academicJournals

Vol. 16(15), pp. 819-825, 12 April, 2017
DOI: 10.5897/AJB2016.15504
Article Number: 11F9C6063697
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Effects of activated charcoal on medium-term conservation of yam (*Dioscorea* spp.) cultivated in Benin

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Received 1 June, 2016; Accepted 15 February, 2017

The effects of activated charcoal were tested on medium term conservation of yams cultivated through tissue culture techniques. Galzy glutamine basic medium and that supplemented with 3 g/l activated charcoal were used. Growth parameters such as number of leaves, height of stem, number of nodes and length of the main root are evaluated on plantlets after 20 months. The test T of Student Newman and Keuls (SNK) with two independent variables was used for the comparison of means with Minitab 16 software. Plantlets obtained in the control medium faded and presented a high number. The length of internodes obtained from the medium treated with activated charcoal had a length relatively greater than the control medium with increase of 33%. Probabilities (P = 0.001) associated with the T-test of equality of means of leaves, the height of stem and the length of internodes are lower than 0.05 but there was no significant difference between the number of nodes, length of internodes, number of roots, and length of the main root of plantlets obtained in both media at 5%. In addition, tests of correlation (Pearson) in the control medium revealed the existence of a highly significant link (P <0.001) between the number of nodes and number of leaves. In treated medium, there was a highly significant link (P <0.004) between the length of the stem and number of leaves; the height of the stem and length of the main root (P <0.001). Activated charcoal facilitates conservation and constitutes an alternative of conservation of yam. These results may be used to realize *in vitro* collection of different genotypes of yam cultivated in Benin.

Key words: *In vitro* conservation, plantlets, yam, activated charcoal.

INTRODUCTION

Yam (*Dioscorea spp.*) has a large morpho-ecological, physiological adaptation and is found in all continents (Degras, 1993). It is the second root and plants tuber after cassava with a global annual production estimated at 50 million tons (FAOSAT, 2012). This crop is a major source of food for more than 50 million people in west and central Africa (Asiedu and Sartie, 2010). In West Africa, it occupies an important place in food security and accounts for 95% of production. This tuber is an important source of income for farmers. In the savannas

extending from Cameroon to Ivory Coast, it is always considered as sacred food whose first harvest must be offered to gods and ancestors before yam consumption. So the royal powers associate with their diets, a tradition that is profoundly registered in the cultural and religious heritage of the populations (Degras, 1998). Nowadays, this older and secular plant which is promising crop for Africans (Degras, 1998) remains a major basic food in Africa. Benin is the fourth country producing yam in Western Africa after Nigeria, Ivory Coast and Ghana (FAOSTAT 2014). Despite the importance of this crop in the alimentary, cultural, religious and socioeconomic plan, it has not sufficiently drawn the attention of scientists and institutions, especially its conservation. Indeed, yams produced by vegetative propagation are submitted to viral and other pathologic infections that cause enormous damage for its conservation and generate genetic erosion for different varieties and species. More than 30% of yield tubers are lost (Adeniji, 1970; Okigbo and Ogbonnay, 2006). Due to erosion risks, conservation of genetic resources of cultivated and wild yams becomes more necessary. It is essential to implement appropriate conservation strategies to promote the accessibility of these species and varieties of yam. *In vitro* tissue culture of yams proves to be effective for the conservation of genetic resources (Ovono et al., 2010; Ahanhanzo et al., 2010). Malaurie et al. (1993) specified techniques to be applied in *in vitro* conservation of cultivated yam and the use of plantlets as planting materials. Several techniques used for conservation such as low temperature (Grout, 1995), low level of mineral nutrients, vitamins and carbohydrates, growth regulators as abscissic (Jarret and Gawell, 1991; Engelmann and Engelmann, 2014), activated charcoal (Agbidinoukoun et al., 2013; Polzin et al., 2014) and long term conservation techniques such as cryogenic conservation can be used to achieve high regeneration rates of the different species of *Dioscorea in vitro* (Das et al., 2013). In 2000, Central Laboratory of Plant Biotechnologies and Breeding, Faculty of Science and Technology (FAST), University of Abomey-Calavi applied some of these techniques in *in vitro* morphogenesis (Ahanhanzo et al., 2010) or in *in vitro* conservation of certain accessions of yam. The present work was a continuation of the previous studies and aimed to contribute to the genetic resources management through the verification of the influence of activated charcoal on *Dioscorea cayenensis-rotundata* in *in vitro* conservation.

MATERIALS AND METHODS

The plant materials are composed of healthy plantlets of yam of complex *Dioscorea-Cayenensis – rotundata.* They were obtained from Central Laboratory of Plant Biotechnologies and Breeding, Faculty of Science and Technology (FAST), University of Abomey-Calavi (UAC) located approximately 16 km North of Cotonou in the municipality of Abomey – Calavi, Republic of Benin. The plantlets were twenty-months old**.**

The plantlets are cultivated in two different media: The medium of Galzy glutamine (2GG) (Doukoure, 2000) and the medium (2GG) supplemented with 3 $g.l^{-1}$ activated charcoal. Cultures were placed in an air conditioned chamber at 28 ± 1°C, according to the standard proposed by Hunter. The relative humidity of the culture chamber is maintained at 80%. The culture chamber is equipped with lamp of types Philips TLD18W and Sibalec FL18W that ensure illumination of about 5000 luxes with a photoperiod of 12 h. 30 (15 × 2) healthy plantlets of yam of complex *D.-Cayenensis – rotundata* are used for each experiment repeated two times**.**

The following growth parameters were studied in order to evaluate the effect of activated charcoal on the conservation of yam plantlets concerned: number of leaves, number of roots, number of nodes, height of stem, the mean length of internodes and the mean length of main roots. Height of the stem and length of the main root were measured using a caliper with a precision of ± 0.1 mm. As for the length of internodes, it was taken with a ruler. The test T of Student Newman and Keuls (SNK) was used for the comparison of means with Minitab 16 software. The means were compared by using least significant difference method at 5% threshold. Pearson correlation test was performed to evaluate the relationship between the different growths parameters studied.

RESULTS

Effects of activated charcoal on the growth parameters

The main results indicated the influence of activated charcoal on plantlets of yam. Plantlets obtained in control medium present a high number of leaves (28 against 13) more than those presented in the medium containing activated charcoal (Figure 1).

The P value of 0.001 for the T-test of equality of means indicated that the leaves development was highly significant in the presence of activated charcoal at 5%.

Plantlets obtained in the control medium had height greater than those obtained in medium containing activated charcoal (Figure 2).

The P value of 0.022 for the T-test of equality of the average height of stem was lower than 0.05. There was a significant difference between the means length of stems obtained in both media on the threshold of 5%.

Figure 3 shows the number of nodes obtained in the two media: plantlets placed in the activated charcoal medium had eight nodes while those placed in control medium had seven nodes.

The P value of 0.582 for the T-test of equality of the two media showed that their nodes were more than 0.05; so there was no significant difference between the number

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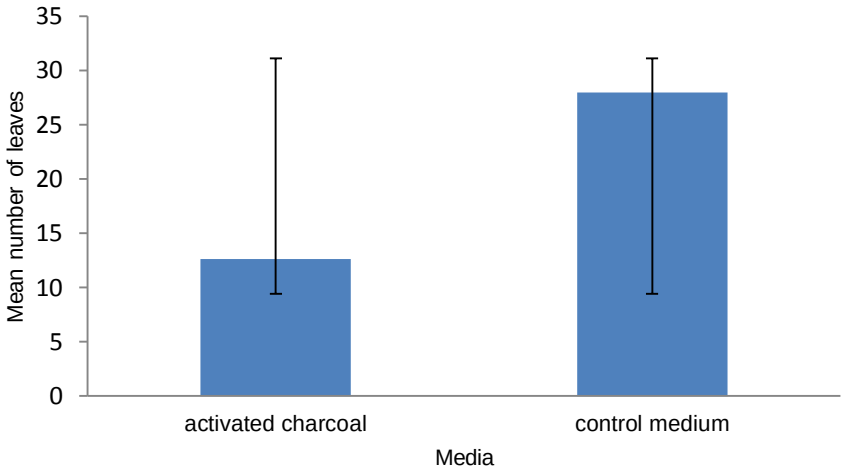

Figure 1. Effect of activated charcoal on leaves of yam.

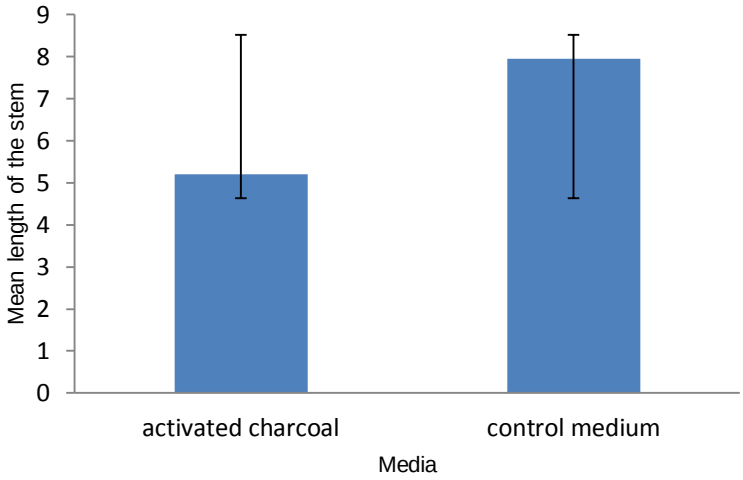

Figure 2. Effect of activated carbon on the length of the stem of yam.

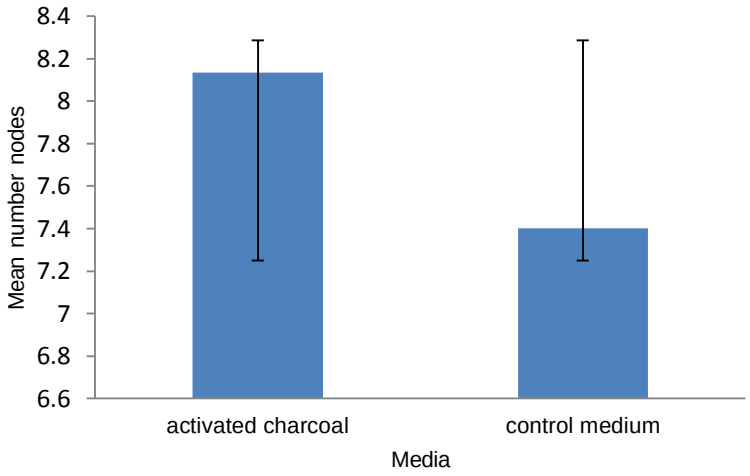

Figure 3. Effect of activated charcoal on the number of nodes of the yam.

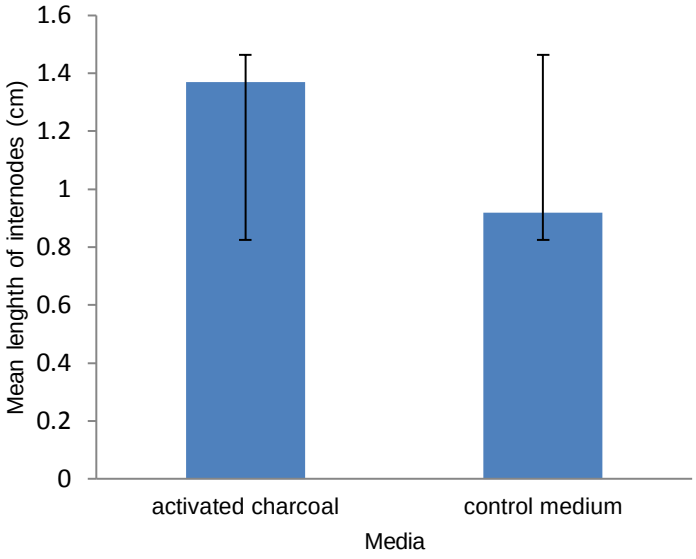

Figure 4. Effect of the activated charcoal on the length of internodes yam.

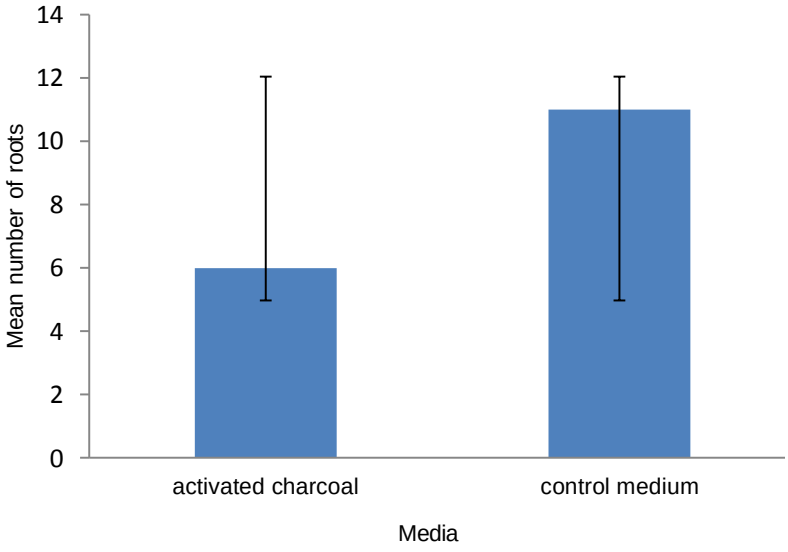

Figure 5. Effect of activated charcoal on the number of roots of yam.

of nodes obtained in both media at 5%.

The length of the internodes of the plantlets obtained from the medium treated with activated charcoal had a length relatively greater than those in control medium with an increase of 33% (Figure 4).

The P value of 0.001 for the T-test of equality of the mean of the lengths of the internodes of both media was lower than 0.05. There was a highly significant difference between the mean length of the internodes obtained in both media at 5%.

Plantlets of the control medium had a relatively high number of roots than those issued in the second medium (Figure 5).

The P value of 0.085 for n the T-test of equality of the two means of roots was greater than 0.05. At the threshold of 5%, there was no a significant difference between the mean number of roots obtained in both media.

In the same way, height of the main root of plantlets obtained in the medium without activated charcoal was

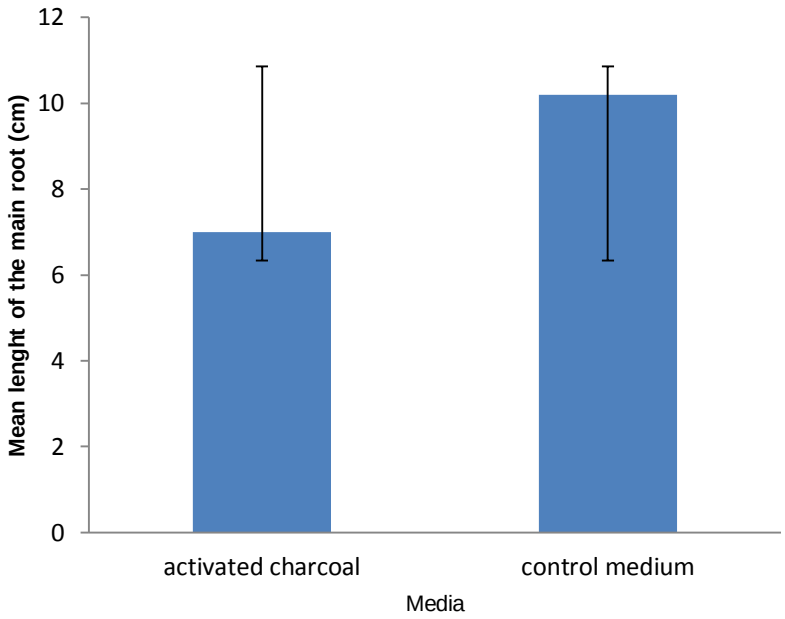

Figure 6. Effect of activated charcoal on the length of the main root of the yam.

Table 1. Pearson correlation and value of P established between the various parameters of the yam plantlets from the medium without activated charcoal.

Parameter	NL CG	NR CG	NN CG	LS CG	LEN CG	LR CG
NL CG	1					
NR CG	0.239^{NS}	1				
NN CG	0.001**	0.422^{NS}	1			
LS CG	0.406^{NS}	0.228^{NS}	0.285^{NS}	1		
LEN CG	0.743^{NS}	0.371^{NS}	0.117^{NS}	0.128^{NS}	1	
LR CG	0.690^{NS}	0.689	0.410^{NS}	0.668^{NS}	0.148^{NS}	1

^{NS}Not Significant; *significant; **highly significant; GG,Galzy glutamine medium; NL, number of leaves; NR, number of roots; NN, number of nodes; LS, length of the stalk; LR-P, length of main root; LEN, length internodes;a highly significant link (P <0.004) between the length of the stalk and the number of leaves; the length of the stalk and that of the main root (P <0.001).

relatively greater than those of the treated medium with an increase of 14% (Figure 6).

The P value of 0.521 for the T-test of equality of the average length of the main root was more than 0.05. There was no significant difference between the mean lengths of the main root of plantlets issued in the two media on the threshold of 5%.

Relations between the parameters studied on the medium with/ without activated charcoal

Analysis of Table 1 revealed a highly significant link (P <0.001) between the number of nodes and the number of leaves in the control medium.

In the treated medium, there was a highly significant link (P <0.004) between the height of stem and number of leaves, on one hand; and the height of the stem and length of the main root (P <0.001), on the other hand (Table 2). We noted also a significant relationship (P <0.043) between the length of the stem and length of internodes in this medium.

DISCUSSION

Plantlets obtained in the medium control presented faded leaves contrary to those in activated charcoal medium, which had quite green leaves. This state of fading plant may be explained by a temporary availability of nutrients

Table 2. Pearson correlation and P value established between the different parameters in vitro plantlets from the medium supplemented with activated charcoal.

Parameter	NL CG+CA	NR CG+CA	NN CG+CA	HT CG+CA	LEN CG+CA	LR CG+CA
NL CG+CA	1					
NR CG+CA	0.446^{NS}	1				
NN CG+CA	0.065^{NS}	0.918^{NS}	1			
HT CG+CA	0.004**	0.584^{NS}	0.163^{NS}	1		
LEN CG+CA	0.673^{NS}	0.968^{NS}	0.379^{NS}	0.043*	1	
LR CG+CA	0.042^{NS}	0.634^{NS}	0.476^{NS}	0.001**	0.120^{NS}	1

^{NS}Not significant; *significant; **highly significant; CG + AC, Galzy glutamine medium supplemented with activated charcoal NL, number of leaves; NR, number of roots; NN, number of nodes; LS, length of the stalk; LR-P, length of main root; LEN, length internodes; a highly significant link (P <0.004) between the length of the stalk and the number of leaves; the length of the stalk and that of the main root (P <0.001).

for plantlets' rapid growth. Long term conservation made the medium to become impoverished in nutrients, which deficiency is pronounced on the plantlets. The activated charcoal seems to trap nutrients which were slowly absorbed by plantlets thus allowing continuous presence of those nutrients in the medium. A similar observation was made by Borges et al. (2004) who reported that the activated charcoal used in the preservation favors healthy tissue viability resulting in a high rate of shoots unlike the medium devoid of activated charcoal. Activated charcoal slowed the growth of plantlets through the mean number of leaves; the mean height of stem, the mean length of internodes except the number of node, the number of roots and the length of roots. These results were similar to those obtained by Agbidinoukoun et al. (2013) on yam. It was found that activated charcoal slowed down the formation of the shoots and leaves, but it led to a better rooting in all the accessions tested. Plantlets' growth can be slowed down in medium - term conservation of genetic resources. Our results confirmed those of Gomes and Canhoto (2009) in *Arbutus unedo* L., where the addition of charcoal did not improve root formation. In addition, the addition of activated charcoal had a positive effect on the growth of the internodes (Polzin et al., 2014). On the other hand, those results had been obtained (Etse et al., 2011) on *Zanthoxylum zanthoxyloides* Lam. Gübbük and Pekmezci (2004), working on Musa, revealed that activated charcoal had a favorable effect on roots of plantlets. Activated charcoal influenced significantly the increase in fresh matter (Agbidinoukoun et al., 2013). The same authors also found that from the sixth months, shoots of *Dioscorea* spp. cultivated on the control medium or with a low concentration (1 g.l^{-1}) of activated charcoal began a phase of fading while those cultivated on the medium rich in activated charcoal (2 and 3 g.l^{-1}) had good vigor. Damiano (1980) observed that the addition of 1 to 2 g. l^{-1} of activated charcoal in the medium reduces necessarily its transmission in strawberry roots. So activated charcoal action depends on its concentration in the medium and crop species. It sometimes has positive effects on the slowing growth of plantlets and is used *in vitro* for his antioxidant role. It results in a considerable reduction in the browning of the medium caused by phenolic compounds, and reduces the effect of these phenolic compounds present in the medium (Shukla and Shukla, 2014).

On one hand, this study indicated significant relationship between the height of stem and length of root and on the other hand between the number of leaves and height of the stem on medium supplemented with activated charcoal. In addition, the plantlets on the medium with activated charcoal have more and longer nodes and they had shorter height. This antioxidant reduced the height in order to facilitate the conservation of plantlets with more potential nodes. It appeared that activated charcoal has an inhibitory action on the morphogenesis of the variety of yam tested. However, the age and physiological state of explants have an important role in the possibilities of conserving the material.

Conclusion

At the end of this study, it is clear that activated charcoal in the medium increases the conservation of the yam cultivars through *in vitro* tissue culture. The addition of this antioxidant has been favorable to plantlets in slowing growth. With this method of medium - term conservation, it is possible to proceed to massive production of this plant tuber culture at low -cost.

CONFLICTS OF INTERESTS

The authors have not declared any conflict of interests.

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