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REPORT FORMAT FOR COMPLETED PROJECTS

A. Basic Information

1. Project Title: **COST EFFECTIVE MICROPROPAGATION TECHNOLOGY FOR GINGER, *ZINGIBER OFFICINALE* ROSC.**

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B. TECHNICAL REPORT

TITLE: COST EFFECTIVE MICROPROPAGATION TECHNOLOGY FOR GINGER, *ZINGIBER OFFICINALE* ROSC.

ABSTRACT

An attempt to reduce the cost of micropropagation through the use of alternative gelling agents and reducing the volume of culture media in *in vitro* production of ginger, Native variety was done. The commonly found seaweeds along the coastal areas of the Ilocos (gracilaria and eucheuma), and the commercially available agar bar were compared with that of tissue culture grade powdered agar. Likewise, the volume of culture media was reduced by one-half the standard volume.

Results indicated that the different gelling agents did not significantly affect the number of shoots, shoot length, number of roots and length and width of leaves. However, the number of leaves and length of roots were significantly affected in which cultures grown in gracilaria-gelled media produced the least number of leaves and shortest roots. Though proliferation rate was similar, regardless of the solidifying agent used, it is recommended that the commercial agar, eucheuma or gracilaria can be a good substitute for powdered agar, giving a reduction in cost of gelling agent of about 61-67%.

Reducing the volume of media from 20 ml per 60 ml capacity culture vessel to 10 ml showed similar performance of ginger cultures. With this, a substantial reduction of 50% in cost of culture media was realized.

I. RATIONALE

Ginger is normally vegetatively propagated, using rhizomes as planting material. This method is slow and a farmer has to set aside a portion of his produce as planting materials for the next planting season. In addition, contamination of seed material by pathogens (nematodes) can cause severe reduction in yield. That is why, despite tremendous efforts, little success has been achieved in conventional seed ginger production scheme. In this event plant biotechnology offers a great potential to complement conventional breeding methodology for ginger improvement and production via plant tissue culture techniques (mass production of pathogen free ginger material through micropropagation). However, lack of budget, limited resource

allocation and relatively high recurrent cost (chemical expenses) of this technology in developing countries particularly, in the Philippines, remains to be a challenge.

The only alternative is to develop cost effective technology for in vitro clonal propagation of ginger. It has been demonstrated that in vitro propagation is a potential method of rapid multiplication of ginger. Media for in vitro propagation may either be liquid or solid. Most cultures are gelled by agar at a concentration of 5 – 8 g/li. The wide use of agar as a gelling agent has been attributed to its stability, high clarity, resistant to metabolism (not digested by plant enzymes and remain stable at all feasible incubation temperatures), limited diffusion of medium components and water (McLachlan, 1985; Henderson and Kinnersly, 1988) and gel strength (Debergh, 1983). However, agar represents one of the most expensive and commonly used media components, contributing about 70% of the total production cost (Prakash, 1993). Pierik (1989) also stated that the most expensive and extensively used component of semisolid nutrient media for in vitro micropropagation is agar.

In the present study an attempt was made to determine the feasibility of using gracillaria and eucheuma seaweeds, which are abundantly growing in the wild along the shores of the Ilocos, as a gelling agent for micropropagation of ginger. Ultimately, the study aimed to select a cheaper substitute for tissue culture grade powdered agar, thereby developing a cost effective micropropagation for ginger. Likewise, reducing the volume of culture media was done.

Objectives:

1. To compare the effects of different gelling agents in *in vitro* production of ginger.
2. To determine the effects of volume of media on micropropagation of ginger.
3. To determine the economic analysis of ginger micrropropagation using different gelling agents and different volumes of culture media.

II. REVIEW OF LITERATURE

Ginger has been found to have potential for multiplication in vitro using gelled medium. The conventional gelling agents (agar and gelrite) for in vitro micropropagation are presently imported, expensive and not readily available. Therefore, there is a need for cheaper, easily available local alternative for generating clean propagules of ginger (Nkere, et al).

Media for in vitro cultures can be classified as liquid or solid. Gelling agents are added to culture medium to increase viscosity wherein explants are not submerged in the medium (Prakash et al., 2000).

Agar is the most commonly used gelling agent in most tissue culture media. The wide use of agar as a gelling agent has been attributed to its stability, high clarity, resistant to metabolism (not digested by plant enzymes and remain stable at all feasible incubation temperatures), limited diffusion of medium components water (McLachlan, 1985; Henderson and Kinnersly, 1988) and gel strength (Debergh, 1983). However, agar is very expensive that it represents about 70% of the total production cost (Prakash, 1993).

Macroalgae, known also as seaweeds, represent 23.4% of the tonnage and 9.7% of the value of the global (marine, brackish water, and freshwater) aquaculture production, estimated at 59.4 million tonnes and $ 70.3 billion in 2004. They are used as food, fodder, feed and fertilizer and many of the bioactive compounds produced by the macroalgae are known to have potential beneficial use in healthcare (Ghosh, 2012). Gracilaria Greville genus (Gracilariales, Rhodophyta) is represented by more than 300 species of which 160 have been accepted taxonomically. The macroalgae belonging to this genus are important for industrial and biotechnological uses and are considered economically valuable resources, because of their ability to achieve high yields of commercially valuable biomass. In fact, they contain, besides other compounds, phycocolloids, the main source of agar-agar, which is a gelatinous non-toxic colloidal carbohydrate present in the cell wall and intercellular spaces of the algae (Capo, 1999).

Gracilaria is a genus of red algae (Rhodophyta) notable for its economic importance as an agarophyte, as well as its use as a food for humans and various species of shellfish. In the Philippines, it is called gulaman and used to make gelatin, also called gulaman (Davidson, 2004). Gracilaria sp. polysaccharides have been shown to be an effective prophylactic agent during in vitro and in vivo experiments.

Kappaphycus and eucheuma are sources of carrageenan used in many food processess because of its hydrocolloidal properties (Tye as cited by Junio 2013). Recent researches show that Carrageenan is a valuable substance used in gelling, suspending, thickening or water holding properties in various products and is one of the world's foremost consumed food and industrial additives today. Kappaphycus spp. and eucheuma spp. grow abundantly in the coastal areas of the Philippines (Junio 2013)

Agar has long been used to solidify media for plant tissue culture. The type of agar or gelling agent used can influence the growth of the tissue in culture. Both purity and cost of the gelling agent are important factors in any research or production operations (PhytoTechLab, 2014).

The in vitro cultivation of plant tissues is generally carried out in a solid or semi-solid nutrient medium, using gelling agents. Traditionally, agar is used, which is a polysaccharide extracted from seaweeds. This hydrocolloid is composed of agarobiose (3 B-D galactopyranosyl-(1,4)-3,6- anhydro A L-galatose (Lucyszyn, et al., 2006). The main differences among different agar-products are due to the impurities, their level and composition, which can vary according to manufacturers. Agar has been widely used since it has convenient gelling properties and stability during tissue culture. In all media used for in vitro culture of plants, agar is the major source of unknown variations (Scholten and Pierik, 1998), besides it's being costly.

Many gelling agents are used for plant tissue culture media, such as agar technical Oxoid, agarose, phytagel and gelrite (Debergh, 1983). Agar is the most commonly used as gelling agent for media preparation (Afrasiab and Jafar, 2011). Henderson and Kinnersely (1988) also stated that agar has been widely used as a gelling agent in plant tissue culture technique because of its stability, high clarity and non toxic nature.

MATERIALS AND METHODS

This experiment was carried out at the Tissue Culture Laboratory of DMMMSU, Bacnotan, La Union. Rhizomes of ginger (*Zingiber officinale* Roscoe), Native variety were used. Buds from the rhizomes measuring about 2-3 cm were used as source of initial explants. These were washed in running water, then soaked in water plus detergent for 10 minutes. These were rinsed 4x with tap water, then soaked again for another 10 minutes in Benlate, ascorbic acid and citric acid. Final step in surface sterilization was by soaking in solution of 10 % bleach plus 25 drops of Tween 20 for 5 minutes. The buds were finally rinsed three times with sterile distilled water.

The Murashige and Skoog's (MS) basal medium supplemented with 2 mg L-1 glycin and 40 g L-1 sucrose. MS vitamins (MS 1962, as cited by Villamor, 1999) was used. The pH was adjusted to 5.8+/-0.1 by either NaOH or HCl. About 20 ml culture media was poured out in each culture vessel prior to sterilization in pressure cooker at 121^0C for 20 minutes. Two explants were

placed in each vessel. For multiplication purposes, cultures were transferred to fresh medium every after 2 weeks until enough cultures were produced to be used in the two experiments.

For Experiment 1, 20 ml culture media (MS basal medium + MS vitamins), solidified with different gelling agents (gracilaria, eucheuma, commercial agar bar and Tissue Culture grade powdered agar) as specified in the treatments, was poured out in each culture vessel. In micropropagation, the most commonly used volume of media was 20 ml per 60 ml capacity ampicillin bottle. One plantlet was placed upright in every culture vessel.

The seaweeds, eucheuma and gracilaria, were collected from along the seashores of Balaoan and Sto. Tomas, respectively. These were washed thoroughly in running water to get rid of salts, then sundried. One-hundred grams seaweeds were placed in a casserole containing 3 li water, then allowed to boil for 30 minutes, with continuous stirring. These were then cooled to about 40^{o}C, and osterized in a blender until homogenous sticky solution was formed. The solution was allowed to pass through cotton mess sieve to separate the fiber component of the seaweeds from the gel and finally was allowed to cool in a moulder to solidify. The final agar was then added to the MS media as gelling agent (24 g/li gracilaria and 14 g/li eucheuma). These were cooked separately in a casserole until the solution becomes homogenous. The flowchart of step-by-step process is shown in Fig. 1.

The commercial agar bar was purchased from the local market. This was washed with distilled water, then thinly cut to about 0.5 in^3 in which 10g was mixed with the newly prepared culture media then allowed to boil with continuous stirring until the solution becomes homogenous.

The High Media tissue culture grade powdered agar was mixed with the culture media at a concentration of 5 g/li, then cooked with continuous stirring until the media becomes clear.

All the media were allowed to cool to about 40^{0}C before adjusting the pH to 5.6 +/-2 by adding NaOH to raise the pH, or HCl to lower down the pH. The media were dispensed in 60 ml capacity clear culture vessels.

For Experiment 2, MS basal media + MS vitamins solidified with tissue culture grade agar (High Media powdered agar) was used. The techniques employed in Experiment 1 was the same with that of Experiment 2. Only the volume of media differed, as can be seen in the treatments (10 ml and 20 ml).

The experiments were set up in Completely Randomized Design with three replications.

The treatments were the following:

Experiment 1. Effects of different gelling agents on the performance of ginger cultures in vitro

Treatments:

T^1 – Gracilaria
T_2 – Eucheuma
T_3 – Commercial agar bar
T_4 – Tissue culture grade powdered agar

Experiment 2. Effects of volume of culture media on the performance of ginger cultures

Treatments:

T_1 – 10 ml
T_2 – 20 ml

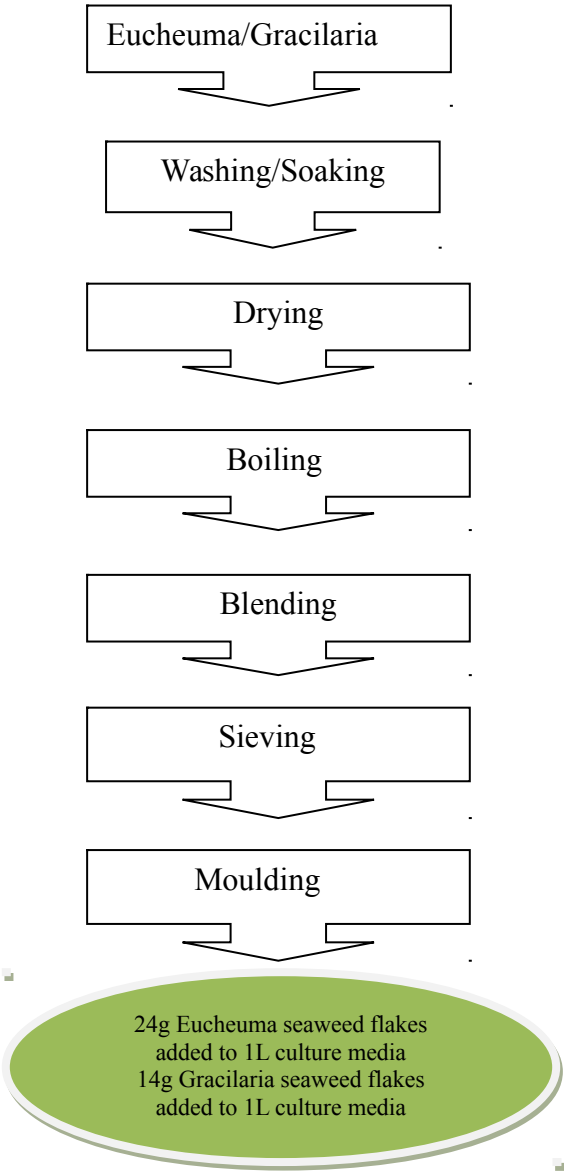

Figure 1. Flowchart of step-by-step process of making seaweed flakes as gelling agent.

RESULTS AND DISCUSSION

Table 1 shows that the different gelling agents did not significantly affect the performance of cultures in terms of shoot proliferation, length of shoots, number of roots and length and width of leaves. However, the number of leaves and length of roots were significantly affected by the gelling agents used.

Like the tissue culture grade powder agar, it was observed that the culture media gelled with any of the other gelling agents used (gracilaria, eucheuma or commercial bar agar) remained stable and did not show any signs of softening throughout the culture duration. This conforms to the study of Shah, et al (2003) where he used sphagol *(Plantago ovata)* husk as gelling agent for potato. He stated that ispaghol gelled media did not soften throughout culture period, which indicated that ispaghol did not metabolize during culture.

The insignificant difference on the number of shoots indicates that the use of either gracilaria or eucheuma seaweeds which are abundantly available and can be collected in the wild, showed that the former had no adverse effects on shoot formation. This further indicates that the locally available seaweeds (gracilaria and eucheuma) or the commercially available bar agar can be as effective as using the tissue culture grade powdered agar which is the commonly used gelling agent for tissue culture.

Table 1. Effects of different gelling agents on the performance of ginger cultures in vitro.

Treatments	No. of shoots	Shoot length, cm	No. of leaves	No. of roots	Length of roots, cm	Length of leaves, cm	Width of leaves, cm
T_1-Gracilaria	2.75	5.08	3.75c	15.75	0.80b	1.6	0.38
T_2-Eucheuma	4.25	4.63	6.25b	12.5	2.78a	1.83	0.53
T_3–Bar agar	4.0	7.0	7.5ab	19.5	2.85a	2.23	0.58
T_4-Powdered agar	3.25	7.10	8.25a	18.75	2.85a	2.30	0.65
Significance	ns	ns	*	ns	*	ns	ns

In a column, means followed by the same letter are not significantly different at 5% level of significance, DMRT.

It was shown in Table 2 that the most expensive gelling agent was the agar powder (28.80/li media) which is about 3x much higher as the price of the other solidifying agents

(gracilaria (P9.59), eucheuma(P11.20) and commercial agar bar (P10.10). Though proliferation rate was similar, regardless of the solidifying agent used, results suggest that the commercial agar, eucheuma or gracilaria can be a good substitute for powdered agar, which is the most commonly used gelling agent for tissue culture. A reduced cost or saving cost of about P17.60–P19.21per liter media can be realized when using eucheuma or gracilaria seaweeds or commercial agar bar instead of powdered agar. Based on the results, it is therefore interesting to note that commercial agar bar, eucheuma or gracilaria can be used as cheaper substitute for powdered agar, thereby developing a cost effective micropropagation technology for ginger.

Table 2. Comparative costs of gelling agents for in vitro propagation of ginger using different gelling agents.

Gelling Agent	Cost per unit (P)	Concentration (g/li)	Cost/L media (P)	Reduced Cost (P)	Cost Saving (%)
T_1 – Gracilaria	400/kg	24	9.59	19.21	66.7
T_2 – Eucheuma	800/kg	14	11.2	17.60	61.1
T_3–Commercial agar bar	20/bar	10	10.10	18.70	64.9
T_4–Powdered agar (tissue culture grade)	2,880/ 500g	5	28.80	-	-

Again, an attempt to reduce the cost of micropropagation was done by reducing the volume of culture media in each culture vessel. Table 3 shows that ginger plantlets performed similarly regardless of the volume of media in the culture vessels. Results indicate that reducing the volume of media by 50 percent did not cause any drastic effects in terms of shoot, root and leaf production and development. It is interesting to note that even if shoot proliferation rate was not improved in reducing the volume of culture media, the cost of production is reduced brought about by the reduction in the volume of media (Table 4). With this, a substantial reduction of about 50% in cost of culture media was realized.

Table 3. Effects of volume of media on the performance of ginger cultures.

Treatments	No. of shoots	Shoot length, cm	No. of leaves	No. of roots	Length of roots, cm	Length of leaves, cm	Width of leaves, cm
T_1 – 10 ml	2.67	9.4	4.36	7.98	3.70	3.53	0.72
T_2 – 20 ml	2.58	10.15	4.23	6.97	3.55	3.82	0.69
cv (%)	17.5	3.08	21.55	21.56	13.77	6.30	6.43

Table 4. Comparative costs of culture medium for in vitro propagation of ginger using different gelling agents.

Treatments	Cost/L (P)	No. of bottles/L	Cost/bottle (P)	Reduced Cost (P)	Saving Cost (%)
T_1 – 10 ml	247.20	100	2.47	2.47	50
T_2 – 20 ml	247.20	50	4.94	-	-

Conclusions

Based on the results of the study, the following conclusions were derived:

1. The different gelling agents significantly affected the number of leaves and the length of roots, but not on shoot proliferation, length of shoots, number of roots and length and width of leaves.
2. The most expensive gelling agent was the agar powder (28.80/li media). This was about 3x much higher as the price of the other solidifying agents (gracilaria (₱9.59), eucheuma (₱11.20) and commercial agar bar (₱10.10).
3. The use of either 10ml or 20 ml culture media showed similar performance of the ginger cultures in terms of shoot proliferation, shoot length, number, length and width of leaves, and number and length of roots.
4. A 50% reduction in the cost of culture media was realized when the volume of media was reduced by 50 %.

Recommendations

1. Though proliferation rate was similar, regardless of the solidifying agent used, it is recommended that the commercial agar, eucheuma or gracilaria can be a good substitute for powdered agar, which is the most commonly used gelling agent for tissue culture.
2. Reducing the volume of culture media from 20 ml per 60ml capacity ampicillin bottle (commonly practiced) to 10 ml is recommended because the performance of the ginger cultures were similar in both volumes.

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