Biological Sciences Section

Pak. j. sci. ind. res., vol.40, nos.1-4, January-April 1996

EFFECT OF INITIAL CALLUS SIZE/QUANTITY, AGAR, PHYTAGEL ANDTHIAMINE ON FURTHER GROWTH OF LEAF AND PETIOLE-DERIVED CALLUS CULTURES OF *GOSSYPIUM HIRSUTUM* L.

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(Received August 19,1996; revised August 19,1996)

Effects of initial size of cotton callus on its further growth *in vitro* were investigated. It was observed that reduction in initial size/quantity of cultured callus resulted in reduced growth rate. Phytagel was found to be a superior gelling agent compared to agar. Thiamine at its higher concentration promoted the callus growth compared to the lower concentration.

Key words: Callus size, Gelling agent, Thiamine, Cotton.

Introduction

Callus growth is affected by many factors like the culture medium, incubation temperature, thiamine and the gelling agent. The initial inoculum (size) also affects growth of the callus cultured *in vitro*. Dixon [1] mentioned that if the size of the callus tissue separated from the explants was small, further growth could be slowed/reduced. Akhtar [2] also found that further growth of callus was reduced if the initial inoculum callus size was reduced. Akhtar [2], Zimmerman and Robacker [3] found that phytagel was a superior gelling agent than agar and thiamine, a callus growth promoter. The present studies were, therefore, carried out to investigate the effect of the initial size/quantity of callus on further growth of cotton.

Materials and Methods

Callus induction. Callus was induced from leaf and petiole tissues of *Gossypium hirsutum* L. cv. Acala SJ2 on a medium containing Murashige and Skoog [4] basal salts plus 2,4-D and BAP (0.1 mg l^{-1}), *myo*-inositol (100 mg l^{-1}) and glucose (30 g l^{-1}). These calli were subcultured every 28 days on the same medium for ten months to provide sufficient material for experimentation. The experiments described here were carried out using the same medium as used for the callus induction to minimize the growth differences due to carry over effects. The callus used was fast growing.

Measurement of growth. Pre-weighed disposable sterilized petri dishes containing 15 cm^3 culture medium were inoculated with callus tissues and the inoculated dishes were re-weighed to obtain the initial weight of the callus inoculum. The cultures were incubated at 28 ± 2° C for 28 days. Relative Growth Rate (RGR) (per week) was recorded according to the following formula.

$$R.G.R. = [\ln (\text{Final wt.}) - \ln (\text{Initial wt.})] / 4 \text{ weeks}$$

where In is natural log

Experiment 1. This experiment was designed to investigate the effect of size of leaf callus pieces on growth of callus. An equal quantity (about 1g) of the callus was used in all treatments/replications. Agar and phytagel were used at concentrations of 0.8 and 0.2%, respectively. All treatments had 5 replications. The callus was placed with petri dishes as described in Table 1.

Each litre of the culture medium contained Murashige and skoog [4] salt, myo-inositol (100 mg), 2,4-D, BAP (0.1 mg) and glucose (30 g.)

Experiment 2. In this experiment the first 10 treatments of experiment no. 1 were tested for petiole callus growth having thiamine 20 mg l^{-1} only. All other conditions were the same as in experiment no. 1. All treatments consisted of 5 replications.

TABLE 1.

Tr.	Gel	Thiamine	Placement of callus
T1	Agar	20 mg l^{-1}	whole quantity of callus kept at one place in the petri dish
T2	"	"	whole quantity divided equally and kept at two places
T3	"	"	whole quantity divided equally and kept at three places
T4	"	"	whole quantity divided equally and kept at four places
T5	"	"	whole quantity divided equally and kept at five places
T6	Phytagel	"	whole quantity of callus kept at one place in the petri dish
T7	"	"	whole quantity divided equally and kept at two places
T8	"	"	whole quantity divided equally and kept at three places
T9	"	"	whole quantity divided equally and kept at four places
T10	"	"	whole quantity divided equally and dept at five places

T11-T15 were like T1-T5 but had 0.4 mg thiamine l^{-1}

T16-T20 were like T6-T10 but had 0.4 mg thiamine l^{-1}

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Data analysis. Data were subjected to analysis of variance using minitab statistical package and means and standard error were computed. Means were compared by Tukey's method [5].

Results and Discussion

Leaf callus cultures. Statistical analysis of data obtained showed that callus growth rate was affected by the size of the initial inoculum. RGR was highest for large sized callus while it reduced in case of small sized callus (Fig. 1a). The mean RGR value for large sized callus was significantly different from all other means ($P<0.01$) while all other sizes gave the same results.

Phytagel gave higher RGR particularly for smaller sizes compared to agar at all sizes ($P<0.05$)(Fig. 1a).

The two levels of thiamine produced highly significant effects on RGR (Fig. 1b) of the callus ($P<0.01$). Thiamine at 20 mg l^{-1} gave higher RGR for the biggest and the smallest sizes than for the intermediate ones.

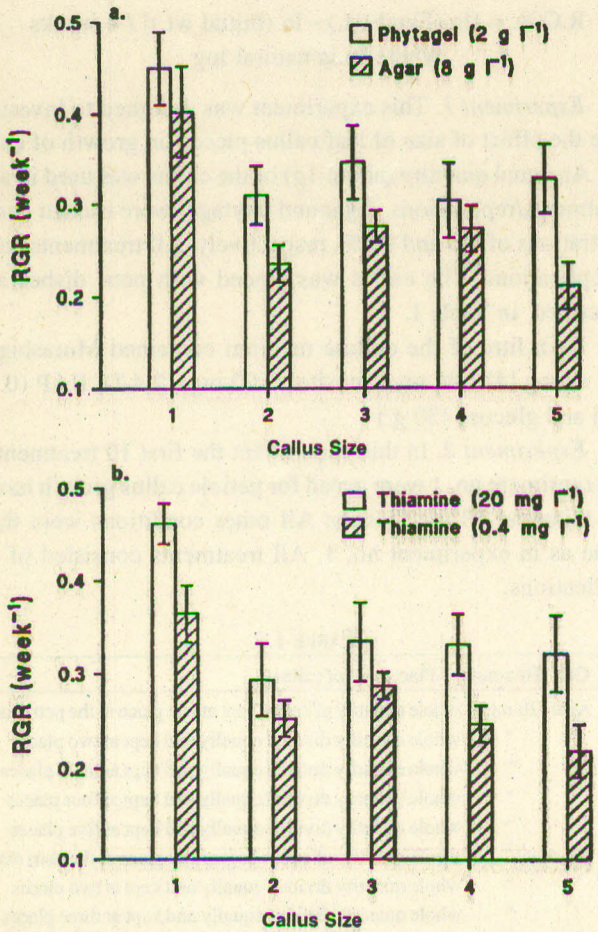

Fig. 1ab. Effect of size of initial inculum, gelling agents (a) and thiamine (b) on relative growth rate of leaf callus (n=6). The vertical bars represent standard error of means (experiment No.1.

Petiole callus cultures. The results obtained (Fig.2) showed a similar trend in RGR giving the highest RGR for the largest size of callus. Reduction in callus size resulted in reduced callus growth rate on either of the medium. Mean values for different sizes were statistically different from each other ($P<0.01$).

Gelling the medium with phytagel gave a higher RGR (0.374) as compared to agar solidified medium (0.270) (Fig. 2). The phytagel gave higher values especially for smaller sizes. Analysis of variance of the data showed significant differences between the two gelling agents ($P<0.05$).

The results obtained in the present investigations revealed that callus growth was affected by the initial size/quantity of callus. The best callus size/quantity of those evaluated was found to be about 1 g . Reduction in this quantity resulted in reduced growth rate. These results do not agree with those of Williams [6] who used the same cotton species (*Gossypium hirsutum* L.) but a different genotype. He reported maximum stem callus tissue proliferation using 50-127 mg inoculum. The disagreement may be due to different plant material used (leaf and petiole callus in the present case). It has been well documented that the response of various explants even from the same genotype vary *in vitro* [2]. Akhtar [2] carried out detailed studies regarding the nutritional and cultural requirement of callus tissue derived from leaf and petiole explants of Acala SJ2 and found that callus from both explants had different nutritional requirements. The superiority of phytagel over agar may be due to higher calcium concentrations in phytagel [7,8] because calcium has been found to promote growth of callus of the same genotype [1]. El-Hadrami *et al.* [9] also found phytagel to be superior over agar. Akhtar [2] and Williams [6] found thiamine to promote callus growth in *Gossypium hirsutum* L.

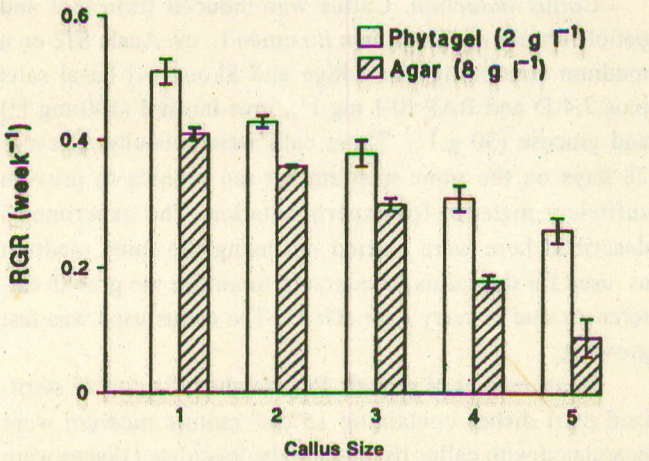

Fig.2. Effect of size of initial inoculum and gelling agents on relative growth rate of petiole callus (n=3). The vertical bars represent standard error of means (Experiment No.2.

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