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Effects of Plant Growth Regulators and Explant Types on Callus Formation in African Yam Bean (*Sphenostylis stenocarpa* (Hochst. Ex A. Rich) Harms)

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ABSTRACT

This study was conducted to investigate the effect of explant types and plant growth regulator regimes on callus induction in African yam bean, with a view to developing protocols for regeneration. Leaf, stem and root explants of African yam bean were cultured in media containing different types and concentrations of plant growth regulators (PGR). The explants were observed for callus formation after three months. The main effects of variety, explant and plant growth regulator regimes and their interactions were highly significant. Highest callus formation was recorded in stem explant while the root explant had the lowest. The accession, SSSWN56 (brown seed) had higher percentage callus formation than SSSWN75 (grey seed). No callus was formed in hormone-free medium and the media containing indole acetic acid (IAA). One- hundred-percent callus formation was recorded for both accessions in the medium containing 1.5 mg/l each of kinetin and Naphthalene acetic acid (NAA). This medium is recommended for callus induction using stem explant and it will be useful in perfecting regeneration protocols for African yam bean.

Key words: African yam bean, callus, plant growth regulators, explants

INTRODUCTION

African yam bean (*Sphenostylis stenocarpa* Hochst. (Ex A. Rich) is an indigenous grain legume in Nigeria. It is a good source of protein, carbohydrate, vitamins and minerals. It also contains high concentrations of anti-nutritional factors such as trypsin inhibitor, phytate, tannin, oxalate and alkaloids (Nwokolo, 1987; Ajibade *et al.*, 2005; Fasoyiro *et al.*, 2006). It is sometimes used to replace cowpea in most food preparations particularly in rural areas where the crop is still being cultivated. A lectin from African yam bean seeds has also been reported to have insecticidal effects on the cowpea beetle (*Callosobruchus maculatus*) and pod-sucking bug, (*Clavigralla tomentosicollis*). The two insects are the major pests of cowpea in Nigeria and other African countries (Okeola *et al.*, 2002).

However, constraints to African yam bean cultivation and utilization include the presence of anti-nutritional factors, long cooking

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time (Fasoyiro *et al.*, 2006) and low seed yield (Saka *et al.*, 2004). The germplasm base available to breeders for genetic improvement is also narrow. There has not been sufficient effort to collect African yam bean germplasm from the areas where it is cultivated and so most of the genetic resources of the crop are in the hands of aged farmers (Saka *et al.*, 2004). There is, therefore, a dearth of information on the genetic improvement of this crop through conventional and biotechnological methods. Genetic transformations via tissue culture techniques offer viable crop improvement options through somaclonal variation wherein desirable changes are manifested by plantlets regenerated from different explants via a callus phase (Ogunbodede and Novak, 1998). These changes can widen the genetic base and can also be incorporated into breeding programmes (Opabode and Adebooye, 2005). However, one of the prerequisites for genetic improvement of crop plants through genetic transformation is the availability of a reliable protocol for regeneration (Yadav and Padmaja, 2003). This is lacking for African yam bean. Hence, the objectives of this study were to investigate the effect of explant types and plant growth regulator regimes on callus induction in African yam bean, with a view to developing protocols for regeneration.

MATERIALS AND METHODS

Seeds of two accessions of African yam bean, SSSWN56 (brown seed) SSSWN75 (grey seed) were collected from germplasm at the Nigerian Institute of Agricultural Research and Training. The seeds were disinfected in 70% methylated spirit followed by 35% commercial bleach and rinsed in three changes of sterile distilled water. The disinfected seeds were cultured in Murashige and Skoog (1962) medium containing 30g sucrose, 0.1g myo-inositol and 7g agar (per litre) set at a pH of 5.7. When the seeds had germinated (three weeks after culturing), the leaf, stem and the root were cut into portions 1 cm in length and cultured in petri plates on 16 media with the same constituents as above but differing in type and concentration of plant growth regulators. The cytokinins used were benzylaminopurine (BAP) and kinetin (KIN), while naphthalene acetic acid (NAA) and indole acetic acid (IAA) were the auxins used. The plant growth regulator regimes of the 16 media are shown in Table 1.

The experiment was a 3 (explants) x 16 (PGR regimes) x 2 (genotypes) factorial in a completely randomized design. There were three replicates per treatment combination. Three months after culturing, data were collected on length and width of callus from which percentage callus formation was calculated. The longest distance between one end of the callus and the other in the petri plate was taken as the length, while the shortest distance was taken as the width in centimeters. The calluses were also observed for friability. Analysis of variance was carried out

Table 1 Plant growth regulator regimes of the 16 media used for callus induction in African yam bean.

Media	Plant growth regulator regime
1	Control: No plant growth regulator
2	1.5 mg/l BAP
3	1.5 mg/l BAP, 1.5 mg/l IAA
4	1.5 mg/l IAA
5	3.0 mg/l BAP, 1.5 mg/l IAA
6	1.5 mg/l BAP, 3.0 mg/l IAA
7	3.0 mg/l BAP
8	1.5 mg/l NAA
9	1.5 mg/l NAA, 1.5 mg/l BAP
10	3.0 mg/l BAP, 1.5 mg/l NAA
11	1.5 mg/l BAP, 3.0 mg/l NAA
12	1.5 mg/l Kinetin
13	3.0 my/l Kinetin
14	1.5 mg/l Kinetin, 1.5 mg/l NAA
15	3.0 mg/l Kinetin, 3.0 mg/l NAA
16	1.5 mg/l Kinetin, 3.0 mg/l NAA

using SAS (SAS, 1997) and means were separated at P=0.05 using Duncan's multiple range test.

RESULTS AND DISCUSSION

The main effects of variety, explant and plant growth regulators (PGR) regimes and their interaction were highly significant for all the parameters except for callus width where variety x explant x media interaction had no significant effect (Table 2). Naphthalene acetic acid (NAA) in combination with kinetin (media 14 and 15) enhanced callus initiation in African yam bean as all the explants of both varieties had formed calluses in these media as early as two weeks after culture. Also, compared with the other explants, callus initiation was faster with stem explant as it had formed calluses on all the media except media 1, 3, 4 and 6 at this age. On average, stem explant formed significantly longer and wider calluses than leaf explant. Root explant had the lowest mean values for callus length and width (0.14 and 0.06cm respectively) (Tables 3 and 4). In addition, parts of the calluses formed from stem and leaf explants were white-to- cream, soft-textured and friable (Figure 1), while the calluses formed from the root explant were compact, brownish and non friable (Figure 2). Studies have suggested that the organogenic potential is related to callus structures (Shimamoto *et al.*, 1993; Gurel *et al.*, 2001). Gurel *et al.* (2001) reported that white and friable callus consisting of large cells was able to produce roots and shoots in sugar beet, while green and compact callus with small cells showed no organogenic

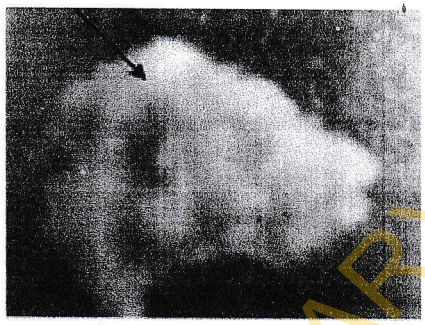

Figure 1 Callus induced from stem explant of African yam bean (SSSWN56) showing friable part (arrow).

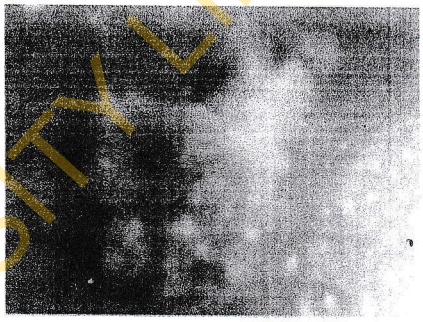

Figure 2 Non friable compact callus derived from root explant of African yam bean (SSSWN56).

Table 2 Mean square values of leaf, stem and root explants of two accessions of African yam bean in 16 media.

Source of variation	Length	Width	Percentage
Variety	6.12**	8.72**	1.00**
Explant	21.94**	15.47**	5.73**
Variety x Explant	3.06**	2.28**	0.94**
Media	6.57**	3.94**	1.88**
Variety x Media	0.65**	0.87**	0.52**
Explant x Media	1.71**	1.70**	0.38**
Variety x Explant x Media	0.62**	0.61^{ns}	0.32**
Error	0.03	0.51	0.003

*, **, significant at 0.05 and 0.01% probability levels, respectively.

Table 3 Length of callus formed (cm) by leaf, width and root explants of two African yam bean accessions cultured in 16 media.

Media	Leaf			Stem			Root			Overall Mean
	Accession			Accession			Accession			
	1	2	Mean	1	2	Mean	1	2	Mean	
1	0	0	0	0	0	0	0	0	0	0k
2	1.90	0	1.95	1.0	1.80	1.4	0	0	0	0.78ef
3	0	0	0	0	0.0	0	0	0	0	0k
4	0	0	0	0	0	0	0	0	0	0k
5	0.80	0	0.40	1.43	1.0	1.22	0	0	0	0.54hi
6	0.60	0	0.30	0	0	0	0	0	0	0.10k
7	0	0.30	0.15	1.77	0.60	1.18	0	0	0	0.49ij
8	1.90	0	0.95	1.83	1.23	1.53	0	0	0	0.83e
9	2.20	0.70	1.45	1.70	2.13	1.92	0.27	0	0.13	1.17d
10	0.60	0	0.30	1.53	0	0.77	0	0	0	0.36j
11	0.70	0.30	0.50	1.17	1.27	1.22	0	0.67	0.33	0.68fg
12	0	0	0	1.20	0.77	0.98	0	0.40	0.20	0.39j
13	2.10	0	1.05	1.67	0	0.83	0	0	0	0.63gh
14	2.80	2.00	2.40	1.67	1.93	1.80	0.67	0.67	0.67	1.62b
15	2.70	2.10	2.40	1.50	1.67	1.58	0	0.83	0.42	1.47c
16	3.50	3.70	3.60	1.83	1.93	1.88	0.97	0.0	0.48	1.99a
Mean	1.24	0.57	0.90b	0.90	0.76	1.02a	0.11	0.16	0.14c	0.69

Mean values on the same row/column with different letters are significantly different at 0.05 probability level; Column headings 1 and 2 are accessions SSSWN56 and SSSWN75, respectively.

Table 4 Width of callus formed (cm) by leaf, width and root explants of two African yam bean accessions cultured in 16 media.

Media	Leaf			Stem			Root			Overall Mean
	Accession			Accession			Accession			
	1	2	Mean	1	2	Mean	1	2	Mean	
1	0	0	0	0	0	0	0	0	0	0e
2	2.0	0	1.0	3.10	1.20	2.15	0	0	0	1.05bc
3	0	0	0	0	0	0	0	0	0	0e
4	0	0	0	3.0	0.	1.5	0	0	0	0.50de
5	0.60	0	0.30	0.70	0.47	0.58	0	0	0	0.29de
6	0.40	0	0.20	0	0	0	0	0	0	0.07e
7	0	0.20	0.10	0.80	0.20	0.50	0	0	0	0.20de
8	1.40	0	0.70	1.23	0.73	0.98	0	0	0	0.56cde
9	1.80	0.50	1.15	1.23	0.73	0.98	0.13	0	0.07	0.73bcd
10	0.60	0	0.30	0.67	0	0.33	0	0	0	0.21de
11	0.70	0.13	0.42	1.0	0.57	0.78	0	0.20	0.10	0.43de
12	0	0	0	0.50	0.30	0.40	0	0.13	0.07	0.16e
13	1.80	0	0.90	0.70	0	0.35	0	0	0	0.41de
14	2.0	1.3	1.65	0.67	1.33	1.0	0.60	0.27	0.43	1.03bc
15	1.90	2.1	2.0	0.80	1.67	1.23	0.00	0.30	0.15	1.13b
16	3.70	2.90	3.30	1.17	1.57	1.37	0.30	0	0.15	1.61a
Mean	1.06	0.45	0.75a	0.97	0.55	0.76a	0.06	0.06	0.06b	0.52

Mean values on the same row/column with different letters are significantly different at 0.05 probability level; Column headings 1 and 2 are accessions SSSWN56 and SSSWN75, respectively.

Table 5 Percentage callus formation by leaf, stem and root explants of two African yam bean accessions cultured in 16 media regimes.

Media	Leaf			Stem			Root			Overall Mean
	Accession			Accession			Accession			
	1	2	Mean	1	2	Mean	1	2	Mean	
1	0.0	0	0	0.0	0	0	0	0	0	0g
2	100	0	50	100	100	100	0	0	0	50d
3	0.0	0	0	0.0	0	0	0	0	0	0g
4	0.0	0	0	0.0	0	0	0	0	0	0g
5	100	0	50	100	100	100	0	0	0	50d
6	100	0	50	0.0	0	0	0	0	0	16.67f
7	0.0	100	50	100	100	100	0	0	0	50d
8	100	0	50	100	100	100	0	0	0	50d
9	100	100	100	100	100	100	66.67	0	33.33	77.78c
10	100	0	50	100	0	50	0	0	0	33.33e
11	100	100	100	100	100	100	0	100	50	83.33b
12	0.0	0	0	100	100	100	0	100	50	50d
13	100	0	50	100	0	50	0	0	0	33.33e
14	100	100	100	100	100	100	100	100	100	100a
15	100	100	100	100	100	100	0	100	50	83.33b
16	100	100	100	100	100	100	100	0	16	83.33b
Mean	68.75	37.5	53.12b	75	62.5	68.01a	16.67	25	20.83c	0.48

Mean values on the same row/column with different letters are significantly different at 0.05 probability level; Column headings 1 and 2 are accessions SSSWN56 and SSSWN75, respectively.

capacity. The highest value among the media for the length of callus formed by leaf, stem and root explants across varieties was 3.60, 1.92 and 0.67cm respectively. These results showed that although callus from the stem explant had the highest mean value for length, the longest callus in this study was recorded in the leaf explant (Table 3). Godwin *et al.* (1987) reported that leaf blades were better explant for callus induction and growth than either petioles or stems in the tropical legume, *Stylosanthes scabra*, while Gowda and Satyan (1988) reported that hypocotyl explants of cowpea exhibited the highest mean callus performance on MS medium containing only 0.01 mg/l kinetin.

Among the different media, 100% callus formation was recorded for the three explants of the two African yam bean accessions in the medium containing 1.5 mg/l each of kinetin and NAA (Medium 14) (Table 5) indicating the positive response of African yam bean to NAA. No callus was formed in hormone-free medium or in the media containing IAA except on media 5 and 6 with 50% and 16.67% callus formation, respectively (Tables 1 and 5), indicating the sensitivity of African yam bean to IAA. Gowda and Satyan (1988) also reported that cowpea was sensitive to 2,4-D (another auxin): they observed that as the concentration of 2,4-D increased in the medium, there was a gradual reduction in the quantity of callus production. Sairam *et al.* (2003) however, observed that a combination of two auxins at high concentrations slightly promoted the frequency of callus formation over the control (2.26 µM 2,4-D) in soybean.

Of the two accessions, SSSWN56 (brown seed) had higher mean values for length, width and percentage callus formation across media and explants than SSSWN75 (grey seed) (Figure 3). Percentage callus formation for

Table 5 Percentage callus formation by leaf, stem and root explants of two African yam bean accessions cultured in 16 media regimes.

Media	Leaf			Stem			Root			Overall Mean
	Accession			Accession			Accession			
	1	2	Mean	1	2	Mean	1	2	Mean	
1	0.0	0	0	0.0	0	0	0	0	0	0g
2	100	0	50	100	100	100	0	0	0	50d
3	0.0	0	0	0.0	0	0	0	0	0	0g
4	0.0	0	0	0.0	0	0	0	0	0	0g
5	100	0	50	100	100	100	0	0	0	50d
6	100	0	50	0.0	0	0	0	0	0	16.67f
7	0.0	100	50	100	100	100	0	0	0	50d
8	100	0	50	100	100	100	0	0	0	50d
9	100	100	100	100	100	100	66.67	0	33.33	77.78c
10	100	0	50	100	0	50	0	0	0	33.33e
11	100	100	100	100	100	100	0	100	50	83.33b
12	0.0	0	0	100	100	100	0	100	50	50d
13	100	0	50	100	0	50	0	0	0	33.33e
14	100	100	100	100	100	100	100	100	100	100a
15	100	100	100	100	100	100	0	100	50	83.33b
16	100	100	100	100	100	100	100	0	16	83.33b
Mean	68.75	37.5	53.12b	75	62.5	68.01a	16.67	25	20.83c	0.48

Mean values on the same row/column with different letters are significantly different at 0.05 probability level; Column headings 1 and 2 are accessions SSSWN56 and SSSWN75, respectively.

capacity. The highest value among the media for the length of callus formed by leaf, stem and root explants across varieties was 3.60, 1.92 and 0.67cm respectively. These results showed that although callus from the stem explant had the highest mean value for length, the longest callus in this study was recorded in the leaf explant (Table 3). Godwin *et al.* (1987) reported that leaf blades were better explant for callus induction and growth than either petioles or stems in the tropical legume, *Stylosanthes scabra*, while Gowda and Satyan (1988) reported that hypocotyl explants of cowpea exhibited the highest mean callus performance on MS medium containing only 0.01 mg/l kinetin.

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Of the two accessions, SSSWN56 (brown seed) had higher mean values for length, width and percentage callus formation across media and explants than SSSWN75 (grey seed) (Figure 3). Percentage callus formation for

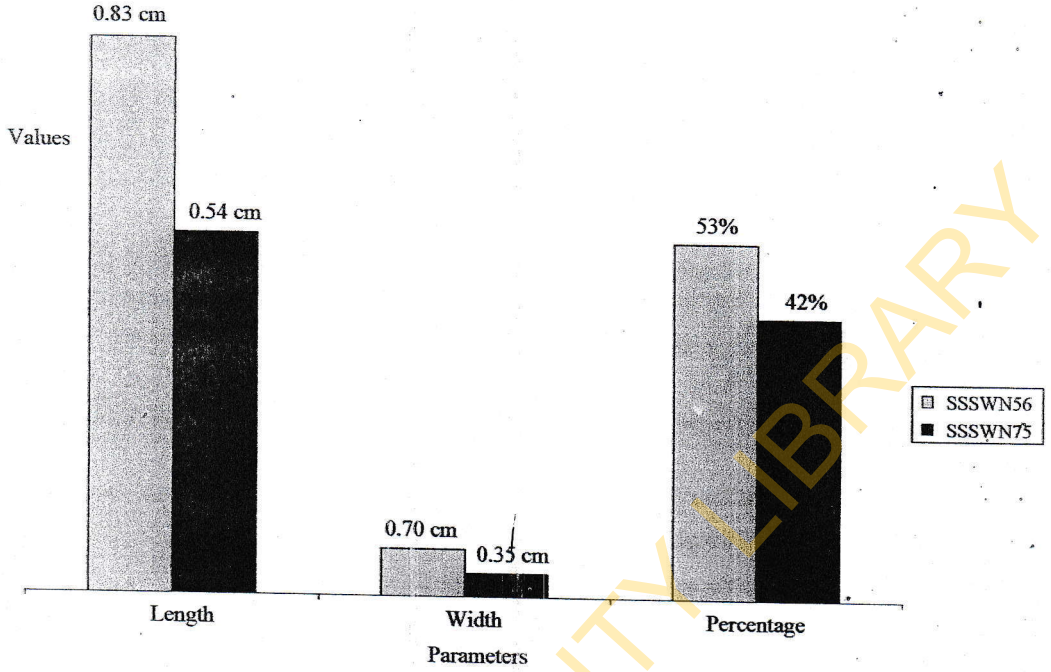

Figure 3 Length, width and percentage callus formation of two African yam bean accessions in 16 media.

SSSWN56 was 53%, while that of SSSWN75 was 42%. Both accessions also responded equally with 100% callus formation in the medium containing 1.5 mg/l each of Kinetin and IAA (medium 14). Therefore, this medium is recommended for callus induction in African yam bean using stem explant. Although the brown-seeded African yam bean accession (SSSWN56) had better callus formation in this study, more genotypes of the crop need to be studied to ascertain the genotypic effect on callus induction with the different combinations of the growth hormones.

In this study, compared with other explants, highest callus formation was recorded in stem explants, while the root explant had the lowest. It was also observed that no callus was formed in hormone-free medium or the media containing indole acetic acid, but 100% callus formation was recorded for both of the African yam accessions used in the medium containing 1.5 mg/l each of kinetin and naphthalene acetic acid. This medium will be useful in developing an optimum protocol for regeneration of African yam bean.

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