure of (5.63 cm) on medium 2, and (4.23 cm) on medium 1duing multiplication. At initiation, a lower value was obtained (2.26 cm). Pona landrace variety responded poorly in terms of shoot in all the three media used. Labariko gave almost the same shoot height of (2.18 cm), (2.15 cm) and (2.12 cm) at initiation and multiplication on media 1and 2 respectively (Fig. 3).

Discussions

Growth regulator (BAP and NAA) combinations are critical for the in-vitro manipulations of yam at initiation. However, growth of cultures after initiation is higher as reported in this experiment where the number of shoots at multiplication was higher for both media 1 and 2. This can be due to the fact that the plants have already been established for in-vitro conditions following initiation. The addition of kinetin, a cytokinin growth regulator to culture medium, has also been proven to be important in *in-vitro* yam tissue culture development. It is a type of cytokinin, and a plant growth regulator that promotes cell division and in this study, it highly enhanced growth of development of yam cultures (Ashun, M., D. 1996; Quain, *et al,* 2015).

At multiplication Dente landrace variety responded well in terms of leave proliferation, shoot and height development. However, the number of leaves developed by "Matches" (another landrace) variety did not differ at both initiation and multiplication. This landrace variety responded considerably well in-vitro in terms of shoot proliferation with an average mean number of shoot (1.96) at initiation, in medium 1 and medium 2. This could be because it is a *D. alata* (Doumbia, *et al.* 2004). However, Pona variety which is a *D. rotundata* and a very popular Ghanaian landrace performed poorly during initiation and multiplication. This response could be due to varietal differences and the fact the growth hormones used was unable to stimulate the cell to elongate and shoot proliferate more shoots. All the landrace varieties responded better on medium 1, whereas the improved Mankrong Pona variety performed best on medium 2 in terms of number of shoots developed and this is similar to report in a previous study (Quain, *et al.*, 2015).

Conclusions

The different varieties responded differently to the two media used in-vitro. There was appreciable growth and development of the various yam varieties used in this study. The positive response to micro propagation recorded will effectively support the yam industry. Hence tissue culture mass propagation can be used to support the yam seed system. Pona variety performed relatively poorly at all developmental stages in the study, therefore, other growth regulators

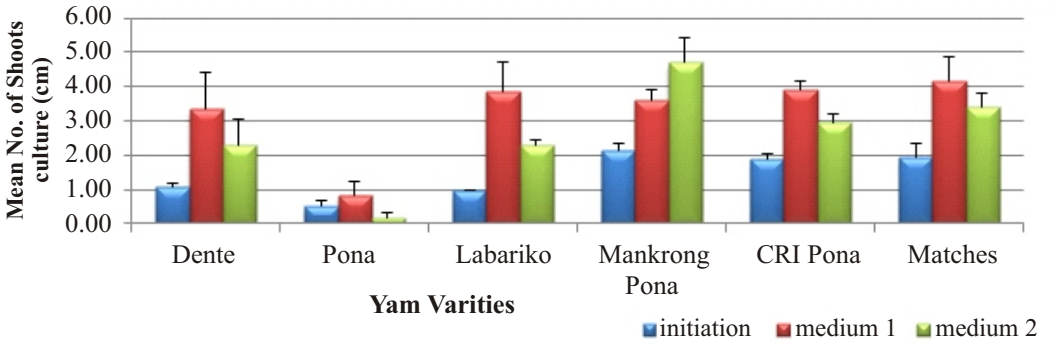


Fig 1 *In-vitro* performance of six yam varieties on shoot development

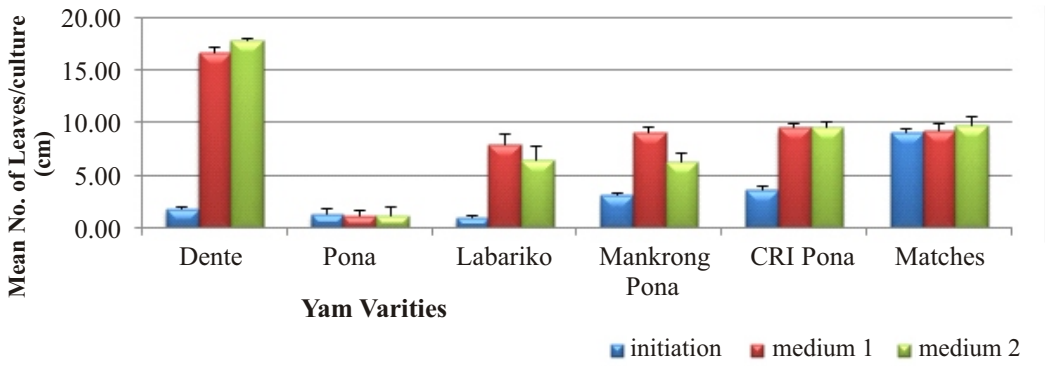


Fig 2. *In-vitro* performance of six yam varieties on leaf development

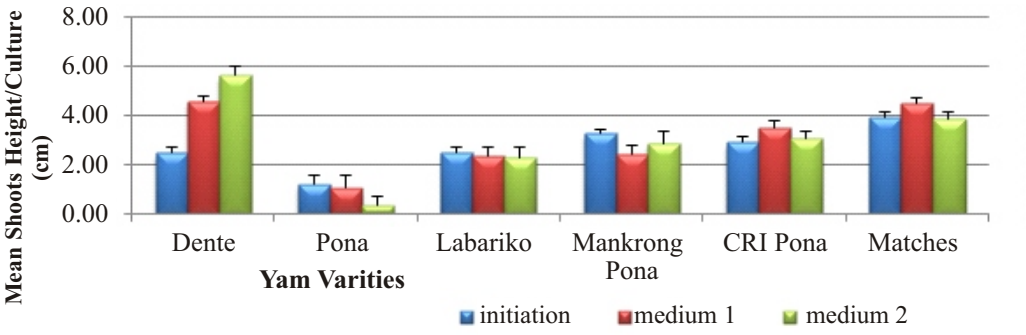


Fig 3. *In-vitro* performance of six yam varieties on Shoot height

available may have to be screened for optimum performance of the variety.

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