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Effect of hormones on in vitro culture of Sesame *Sesamum indicum* (L.) embryo

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The in vitro response of three Sesame *Sesamum indicum* L. genotypes, Alaede, E8 and Ex Sudan obtained from NCRI, Badeggi, Nigeria was studied using tissue culture techniques. Growth media with different concentrations and combinations of hormones were used for callus induction, shoot formation from callus subculture and plantlets development from leaf subculture. Ex Sudan recorded the highest callusing frequency at 0.25mg*L^{-1} 2, 4 D + 0.08mg*L^{-1} Kinetin. It also recorded the highest shoot formation frequency on full strength MS media only and produced the highest mean in seedling development from sub cultured leaves at 0.1mg*L^{-1} Kinetin + 0.2mg*L^{-1} NAA and 0.3mg*L^{-1} Kinetin + 0.1mg*L^{-1} 2,4 D. The latter concentration showed significant difference among the three genotypes. Ex Sudan was found to be more suitable for tissue culture procedures as a result of its responses to the treatments administered, and stand as a candidate for improvement of the other local varieties.

Keywords: calluses, concentrations, hormones, in vitro, sesame

Several opportunities now exist for sesame improvement as a result of recent developments in plant tissue culture and genetic manipulation of crop plants. Tissue culture methods involving a callus phase or regeneration via somatic embryogenesis are known to produce stable variants (Armstrong and Philips, 1988). Somatic embryos have been successfully induced directly from the surface of the zygotic embryos of sesame in culture. Tissue culture method can also be used to facilitate wide crosses using embryo culture techniques. Although, conventional hybrid crosses between cultivated sesame and its wide relatives have been attempted, however in most cases, hybrids were difficult to produce (Nayar and Mehra, 1970; Falusi, *et al*., 2001).

In Nigeria sesame was one of the major foreign exchange earners prior to independence. However, with the rise in demand in production of soybeans, sesame production declined significantly in the late 1960s and early 1970s (Abdullahi, 1998). Sesame seeds are roasted and mixed with groundnut seeds to make soup thickening in Nigeria, and mixed with wheat flour in bakery products to increase its protein contents. It is also used in the production of

paints, soap perfumes, pharmaceutical products and insecticides (Oplinger *et al*., 1997) Sesame oil is one of the best edible oils available as it contains antioxidants sesamolin and sesamin which prevent ageing and malfunctioning of the liver. Sesame oil is as good as olive oil being highly unsaturated and low in cholesterol (Uzo, 1998). After oil extraction, the cake that is left contains 35- 50% proteins, 20% fat and is rich in tryptophan and methionine. The cake is used in production of animal feed (Ram *et al*., 1990), sesame 'ogi' by the Federal Institute of Industrial Research (FIIRO) and has the potentials to be incorporated into infant weaning food (Abdullahi, 1998).

In spite of the various uses to which sesame can be put, large scale production of sesame by conventional means is limited by incidences of diseases and loss of valuable seeds due to the shattering nature of the seeds from pods. Propagation in vitro will however open the door to improvement as tissue culture is the basic procedure for manipulation of plant genome to produce resistance to diseases and also to improve the pod structures to eliminate wastages brought about by shattering.

A number of works have been done on various aspects of sesame development. Taskin *et al*., (1999) studied the *in vitro* regeneration of sesame while George *et al*., (1987) worked on in vitro propagation and shoot tip culture of different cultivars. The protoplasts of sesame were isolated, cultured and induced to form calluses (Bapat *et al*., 1989). Nodal explants from sesame were used for micro propagation by in vitro multiple shoot production by Gangopadhyay *et al*., (1989) while Jeya and Jeyabalan (1997) studied the influence of growth regulators on somatic embryogenesis in sesame. The response of sesame genotypes was studied *in vitro* for callus induction, multiplication, rooting and shooting using different media components (Saravanan and Nadarajan, 2005). However, it was observed that no work has been done on the tissue culture of *Sesamum indicum* in Nigeria. This work, therefore, aims at achieving in vitro germination of sesame embryos, induction of calluses and regeneration of shoots from the embryos, production of seedlings from leaf culture of sesame and establishing appropriate combination of hormones for optimal development of embryo, calluses and seedlings. All these will be linked together to see the responses of the genotypes under study with a view to improving the growth, and hence production and yield of the genotypes.

Materials and Methods

Plant materials

Three genotypes of sesame seeds used for this study were obtained from the National Cereals Research Institute, (NCRI) Badeggi, Niger State, Nigeria. The genotypes were Alaede, E8 and Ex- Sudan.

Culture media

The basal media used for the experiment was Murashige and Skoog (1962) modified with 3% Sucrose, 0.1g*L^{-1} Inocitol and growth hormones concentrations.

Growth hormones

The growth hormones used for the study were obtained from the National Center for Genetic Resources and Biotechnology (NACGRAB), Ibadan, Nigeria. They are abbreviated as follows : 2,4 D (2,4 dichloro - phenoxyacetic acid), CM (Coconut Milk), K (Kinetin), BAP (6,benzylaminepurine) and NAA (Napthalene-1-acetic acid).

Plant Materials

Seeds viability

Viability test was carried out on the seeds before they were used by planting the seeds

on wet filter paper placed inside sterilized Petri dishes. The Petri dishes were covered, sealed and placed in growth room and observed after one week.

Preparation of explants

The explants used are the embryos excised from the seeds. The seeds were placed in separate glass bottles and labeled according to varieties. Each variety was washed with liquid soap and properly rinsed under running water. The seeds were immersed in distilled water, the bottle covered with foil and transferred to the laminar flow bench for sterilization. Prior to use, the UV light of the laminar flow bench was turned on for 15mins, and then turned off. The utility light and the fan were turned on and the surface of the bench and the plant material were swabbed with 70% ethanol solution. Ethanol wash and a glowing splint were prepared for sterilizing the scalpels and forceps at intervals.

Single Disinfection of Seeds

The distilled water in the seed bottles was drained off and the seeds immersed in 70% ethanol solution for 5mins after which the solution was drained off. The seeds were then immersed in 10% Sodium Hypochlorite solution for 20mins and the solution drained out. The seeds were washed in three changes of sterile distilled water and left in the third rinse.

Excising the Embryo

The sterilized seeds were placed on a sterile filter paper inside sterile Petri dish with the aid of sterilized forceps and scalpel; seed coats were carefully removed with the aid of a scalpel. The cotyledons were also gently removed without damaging the embryo.

Culturing of Explants

The forceps used was sterilized in glowing splint after rinsing in ethanol and allowed to cool. The mouth of the medium test – tube was flamed in the glowing splint and the embryo was carefully placed on the medium. The test tube was covered and the forceps sterilized in the glowing splint ready for the next culturing. The media bottle was closed with paraffin to prevent contamination, and the test tubes labeled and dated.

The culture was transferred to the growth room for incubation at 28^0 C ± 2^0 C with a 16/18 hour photoperiod from cool-light fluorescent lights with an intensity of 2000 lux. For each treatment, three replicates with 10 tubes per replicate were maintained. The cultured embryos were scored for the appearance of embryonic callus formation and shoot formation. The calluses derived from the various media compositions were used to produce shoot and the leaves from the shoots were also sub cultured to produce seedlings.

The data obtained were analyzed using CRD (Completely Randomized Design) according to the methods of Steel and Tore (1980). Duncan Multiple Range test was used to test the level of significance among the replicates (Duncan, 1955).

Results and Discussion

Table 1 shows the result of the viability test carried out on the seeds of the different genotypes. Ex Sudan had the highest germination percentage (82.4%) followed by E8 (76.7%) and Alaede (68.9%)

The responses of the three genotypes with regard to callus induction were the same with the exception of Ex Sudan which was lower when treated with 3mg*L^{-1} concentration of 2, 4 D (Table II). The rate of callus induction decreased when the 2, 4D concentration was reduced to 2mg*L^{-1}. The addition of 50ml*L^{-1} of coconut milk (CM) to 2mg*L^{-1} 2, 4 D however tend to reverse the result obtained from 2, 4D only by increasing the rate of callus induction.

Adding 100ml*L^{-1} CM to 3mg*L^{-1} 2, 4D also reduced the rate of callus induction in Alaede and E8 and show no effect on Ex Sudan. The replacement of CM with Kinetin at various concentrations produced a significant increase in the rate of callus induction.

There was significant difference in the responses of the three genotypes with Ex Sudan producing the highest mean of 0.8 at the concentration of 0.3mg*L^{-1} 2, 4D and 0.1mg*L^{-1} kinetin (Table III). The highest callus production was recorded at the combination of 0.08mg*L^{-1} kinetin and 0.25mg*L^{-1} 2, 4D with Ex Sudan producing a mean of 0.9 and E8 and Alaede, 0.7 and 0.5 respectively. Full strength MS produced a significant difference (when subjected to statistical analysis in callus production in the three genotypes). Ex Sudan produced the highest mean of 0.9 and Alaede the lowest of 0.5.

Table I: Percentage germination of Sesame genotypes

Genotypes	Germination Percentage (%)
Alaede	68.9
E8	76.7
Ex Sudan	82.4

Table II: Means of callus induction from embryo culture in Sesame genotypes

Treatment	Genotypes		
	Alaede	E8	Ex Sudan
2,4 D			
3mg*L^{-1}	0.5 ± 0.16	0.5 ± 0.16	0.4 ± 0.15
2mg*L^{-1}	0.1 ± 0.09	0.3 ± 0.14	0.2 ± 0.13
2, 4D + CM			
2mg*L^{-1} + 50ml*L^{-1}	0.5 + 0.16	0.5 + 0.16	0.4 + 0.15
3mg*L^{-1}+ 100ml*L^{-1}	0.1 ± 0.09	0.3 ± 0.14	0.4 ± 0.15
2, 4 D + K			
0.3mg* L^{-1} + 0.1mg *L^{-1}	0.2 ± 0.13^{b}	0.4 ± 0.15^{b}	0.8 ± 0.13^{a}
0.25mg*L^{-1} + 0.08mg*L^{-1}	0.5 ± 0.16	0.7 ± 0.14	0.9 ± 0.09
0.20mg*L^{-1} + 0.06mg*L^{-1}	0.5 ± 0.16	0.5 ± 0.16	0.8 ± 0.13
0.15mg*L^{-1}+ 0.04mg*L^{-1}	0.2 ± 0.13	0.6 ± 0.16	0.6 ± 0.16
0.1mg*L^{-1} + 0.02mg*L^{-1}	0.4 ± 0.15	0.5 ± 0.16	0.7 ± 0.14
MS Only			
Full strength	0.1 ± 0.09^{b}	0.6 ± 0.16^{b}	0.9 ± 0.09^{a}
Half strength	0.2 ± 0.13	0.3 ± 0.14	0.5 ± 0.16

^{ab} Means with different superscripts in the same row are significantly different.

Table III: Means of shoot formation from callus subculture in Sesame genotypes

Treatment	Genotypes		
	Alaede	E8	Ex Sudan
100ml*L^{-1} CM	0.3 ± 0.14	0.4 ± 0.15	0.4 ± 0.15
0.05mg*L^{-1} BAP +0.01mg*L^{-1} IAA	0.1 ± 0.09	0.3 ± 0.14	0.5 ± 0.16
MS Only			
Full strength	0.3 ± 0.14	0.5 ± 0.16	0.7 ± 0.14
Half strength	0.5 ± 0.16	0.5 ± 0.16	0.6 ± 0.16

Table IV: Mean of plantlets development from leaf subculture in Sesame genotypes

Treatment	Genotypes		
	Alaede	E8	Ex Sudan
K(mg*L^{-1}) + NAA(mg*L^{-1})			
0.1 + 0.01	0.4 ± 0.15	0.4 ± 0.15	0.7 ± 0.14
0.1 + 0.02	0.4 ± 0.15	0.7 ± 0.14	0.8 ± 0.13
K(mg*L^{-1})+2,4 D(mg*L^{-1})			
0.1 + 0.3	0.2 ± 0.13	0.3 ± 0.14	0.4 ± 0.15
0.3 + 0.1	0.3 ± 0.14^{b}	0.6 ± 0.16^{ab}	0.8 ± 0.13^{a}
MS Only			
Full strength	0.2 ± 0.13	0.20 ± 0.13	0.3 ± 0.14
Half strength	0.1 ± 0.9	0.2 ± 0.13	0.2 ± 0.13

^{a,b} Means with different superscripts in the same row are significantly different.

In respect of shoot formation from callus subculture, the culture on MS only produced higher means in the three genotypes with Ex Sudan having the highest mean of 0.7 and Alaede the lowest mean of 0.3, the result was reduced with half strength MS. Addition of 100ml*L^{-1} CM reduced the shoot production of Ex Sudan from 0.7 in MS to 0.4, while Alaede was not affected. Modifying the MS with 0.005mg*L^{-1} BAP plus 0.01 mg*L^{-1}NAA however reduced the rate of shoot production from 0.7 in Ex Sudan (MS), to 0.5; and 0.3 in Alaede (MS) to 0.1.

The responses of the genotypes to MS only (full strength and half strength) for the production of plantlets from leaves subculture was low compared to the modified MS. Ex Sudan produced the highest mean of 0.3 with full strength MS, while the least mean in the MS treatment was 0.1 produced by Alaede at half strength MS treatment. Ex Sudan and E8 showed increase in mean of plantlets production as the concentration of NAA was increased from 0.1 to 0.2mg*L^{-1} but Alaede remained unaffected (Table IV). Replacing NAA with 0.3mg*L^{-1} 2, 4D reduced shoot production drastically in the three genotypes. Increasing kinetin concentration to 0.3mg*L^{-1} and reducing that of 2, 4 D to 0.1mg*L^{-1} produced a significantly different increase in plantlets production in the three genotypes with Ex Sudan producing the highest number of seedlings.

The high germination rate of the genotypes, although not significantly different, was an indication that the seeds used were viable. The differences observed in the viability confirm the differences in the genotype of the three samples. The highest percentage of Ex Sudan (82.7%), (Table I), may have arisen from its genetic constitution which confers an advantage on it. The seeds of Ex Sudan (2mm) and E8 (1.5mm) were also relatively bigger than that of Alaede (1.3mm) in length. This could have also contributed to the performances as Alaede responded least in all the treatments.

Auxins are generally known to positively influence cell enlargement and division (Strivastava, 2002). This was responsible for the reduction in callus induction when the concentration of 2, 4 D (an auxin) was reduced from 3mg*L^{-1} to 2mg*L^{-1} (Table II). Jeya and Jeyabalan, (1997) reported that 2, 4 D was the most effective auxin in callus induction, hence its adoption for this work. The increment

observed in callus induction when 50ml*L^{-1} Coconut Milk was added to 2mg*L^{-1}2, 4 D conformed with the observation of Shrawat, *et al*., (2001) that Coconut Milk had the same effect as auxins. The reduction in callus production of Alaede and E8 and its non effect on Ex Sudan when 100ml*L^{-1} Coconut Milk was added to 3mg*L^{-1} 2, 4 D showed that the auxin-like effect of Coconut Milk was a function of both genotype and the volume of Coconut Milk added.

The increment observed in three genotypes when Coconut Milk was replaced with Kinetin showed that Kinetin was more suitable for callus production in the sesame genotypes under study. The ratio of auxins to cytokinins suitable for callus induction in these three genotypes is approximately 3:1; this is different from the general ratio of 1:1 given by Esiobu (2008).

The reduction in the mean of callus production in half strength MS can be attributed to the reduction in the composition of the normal growth medium required by plants which is complete in full strength MS but reduced to half in half strength MS. The significant difference observed in the mean of the three genotypes showed that the three would respond differently to the same treatment owing to their different genotypes.

As the calluses were sub cultured to produce leaves, even though Ex Sudan produced a higher mean on MS in comparison with the others, the means were not significantly different when subjected to statistical analysis. The same applied to half strength MS. Reduction in the means of the three genotypes when compared to full strength MS is as a result of the reason given in callus production. MS modified with 0.05mg*L^{-1} BAP and 0.01mg*L^{-1}IAA produced higher mean in Ex Sudan and less in Alaede and E8. Since the increment is not significantly different, it can be deduced that BAP and Coconut Milk had the same effect on shoot production in the three genotypes. The ratio of BAP: IAA (Cytokinin to Auxin) in this work was 5:1; this is close to 5.5:1 obtained by Saravanan and Nadarajan (2005) in their shoot multiplication experiment in sesame culture.

In leaf subculture to produce plantlets, Ex Sudan responded best of all the three genotypes to all the treatments they were subjected to. The responses were generally low with MS only. This means that modifying the media was important for shoot production from leaves in these genotypes. Increasing the ratio of cytokinin (K) to auxin (NAA) to 1:20 from 1:10 (i.e. from 0.1mg*L^{-1}K + 0.01mg*L^{-1} NAA to 0.1mg*L^{-1} K + 0.02mg*L^{-1} NAA) increased the responses of Ex Sudan and E8 without any effect on Alaede (Table IV). It can be deduced therefore that the three genotypes would produce plantlets from leaves at K:NAA ratio of 1:20, and that the response of Alaede is as a result of its genotype. The reduction in plantlet production in the three genotypes when NAA was replaced with 2, 4 D (Table IV) implies that hormones belonging to the same class may not necessarily produce the same effect on the same plant species/genotypes. The significant difference observed in the means when K was increased to 0.3 and 2, 4 D reduced to 0.1 shows that plantlets could be regenerated faster from leaves at that concentration and combination. It also showed that the three genotypes responded differently to that treatment.

Conclusion

The response of Ex Sudan is higher in almost all the studies carried out when compared to the other two genotypes. This could be traced to its performance in the viability test in which it gave the highest germination rate. It has been observed in this study that Kinetin is a better cytokinin

for callus development in the three genotypes under study than coconut milk. Also, BAP and coconut milk have been found to produce similar effects on plantlets production in the three genotypes. Plantlet development from leaf subculture was found to improve significantly when MS was modified with hormones. Individual hormone rather than class produced different effects on the three genotypes.

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