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IN VITRO CALLUS CULTURE OF SUNFLOWER BY USING ISUBGOL (*PLANTAGO OVATE*)

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ABSTRACT

'Isubgol', the mucilaginous husk, derived from *Plantago ovate* a stem less herb of the plantaginaceae family, is used as emollient, demulcent and laxative and in the treatment of dysentery and diarrhoea. Attempts have been made to find a substitute for agar as gelling agent. The study was conducted on commercially important crop plants like sunflower. Isubgol was successfully used as a gelling material in tissue culture media. The price of 'Isubgol husk' is cheaper than the conventionally used agar and it had reduced the price of gelling agent approximately by 47.5 % in plant tissue culture media. The use of 'Isubgol husk' along with agar can reduce the cost of gelling agents. The response from media gelled with Isubgol husk in sunflower was similar to that from media solidified with agar.

KEYWORDS: Isubgol, *Plantago ovate*, Callus culture, Gelling agent, Sunflower, Explant

INTRODUCTION

The 'Isubgol husk' derived from the seeds of *Plantago ovate* a stem less herb of the plantaginaceae family. The efficacy of 'Isubgol husk' is entirely due to the large quantity of mucilage present in the husk. Like agar, 'Isubgol' mucilage is colloidal and polysaccharide likes in nature and is mainly composed of xylose, arabinose and galactouronic acid, rhamnose and galactose.[2] Agar has mostly been used as solidifying agent in tissue culture media because of its stability, high clarity, non-toxic nature and resistance to metabolism during culture.[6,4] Some investigations have, however, raised doubts about the biological inertness and non-toxic nature of agar.[7,3,5,1] Commercially, agar is extracted from species of red algae genera Gelidium, Gracillaria and Pterocladia.[6] The almost exclusive use of agar is resulting

in over-exploitation of its sources. Because of the above-mentioned reasons and the exorbitant price of tissue culture grade agar, attempts have been made to identify a suitable alternate gelling agent which will economically be feasible and easily available in the country. Many attempts have been made to identify suitable alternative gelling agents. Through this experiment, 'Isubgol husk' was used as a gelling agent to reduce the cost of plant tissue culture media. Local price of 'Isubgol husk' is about one twentieth of the price of good quality agar. Through this experiment, an attempt was taken to reduce the cost of gelling agent in the media. The aim of the present investigation, therefore, is to establish a suitable protocol for using 'Isubgol' as gelling mixture of Isubgol husk together with conventionally agent combined with agar in plant tissue culture media.

MATERIALS AND METHODS

The present investigation was carried out at Vilasrao Deshmukh College of Agricultural Biotechnology, Latur (M.S) during the year 2014-2015.

MATERIALS

Sunflower leaves were used for their callus formation on 'Isubgol' as a gelling agent together with conventional agar media. The plants were certified as disease free. The media used for this present investigation were 'Isubgol husk' in combination with agar (Sigma type A, composed of 70% agarose and 30% agaropectin).

METHODS

Preparation of stock solutions of media and hormones

Table 1: Chemical composition of MS media.

Constituents	Quantity (mg/lit)
Macronutrients	
$MgSo_4.7H_2O$	7400
KH_2PO_4	3400
KNO_3	38000
NH_4NO_3	33000
$CaCl_22H_2O$	8800
Micronutrients	
H_3BO_3	1240
$MnSO_4.4H_2O$	4460
$ZnSO_4.7H_20$	1720
$Na_2MoO_4.2H_2O$	50
$CuS0_4.5H_2O$	5
$CoCl_2.6H_2O$	5
Chelating agent	

$FeSO_4.7H_2O$	5560
$Na_2EDTA.2H_2O$	7460
Vitamins	
Inositol	20000
Thiamine HCl	100
Pyridoxine HCl	100
Nicotinic Acid	100
Glycine	400

Table 2: Different combinations of 'agar and Isubgol husk' as gelling agent.

Culture Group MS media	Hormones concentration	Agar (%) + Isubgol husk	Agar + Isubgol husk (gm)/250ml
Group A	-	100+0	1.75 +0.0
Group B	0.5 mg/l 2,4-D + 1.5 mg/l BAP	50+50	0.85+0.85
Group C	0.5 mg/l 2,4-D + 1.5 mg/l BAP	30+70	0.52+1.23
Group D	0.5 mg/l 2,4-D + 1.5 mg/l BAP	100+0	1.75+0.0
Group E	0.5 mg/l 2,4-D + 1.5 mg/l BAP	0+100	0.0+1.75

Media Preparation

The media contained macronutrients, micronutrients, chelating agent and supplement with BAP and 2,4-D hormone, sucrose (30g/l), agar + Isubgol husk. The PH was adjusted to 5.8 and sterilized by autoclaving at 121^0C and 15ib/inch pressure for 15-20 min.

Preparation of explants

Freshly collected explants were washed under running tap water. Then it was washed with soft detergent for three times followed by washing with running tap water for 30 minutes. The explants were then washed with 70% ethanol followed by rinsing with distilled water for three times with 0.1% $HgCl_2$ in a Laminar airflow, finally the explants were rinsed with double distilled water for three times in order to remove the last drop of $HgCl_2$.

Inoculation of explants

The sterilized explants were taken in a sterilized Petri dish and cut into pieces two or three leaves attaining 1-2 cm in length were cut from the explants and were then inoculated aseptically on medium containing different concentrations and combinations of growth regulators. All cultures were grown in the growth chamber illumination by 40W (~2500 lux) white fluorescent tubes fitted at a distance 25 cm from culture shelves and controlled

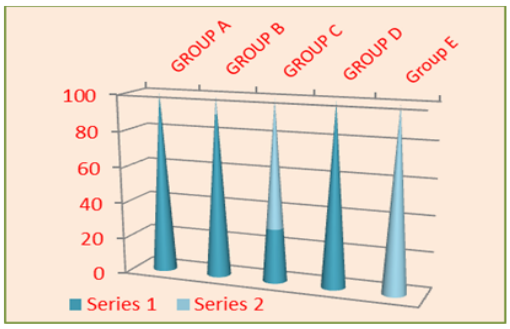
temperature maintain 25 ± 2°C with air cooler. Fifteen cultural bottles, on average, were taken in the study for all fifteen groups. Equal amount of cultural bottle (Fifteen) were taken to study of sunflower variety explants. Fifteen explants of sunflower were sub cultured into each bottle.

RESULTS AND DISCUSSION

Gelling performance of used combinations was compared (Figure-1). Culture media contained Group C gelling agent did not gel properly. Height and health of plantlets grown in Isubgol husk + agar gelled media was almost the same as plantlets grown in agar media (Table 2). Hormones for callus culture were applied in Group B culture media but not in Group A. Callus were observed only in the explants on group B culture media (Table 2). So it could be concluded that 'Isubgol' does not hamper the effect of hormones. It was also observed that culture media contained Group C gelling agent was not appropriate for the growth of sunflower. Probably this combination of 'Isubgol husk' and agar was not sufficient for gelling of the culture media resulting in improper growth of callus (Table 2). So, Group C was excluded from the experiment later. Later Group B & D culture media were prepared with combination of equal amount of agar and 'Isubgol husk'. Concentrations of 2, 4-D and BAP applied to that culture media were same as Group-B. Growth of callus in both media was almost the same. The growth of callus in group B and group D culture bottle same due to same concentration of hormones and gelling of media. So Isubgol husk + agar gelled media did not hamper hormone activity. Group E contain only Isubgol as gelling agent. This combination was not appropriate for the growth of sunflower. The genotypic changes in sunflower were not expected because callus were grown with normal life regulatory factors such as same levels of plant growth regulators and nutrient medium, light source, and growth temperature, during the course of experiment. Different types of sugars in agar and Isubgol variable hardness of respective gels due to biochemical and structural differences was expected to affect the molecular diffusion of growth regulators and nutrients through the medium, resulting in quantitative variations in the number of shoots of the cultured explants (Table 2). Their subsequent growths were comparable on agar and 'Isubgol'-gelled media (Table 2). Thus, the development of callus on 'Isubgol husk' medium did not appear to have any adverse effect. In this observation, there was no softening of the 'Isubgol'- gelled medium during the entire course of culture, indicating that it is not metabolized during culture.

Culture media with Group C and E gelling agent did not gel properly. Admixture of Isubgol husk together with conventionally that the response on starch-gelled medium was invariably

better than that of agar medium. According to them the problem of softness of the starch-gelled medium could be eliminated by increasing the concentration of starch up to 10%. It becomes evident that the problems which may prevent the universal acceptance of starch as an alternative gelling agent are its inferior gelling quality, lower clarity than agar and metabolizable nature which leads to softening of the media during the culture period.



Culture media Group A contained gelling agent of combination 1.
Culture media Group B contained gelling agent of combination 2.
Culture media Group C contained gelling agent of combination 3.
Culture media Group D contained gelling agent of combination 4.
Culture media Group E contained gelling agent of combination 5

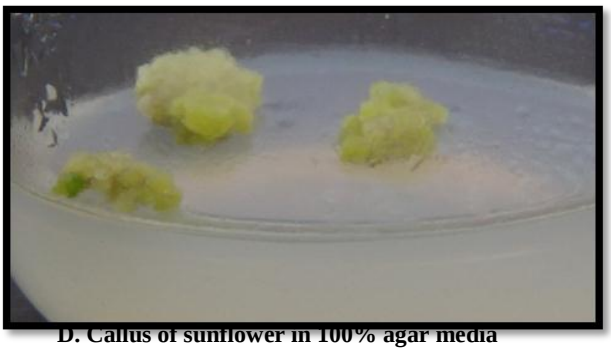
Figure 1: Gelling performance of different combinations of agar and 'Isubgol husk'

Plant	Group	Agar + Isubgol husk (%)	Frequency (%) of callus formation
Sunflower	Group A	100+0	10
	Group B	50+50	90
	Group C	30+70	30
	Group D	100+0	100
	Group E	0+100	0

A. (Agar) (Isubgol)

C. *Callus of sunflower in* 50%agar + 50%Isubgol gelled media

B. Agar and Agar + Isubgol gelling media (50%Agar+50%Isubgol gelling media) (100% Agar gelling media)



D. Callus of sunflower in 100% agar media

Moreover, upon autoclaving, starches yield sugars which will have their own effect, osmotic or metabolic, on the response of cultures. As starches and their hydrolytic products are not biologically inert, they are expected to have limited use and that only for explants whose response is not adversely affected by the presence of starch in the medium.

The properties of 'Isubgol husk', including its polysaccharide like and colloidal nature, reported resistance to enzymatic activity, good gelling ability even in cold water, and reasonable clarity in gelled form, are indicative of its potential to become an universal gelling agent in tissue culture media. The husk even after autoclaving remained suspended and formed a gradient once the medium solidified. Despite this, the media remained reasonably transparent and offered no serious problems to the explants. In comparison, the media gelled with the agar used in the present study appear almost opaque. However, this problem with 'Isubgol' gel can be overcome by using purified mucilage that is devoid of husk. Stickiness of Isubgol increased with the increase of the concentration of Isubgol in combinations with agar even after autoclaving. A frequent problem in case of agar and Isubgol media is cracking. Local price of 'Isubgol' is about one-twentieth that of agar used in this study. 'Isubgol' reduced price of gelling agent approximately by 47.5 % in plant tissue culture media (Table 3). There are lots of commercial tissue culture laboratories in our country. Agar has remained to be the most expensive constituent of culture media anywhere in any time. The use of 'Isubgol' along with agar can reduce the cost of gelling agents in these laboratories. Besides, the study was conducted on commercially important crop plants like sunflower varieties. So, from the local and global perspective, this study shows an economic feasibility of plant tissue culture media.

Table 3: Cost reduction of agar + Isubgol husk gelled Media

Gelling agent	Agar/lit MS medium (gm)	Isobgol/ lit MS medium (gm)	Cost (Rs)	Cost Reduction
Agar	7.0	0.0	70.00	-
Agar+ Isubgol	3.5	3.5	36.75	47.5%

CONCLUSIONS

Gelling performance of used combinations was compared so it could be concluded that 'Isubgol' does not hamper the effect of hormones. It was also observed that culture media contained Group A, C, E gelling agent was not appropriate for the growth of sunflower. Group B and Group D media are suitable for the growth of sunflower. The properties of 'Isubgol husk', including its polysaccharide like and colloidal nature, reported resistance to

enzymatic activity, good gelling ability even in cold water, and reasonable clarity in gelled form, are indicative of its potential to become an universal gelling agent in tissue culture media.

Local price of 'Isubgol' is about one-twentieth that of agar used in this study. 'Isubgol' reduced price of gelling agent approximately by 47.5 % in plant tissue culture media. There are lots of commercial tissue culture laboratories in our country. Agar has remained to be the most expensive constituent of culture media anywhere in any time. The use of 'Isubgol' along with agar can reduce the cost of gelling agents in these laboratories.

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