ISRJ ONLINE
INTERNATIONAL SCIENCE RESEARCH JOURNAL
ISSN: 2006-1730

MEDIA FORMULATION FOR PLANTLET REGENERATION AND CALLUS INDUCTION FROM SESAME SEED

***[1]Mbagwu. F.N., [1]Fabunmi, O.I, [2]Edeoga, H.O**

[1]Department of Plant Science and Biotechnology, Imo State University, P.M.B 2000, Owerri, Nigeria
[2]Department of Biological Science, Michael Okpara University of Agriculture, Umahia, Abia State.

ABSTRACT: Media formulation for plantlet regeneration and callus induction from *Sesamum indicum* seeds were investigated to acertain which of the media will promote plantlet regeneration and callus induction. Plantlets were regenerated from the seed explants of the accession number NG/SA/07/137of Sesame using four media. The outcome of the investigation showed that regenerated plantlets exhibited vigorous growth in Murashige and Skooge {MS}media supplemented with 0.75 mg/l of IAA. Callus was also induced using2,4-D alone and a combination of 2,4-D and casein hydrolysate. The average callus size and percentage of callus inducted varied with different concentrations of hormones. These findings will provide basis for the development of efficient protocol for the production of a high quality callus and regeneration of Sesame plantlets.

KEY WORDS: *Sesamum indicum*, Media formulation, Plantlet regeneration, callus induction, Tissue Culture

INTRODUCTION

Sesame (*Sesamum indicum*.L.) belongs to the family Pedialaceae and is considered to be the oldest oil seed crop known to man (Brar and Ahuja, 1979). It is cultivated for its edible seed and is thought to have originated in Africa (Ram *et al*, 1990). Sesame is an erect annual self pollinating plant with an erect pubescent. It may be highly branched or relatively unbranched depending on the variety (Kinman and Llyod, 1988). The leaves are ovate to lanceolate, while the flowers are tubular, bell shaped and two lipped with a pale purple or rose to white colour. The fruit is an oblong pubescent capsule containing numerous small, oval and yellow, white, red, brown or black seeds.

Nutritionally, Sesame is very good for all ages as it contains trice the value of calcium than a comparable measure of milk, indicating it to be a good meal for children. Also the oil produced from the seed (benne oil) has natural anti-oxidants and is both water and oil soluble and provides a variety of health benefits upon ingestion. Nigeria is ranked 35th in the World in the production of Sesame. Most of the total output is produced in Tiv and Idoma areas of Benue State, Igbira area of Kwara State and Kwali area of Niger State (Agboola, 1979)

Although a major world oil seed crop , Sesame is primarily grown by small scale farmers in developing countries using crude implements. Crop development programs in these countries are either small or non-existent and little progress has been made during the past 20 years. Low yield potential coupled with the problems encountered during harvesting have tended to discourage growers leading to a decline in the total area devoted to its cultivation (Busari and Ajewole, 1993).

It is interesting to note that despite the high demand of Sesame due to its high qualitative oil, there is a decrease in its cultivation and production due to farmer's crude methods of cultivation. Therefore, there is the need to device a method that will enhance its production especially through modern methods like tissue culture with a view to mass propagate the plant to meet up with the increasing demand. Hence, the objectives of this research work is to formulate the best media that will promote the growth and development of Sesame and recommend the best modern and advanced technology that will enhance its growth and development.

MATERIALS AND METHOD

Source of Material:

The seeds used for this study were collected from the seed gene bank unit of the National Center for Genetic Resources and Biotechnology, Apata, Ibadan, Oyo State, Nigeria.

Experimental Design:

The design used for this experiment was the Completely Randomized Design (CRD). Treatment A1 consisted of IAA while A2 consisted of IBA at varying concentrations. Treatment B1 consisted of a combination of BAP +IAA while B2 consisted of Kinetin +IAA. Treatment C1 consisted of 2,4-D alone while C2 consisted of a combination of 2,4-D +CH. The outcome of the experiment was analyzed using the Analysis of Variance (ANOVA) at a probability of 0.05. Treatment A had 10 levels; B had 10 levels while treatment C had 8 levels and 2 levels of MS as control

Treatment A1 (IAA):

(i) 0.5mg/l
(ii) 0.75mg/l
(iii) 1.00mg/l
(iv) 1.25mg/l
(v) 1.50mg/l

Treatment A2 (IBA):

(i) 0.5mg/l
(ii) 0.75mg/l
(iii) 1.00mg/l
(iv) 1.25mg/l
(V) 1.50mg/l

***Corresponding Author:** E-mail: Mbagwu101@yahoo.co.uk

Treatment B2 (BAP +IAA):
(i) 0.8mg/l +0.5mg/l
(ii) 1.0mg/l +0.5mg/l
(iii) 1.2mg/l + 0.5mg/l
(iv) 1.4mg/l +0.5mg/l
(v) 1.6mg/l + 0.5mg/l

Treatment B2 (Kinetin + IAA):
(i) 0.8mg/l + 0.5mg/l
(ii) 1.0mg/l + 0.5mg/l
(iii) 1.2mg/l + 0.5mg/l
(iv) 1.4mg/l + 0.5mg/l
(v) 1.6mg/l + 0.5mg/l

Treatment C1 (2,4-D):
(i) 2.0mg/l
(ii) 2.5mg/l
(iii) 3.0mg/l
(iv) 3.5mg/l

Treatment C2 (2,4-D + CH):
(i) 2.0mg/l +0.1g/l
(ii) 2.5mg/l + 0.1g/l
(iii) 3.0mg/l + 0.1g/l
(iv) 3.5mg/l + 0.1g/l

RESULTS

The result obtained indicated that shoot height of plantlets was highest in 0.75 mg/l IAA enriched media while the lowest value for the shoot height (0.410)was recorded in 1.5mg/l IAA. The mean value of plant shoot height was highest in 1.5mg/l IBA while the lowest mean value for shoot height was 1.25 mg/l IBA. They were found to be significantly different from each other at a probability of 0.05 ($p< 0.05$).This is shown in Table 1a which compared the mean values for shoot height, number of nodes and number of roots between different levels of IAA and IBA.

In Table 1b, the highest value for shoot height (1.100) was produced in 1.6mg/l BAP + o.5mg/l IAA and the lowest value (0.312)was obtained in 1.2mg/l BAP + 0.5mg/l IAA. Similarly, the highest value for shoot height was produced in 1.6 mg/l KIN + 0.5 mg/l IAA while the least value was recorded in 1.4mg/l KIN + 0.5mg/l IAA. Comparing the four levels of media, the tallest shoot growth (2.522) was produced in 075mg/l IAA.

1.6 KIN gave the highest number of roots (1.408) while 1.4mg/l KIN recorded the least number of roots (0.701). The various hormone regimes were found to be significantly different at a probability of 0.05. Similarly, 1.0mg/l BAP, 1.2mg/l BAP and 1.6mg/l BAP produced the same mean number of nodes (3.24) while 0.8mg/l BAP gave the lowest number of nodes (2.56).The highest number of nodes was recorded in 1.00mg/l KIN and 1.20mg/l KIN while 1.4mg/l KIN recorded the lowest number of nodes. They were all not significantly different from each other at a probability of 0.05 ($p<0.05$).

Percentage callus formation and average size of callus induced by various phytohormones are represented in Table 2. The lowest concentration of 2,4-D at 2.0mg/l induced the highest percentage of callus (60%) while the highest concentration at3.5mg/l induced the least percentage of callus(40%). The lowest concentration of2,4-D at 2.0mg/l+o.1g/l CH induced the least percentage of callus(30%) while the highest concentration of 2,4-D at 3.5mg/l +0.1g/l CH gave the higest percentage of callus(50%). MS medium supplemented with 2.0mg/l 0f 2,4-D recorded the biggest callus size of 2.84. The lowest concentration of 2,4-D at 2.0mg/l +0.1g/l CH gave the largest callus with a size of 1.63 .

Table 1a: Mean values of three morphological attributes evaluated on Sesame plantlets grown on IAA and IBA enriched media.

MEDIA	SHOOT HEIGHT (Mean +SE)	ROOT NUMBER (Mean +SE)	NODE NUMBER (Mean +SE)
Control	1.350+0.7221ab	1.16+0.477a	3.32+0.669a
0.50mg/l IAA	1.032+0.5185ab	1.00+1.000a	3.63+1.200a
0.75mg/l IAA	2.501+1.2593ab	4.31+0.232a	3.00+0.573a
1.00mg/l IAA	0.431+0.2323b	2.32+1.200a	2.32+ 0.330a
1.25mg/l IAA	1.239+0.5305b	3.00+1.840a	3.00+0.447a
1.50mg/l IAA	0.399+0.2304ab	4.99+1.730a	3.23+0.790a
0.50mg/l IBA	0.819+0.2474a	4.20+1.064a	4.20+0.582a
0.75mg/l IBA	0.324+0.1884b	1.23+0.249a	3.24+0.944a
1.00mg/l IBA	0.899+0.3382ab	2.14+0.703a	4.16+0.600a
1.25mg/l IBA	0.298+0.2998b	1.00+0.573a	2.25+0.249a
1.50mg/l IBA	1.248+0.7499ab	2.00+1.000a	4.50+0.500a

Table 1b: Mean values of three morphological attributes evaluated on Sesame plantlet grown on BAP and Kinetin enriched media.

MEDIA	SHOOT HEIGHT (Mean+SE)	ROOTNUMBER (Mean+SE)	NODE NUMBER (Mean+SE)
Control	1.6490+0.15434a	1.50+0.220a	3.00+0.443a
0.8mg/lBAP+0.5mg/lIAA	0.7500+0.5950abc	0.50+0.285ab	2.56+0.285a
1.0mg/lBAP+0.5mg/lIAA	0.7000+0.4222abc	0.50+0.500ab	3.24+0.475a
1.2mg/lBAP+0.5mg/lIAA	0.3125+0.22943abc	0.50+0.263ab	3.20+0.411a
1.4mg/lBAP+0.5mg/lIAA	0.6249+0.62500c	0.13+0.124ab	2.87+0.514a
1.6mg/lBAP+0.5mg/lIAA	0.9749+0.60601abc	0.00+0.000b	3.24+0.749a
0.8mg/lKIN+0.5mg/lIAA	1.2331+0.60532abc	1.16+0.473ab	3.00+0.258a
1.0mg/lKIN+0.5mg/lIAA	1.0143+0.27292abc	1.14+0.340ab	4.00+0.534a
1.2mg/lKIN+0.5mg/lIAA	1.0000+0.25491abc	1.19+0.371ab	4.00+0.00a
1.4mg/lKIN+0.5mg/lIAA	0.6000+0.78950abc	0.43+0.202b	2.85+0.401a
1.6mg/lKIN+0.5mg/lIAA	1.3990+0.50543abc	1.75+0.700a	3.61+0.592a

Table 2: Percentage callus formation and average callus size.

Media	No of explants cultured	No of Explants Callused	Percentage callus	Average Callus size
2.0mg/l 2,4-D+0.1g/lCH	10	6	60	2.84
2.5mg/l 2,4-D+0.1g/lCH	10	5	50	1.44
3.0mg/l 2,4-D+0.1g/lCH	10	5	50	1.44
3.5mg/l 2,4-D+0.1g/lCH	10	4	40	1.71
2.0mg/l 2,4-D+0.1g/lCH	10	3	30	1.63
2.5mg/l 2,4-D+0.1g/lCH	10	4	40	1.25
3.0mg/l 2,4-D+0.1g/lCH	10	3	30	1.00
3.5mg/l 2,4-D+0.1g/lCH	10	5	50	1.25

DISCUSSION

In treatment A all MS medium supplemented with IAA and IBA supported shoot, root and node development and their growth was influenced significantly by plant growth regulators.

In trearment B, both BAP and Kinetn were observed to induce morphological growth reponse on the explant. In MS medium supplemented wih varying concentrations of BAP and 0.5mg/l IAA, shoot root and node proliferation was induced. BAP in combination with auxin have being reported by Ogunsola and Ilori(2008) to induce rapid root, shoot and node profeliration on embryo and nodal culture of *Synselpanum dulcificum*

All concentration of 2,4-D and 2,4-D supplemented with 0.1g/l of Casein hydrolysate resulted to callus production. This is in agreement with the work of Xie and Hong (2001) in *Acacia mangium* where calli were reportedly induced from cotyledon explants of mature zygotic embryo in MS basal medium supplemented with 2,4-D and kinetin . The control medium also supported the germination of Sesame seed explant with significant number of roots, nodes and shoots.

CONCLUSION

Seed explants of *Sesamum indicum* L. recorded the highest degree of callus in terms of size and percentage when cultured on MS medium supplemented with lower concentrations of 2,4-D alone than one supplemented with 2,4-D +0.1g/l CH. This suggests that 2,4-D alone at low concentration induce callus better than when supplemented with other phytohormones. All the phytohormones used (IBA, IAA, BAP and Kinetin) supported the growth of whole plantlets of seed explants with significant development of morphological characters after four weeks.

REFERENCES

Agboola, S.A. (1979). Sesame Agriculture. Atlas of Nigeria, Oxford University Press. London.

Brar, G.S. and Ahuja, K.L.(1979).Sesame, Its culture, Genetics, Breeding and biochemistry. Annual Rev. Plant Science. Malik C.P(ed). Kalyani Publishers, New Delhi. Pp245-313.

Busari, .O. and Ajewole, A.O.(1993).Sustainable Development in Agriculture and Environment. University Press, Ibadan.pp 15-20

Kinmann, M.L and Llyod, J.A(1988). Present Status of Sesame Breeding in the United States. *Agran. J. Vol 46* pp24-29.

Ogunsola ,.K.E. and Ilori, C.O.(2008). In-vitro propagation of Miracle Berry (*Synesepalnum dulcificum*) through embryo and nodal cultures. African Journal of Biotechnology Vol33 pp244-248.

Ram, R.P., Catlin,.J. and Cowley.C. (1990) Sesame; New Approaches for Crop Improvement In: Janick .J. (editions), Advances in Crops. Timber Press, Portland pp225-228.

Xie, .D. and Hong,.Y.(2001)..In-vitro regeneration of *Acacia mangium* via Organogenesis, *Plant Cell Tissue Organ Cult* 66, pp 167-173