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COMPARATIVE EFFECT OF DIFFERENT CONCENTRATIONS OF STERILANT (JIK) AND THEIR EXPOSURE TIME DURING IN VITRO PROPAGATION OF ZINGIBER OFFICINALE ROSC.

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A B S T R A C T
The need for alternative sterilant to Hcl in the in vitro propagation of ginger (Zingiber officinale) is of importance, hence the comparative effect of different concentrations of sterilant (JJK) and exposure time during in vitro propagation of Zingiber officinale (Ginger) was conducted, bearing in mind that the improvement and cultivation of ginger shown to be declining. The study showed that higher concentrations of sterilant and exposure time produced pure and healthy cultures. There was a significant difference in the effects of different levels of sterilants at different exposure time. The number of uncontaminated cultures increased as the concentration of the sterilant and exposure time increase. The growth of ginger was more effective at 30% concentration and 30mins exposure time, but not significantly different (P>0.05) from that of 25% concentration at 30mins exposure time. This method of propagation offers an avenue to rapid multiplication and production of disease-free ginger thereby increasing the quantity and quality of ginger to the rapidly growing population. This study also revealed that JIK has a great potential in rapid multiplication of ginger, hence can be used in the place of Sodium chloride that has been commonly used in in vitro propagation.

1. Introduction

Zingiber officinale (ginger) belongs to the family of flowering plants (Zingiberaceae) and of the genus Zingiber, consisting of aromatic perennial herbs with creeping horizontal or tuberous rhizomes. It is a native of Asia, cultivated in the West Indies, Jamaica, and Africa, but naturalized in America. Ginger is the oldest tuberous crop, domesticated as a spice (Anukwuorji, *et al.,* 2013; Sathyagowri and Thayamine, 2011). *Zingiber officinale* is an essential crop in many parts of the world. Medical research has shown that *Zingiber casummunar* and *Zingiber officinale* have anti inflammatory, anti-allergic and pain relieving activity (Eke-okoro, 2005). It is valued as a carminative and aromatic stimulant for the gastrointestinal tract. Cooks use it in soup, stew and often stimulant in tea, biscuit and cookies (Aguoru *et al.*,2008).

However, the rapid decline in *Zingiber* production is threatening the survival and existence of the crop due to low propagation. More so, it is widely perceived that ginger is being threatened by systemic

diseases that affect the rhizomes (Asumugha *et al.*,2006). The reported pathogens include *Phyllosticta zingiber, Fusarium oxysporum, Helix aspersa, Dihochronus punctferalis, Meloidogyne incognita* and *Aspediolla lartic* (Rajan, *et al.*,2002; Scheldon, 2003). This may lead to a total collapse of the plant. Thus, *in vitro* techniques is considered the best alternative method that results to a large number of planting materials for commercial planting (Hamira *et al.*, 2007).

Nevertheless, *in vitro* propagation plays a significant role in producing healthy planting materials. It is the rapid practices of multiplying stock plant material to produce a large number of progeny plant, using the modern plant tissues culture methods (Aguoru *et al.*,2008). There are four stages of *in vitro* propagation method that leads to mass production of plants; Initiation, Subculture, Pre-planting and transfer from culture. More so, there are various techniques used to produce disease free plants such as sterilization, aseptic technique, disinfectants and exposure time (Dobey, 2008). Although many researchers have been done on ginger production, however, documented reports of the comparative effect of different concentrations of sterilant (JIK) and exposure time during *invitro* propagation of *Zingiber officinale* is scanty.

Thus, this research aim is to determine the best concentrations of sterilant (JIK) and the exposure time suitable for *in vitro* propagation of ginger, to ensure the production of disease-free ginger by *in vitro* means and to justify the need of mass output of ginger through *in vitro* means to forestall extinction.

2. Materials and Methods

Study area

This experiment was conducted at the Tissue culture laboratory of The National Root Crops Research Institute Umudike (NRCRI) located between the Latitude 5˚28' North , Longitude 7˚55' South and an Altitude of 122m above sea level in rain forest agro-ecological zone of South-eastern Nigeria.

Source of plant

Healthy ginger rhizomes were collected from the ginger unit at The National Root Crop Research Institute Umudike, Abia State Nigeria. The variety used for this study is the yellow ginger which was labeled as UG1 (Umudike ginger 1). The rhizomes were brought to the Biotechnology unit and were planted in buckets containing sterile soil. After 3 weeks, the sprouted buds were thoroughly washed and were taken into the tissue culture laboratory for *in vitro* propagation.

Media composition

The components of media used include MS basal salt which constitutes of the micro and macro element, Carbon source, Vitamins, Plant growth hormones and gelling agent for the sustenance and nourishment of explants in its artificial environment. And the stock solutions were prepared according to (Murashige and Skoog, 1962).

Preparation of growth regulators

Naphthalene acetic acid (NAA): 10mg of NAA was weighed using analytical weighing balance and dissolved in a few drops of 0.5 NaOH, distilled water was added to make it up to 100ml. The solution was thoroughly mixed and labeled 0.1mg of NAA per ml.

Amino Purine (BAP): 10mg was weighed and dissolved in 95% ethanol, distilled water was added to make it up to 100ml. The solution was labeled 0.1mg of BAP per ml and stored in the refrigerator.

Media preparation

Five hundred mills (500ml) of distilled water was added to a 1litre beaker and placed on a magnetic stirrer plate. A magnetic bar was added to the beaker for thorough stirring. Then the components of medium suitable for ginger were added as follows; MS basal salt(4.33g), Vitamins mix (5ml),

Sucrose (30g), Myo-inositol (0.1g), L- Cysteine (0.01g), BAP (5mg), NAA (0.1mg) and Agar (7g), after the mixture, they were kept in a room temperature of 27ºC, the PH meter was standardized to 7 with 0.5N NaOH after which the PH electrode was inserted into the medium and the PH was adjusted to 5.8 using 0.5N NaOH hence acidic or 0.5N HCL when basic. Finally the 7g of Agar (gelling agent) was added and heated to get a clear mixture. The medium was dispensed into test tubes, covered and autoclaved at 121ºC and pressure 1.05kg/cm² for 15minutes and allowed to cool and solidified in the aseptic room.

Sterilization and culture

The collected buds were washed with running water and brought into the aseptic room and were surface sterilized, trimmed to the shoot tip surrounded by a few leaf primordial and aseptically introduced into the culture medium using sterilized forceps. The cultured vessels were placed on shelves at 28 ± 2°C, illuminated for 16 hours and 8 hours darkness daily.

Experimental design

The experimental design used was one-way analysis of variance (ANOVA) and mean were separated using Fisher LSD ($P < 0.5$) with the aid of a statistical software package, called Statistical Analytical System. 70% ethanol and different concentrations of JIK were termed "X" and the exposure time were termed "Y". That is, 70% ethanol and different concentrations of sterilant were represented by X_1, X_2, X_2, X_4 respectively; the exposure time as Y_1, Y_2, Y_3, Y_4 respectively.

3. Result

The effect of different concentrations of sterilant was significant ($P < 0.05$). Number of the uncontaminated culture increased as the concentration of the sterilant and exposure time increased (15% > 20% >, 25% >, 30%). The effect of different concentrations of sterilant at 15 minutes showed no significant difference ($P>0.05$) in the number of contaminated cultures except that of 15% concentration. Six days and Nine days of treatment showed no significant difference in the number of contaminated cultures at 25% and 30% respectively, while 30% concentration showed the highest number of uncontaminated after 14days (Table1).

Three (3) days of treatment at 20minutes exposure time showed no significant difference ($P>0.05$) in the degree of contamination at 15%, 20%, and 25% respectively. No contamination as recorded in 30%. Six (6) days treatment showed no significant difference ($P>0.05$) in the number of contaminated cultures at different concentrations but 30% showed the highest number of uncontaminated culture. Nine (9) and fourteen (14) days of treatment showed no significant difference ($P>0.05$) in the number of contaminated cultures, thus, 30% showed the highest number of uncontaminated culture followed by 25% (Table 2)

Effect of different concentrations of sterilant at 25 minutes showed that number of contaminated cultures was highest in 15% treatment and Non was recorded in 30%treatment. There was no significant different ($P>0.05$) in the number of contaminated cultures in 15%, 20% and 25% treatment. Six (6) days of treatment showed that number of contaminated cultures were highest in 15% and 20%treatment and Nil in 30%and no significant different ($P>0.05$) in the number of contaminated cultures at 15%, 20% and 25%. Nine (9) and fourteen(14) days of treatment showed the highest number of uncontaminated cultures at 25% and 30% (Table 3)

Effect of different concentrations of sterilant at 30 minutes exposure time after 3 days showed no significant difference ($P>0.05$) in 15% and 20% concentration. And the highest numbers of uncontaminated cultures were recorded in 30% concentration after 14 days, followed by 25% respectively (Table 4)

Table 1 Effect of different concentrations of sterilant at 15 minutes

	DAYS							
	3 days		6 days		9days		14 days	
Treatments	No of contaminated cultures		No of contaminated cultures		No of contaminated cultures		No of contaminated cultures	
X_1Y_1	1.0^{b}	4.0^{a}	2.0^{a}	3.0^{a}	3.0^{a}	2.0^{a}	5.0^{a}	Nil
X_2Y_1	2.0^{ab}	3.0^{ab}	3.0^{a}	2.0^{a}	3.0^{a}	2.0^{a}	5.0^{a}	Nil
X_3Y_1	3.0^{a}	2.0^{b}	3.0^{a}	2.0a	4.0^{a}	1.0^{a}	4.0^{a}	1.0^{b}
X_4Y_1	2.0^{ab}	3.0^{ab}	3.0^{ab}	2.0^{a}	3.0^{a}	2.0^{a}	3.0^{a}	2.0^{a}
LSD (5%)	1.33	1.33	1.63	1.63	1.63	1.33	2.98	0.94

Means down the columns with the same superscript are not significantly different (P>0.05)

X_1Y_1= 15% concentration at 15 minutes exposure time

X_2Y_1= 20% concentration at 15 minutes exposure time

X_3Y_1= 25% concentration at 15 minutes exposure time

X_4Y_1= 30% concentration at 15 minutes exposure time

Table 2: Effect of different concentrations of sterilant at 20minutes

	DAYS							
	3 days		6 days		9days		14 days	
Treatments	No of contaminated cultures		No of contaminated cultures		No of contaminated cultures		No of contaminated cultures	
X_1Y_2	1.0^{a}	4.0^{a}	3.0^{a}	2.0^{a}	3.0^{a}	2.0^{a}	5.0^{a}	Nil
X_2Y_2	1.0^{a}	4.0^{ab}	2.0^{ab}	3.0^{a}	3.0^{a}	2.0^{a}	4.0^{ab}	1.0^{b}
X_3Y_2	1.0^{a}	4.0^{b}	1.0^{a}	4.0a	2.0^{a}	3.0^{a}	4.0^{bc}	2.0^{b}
X_4Y_2	Nil	5.0^{a}	1.0^{b}	4.0^{a}	2.0^{a}	3.0^{a}	2.0^{c}	3.0^{a}
LSD (5%)	0.00	2.49	1.33	2.31	1.88	1.33	1.88	1.63

Means down the columns with the same superscript are not significantly different (P>0.05)

X_1Y_2 = 15% concentration at 20minutes exposure time

X_2Y_2 = 20% concentration at 20minutes exposure time

X_3Y_2 = 25% concentration at 20minutes exposure time

X_4Y_2 = 30% concentration at 20minutes exposure time

LSD = Least significant difference

Table 3: Effect of different concentrations of sterilant at 25minute

	DAYS							
	3 days		6 days		9days		14 days	
Treatments	No of contaminated cultures		No of contaminated cultures		No of contaminated cultures		No of contaminated cultures	
X_1Y_2	2.0^{a}	3.0^{a}	2.0^{a}	3.0^{a}	4.0^{a}	1.0^{a}	5.0^{a}	Nil
X_2Y_2	2.0^{a}	3.0^{ab}	2.0^{a}	3.0^{a}	2.0^{b}	2.0^{bc}	4.0^{a}	1.0^{b}
X_3Y_2	1.0^{ab}	4.0^{a}	1.0^{a}	4.0a	2.0^{a}	3.0^{ab}	2.0^{b}	3.0^{a}
X_4Y_2	Nil	5.0^{a}	$1.0a^{b}$	5.0^{a}	1.0^{a}	4.0^{a}	2.0^{b}	3.0^{a}
LSD (5%)	1.33	2.49	1.33	2.49	1.33	1.63	1.88	1.33

Means down the columns with the same superscript are not significantly different (P>0.05)

X_1Y_3 = 15% concentration at 25minutes exposure time

X_2 Y_3 = 20% concentration at 25minutes exposure time
X_3 Y_3 = 25% concentration at 25minutes exposure time
X_4 Y_3 = 30% concentration at 25minutes exposure time
LSD = Least significant difference

Table 4: Effect of different concentrations of sterilant at 30minutes

	DAYS							
	3 days		6 days		9days		14 days	
Treatments	No of contaminated cultures		No of contaminated cultures		No of contaminated cultures		No of contaminated cultures	
X_1Y_2	1.0^a	4.0^a	2.0^a	3.0^b	3.0^a	2.0^a	3.0^a	2.0^b
X_2Y_2	1.0^a	4.0^a	1.0^{ab}	4.0^{ab}	2.0^a	3.0^{bc}	3.0^a	2.0^b
X_3Y_2	Nil	5.0^a	2.0^a	3.0b	2.0^a	3.0^{ab}	2.0^a	3.0^{ab}
X_4Y_2	Nil	5.0^a	Nil	5.0^a	Nil	5.0^a	1.0^b	4.0^a
LSD (5%)	0.00	1.88	1.33	1.88	1.63	2.49	1.33	1.88

Means down the columns with the same superscripts are not significantly different (P.0.005)
X_1Y_3 = 15% concentration at 30minutes exposure time
X_2 Y_3 = 20% concentration at 30minutes exposure time
X_3 Y_3 = 25% concentration at 30minutes exposure time
X_4 Y_3 = 30% concentration at 30minutes exposure time
LSD = Least significant difference

4. Discusion

This research on the comparative effectiveness of different concentrations of sterilant and their exposure time has a long way to go in the production of ginger *(Zingiber Officinale)* during in-vitro propagation. This agrees with the report of (Thayamini, 2014) on *in vitro* propagation of ginger through direct organogenesis. Based on the results, not all concentrations showed a favorable result, but the results of culture treated with higher levels (25% and 30%) and exposure time (25mins and 30mins) were better than those of lower treatments 15% and 20%. Similar trend were observed by Nirmal (1997) who reported that Ms basal medium with auxin and cytokinin at moderate concentrations gave positive in inducing multiple shoots and roots during in- vitro propagation of vegetative buds, and rhizome bits with auxiliary buds of Ginger. The contamination of the explants may be due to moulds and bacteria this reinforces the documentation of Hamiral *et al.,* (2007) who stated that the contamination of the explants during micro propagation of ginger was as a result of fungi, bacteria, moulds etc, present on the surface or lodged in the cracks. The 30% concentrations were more effective than other levels, 30% concentrations showed the highest number of clean cultures which resulted in good growth of cultures and shoot multiplication followed by 25% concentrations. This does not agree with the report of (Aguoru *et al.*,2008) when they observed that the highest regeneration was produced at levels of 0.5mg/l BAP + 0.03mg/L NAA during *in-vitro* propagation of *Ipomoea batatas*, even though the control (0% concentration at 0mins exposure) time were highly contaminated and showed no sign of growth.

5. Conclusion

In conclusion, this study showed that Jik has a potentials in rapid multiplication of ginger, therefore in the absence of concentrated sodium hypochloride which is commonly used as sterilant, bleach (JIK) can be used as an available sterilant and it has little amount sodium hypochloride.

6. Recommendation

From this study, in the absence or scarcity of concentrated sodium hypochloride, bleach is recommended for *in-vitro* propagation of ginger.

Secondly, more research should be commenced with bleach since it has no adverse effect on the performance of *in-vitro* ginger.

Thirdly, to put more interest on the production of ginger by establishing a method by which it can be rapidly multiplied to increase the availability of ginger due to its different economic importance.

Finally, since this *in-vitro* method of rapid multiplication of propagation gave a successful result, it is recommended that other crops that have very slow vegetative means of propagation can be propagated through the biotechnologically means as a way of ensuring their availability to farmer all time.

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