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Effect of antioxidants on callus browning of *Glycyrrhiza glabra*

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Abstract

Objective: In the present study, adsorbents and antioxidants incorporated in to callus cultures of *Glycyrrhiza glabra* to study their potency in checking callus browning. Methods: Activated charcoal was added as an adsorbent at varying concentrations of 100, 500 and 1000 mg/l. Ascorbic acid and cysteine were added as antioxidants each at concentrations of 50, 100 and 150 mg/l grown on Modified MS basal medium supplemented with growth regulators (BA, Kn, IAA, NAA). Result: Browning was effectively controlled by activated charcoal and ascorbic acid while cysteine did not show any inhibition. Discussion: Browning is a major problem affecting tissue culture studies of medicinal plants containing polyphenolic compounds that cause oxidation reactions leading to browning. Hence browning could be controlled by using adsorbents that adsorb the oxidative by products or by using antioxidants that prevent the oxidation reactions.

Key words: *Glycyrrhiza glabra*, antioxidants, callus browning, necrosis.

1. Introduction

Glycyrrhiza glabra Linn, (Liquorice) is a hardy perennial legume, about 0.6 m tall, reported to be found wild in countries of west Asia and in sub-Himalayan tracks [1]. It contains starch, lignin a brown acrid resin called asparagin, and a characteristic principle known as glycyrrhizin [2]. It is a popular ingredient in almost all cough medicines on account of its soothing properties [3]. Extract of Liquorice is employed almost exclusively as a vehicle for disguising the taste of nauseating medicines, having a remarkable power of converting the flavor of acrid or bitter drugs, such as Mezereon, Quinine or Cascara [4]. It can be safely employed in the treatment of gastric ulcer. It has also been proved to have anti-inflammatory, anti viral, hepato-protective and estrogenic activities [5]. Almost all the activities and uses of the Liquorice are attributed to the presence of glycyrrhizin [6]. When explants of *Glycyrrhiza glabra* were cultured,

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they produced intense brown colored substances in the culture that led to necrosis. This was observed during callus initiation, proliferation and growth. Callus browning has also been observed in other tissue culture studies [7,8]. Several attempts have been made to control this browning by using adsorbents and antioxidants [9,10]. In the present study activated charcoal as an adsorbent and antioxidants like ascorbic acid and cysteine were added in to the culture medium in an attempt to control browning.

2. Materials and Methods

2.1 Plant Material

The explants consisting of leaf and the stem of *Glycyrrhiza glabra* were cut into 1cm long pieces and washed thoroughly with running tap water for 15 minutes followed by tween-60 (1%). They were then washed with distilled water to remove the traces of surfactant, followed by washing with 80% ethanol for 60 seconds. These explants were sterilized by mercuric chloride 0.1% (w/v) for 3-5 minutes and again rinsed thoroughly with sterile distilled water. The explants were inoculated on to the surface of modified MS medium [11].

2.2 Culture Medium

Modified MS medium containing 3% (w/v) sucrose was used in all experiments as basic media. The pH of medium was adjusted to 5.70 ± 0.02 prior to the addition of 0.75% (w/v) agar and autoclaved at 15 psi for 21 minutes.

All the cultures were incubated in culture room maintained at 25 ± 20C temperature, 16 hours photoperiods under fluorescent light (1600 lux) at relative humidity of 50 to 60 %. The medium was dispensed in 20ml aliquots into 25x150 mm culture tubes and 40 ml in 100 ml conical flask. Activated charcoal was added as an adsorbent at varying concentrations of 100, 500 and 1000 mg/l. Ascorbic acid and cysteine were added as antioxidants each at concentrations of 50, 100 and 150 mg/l.

Table 1: Effects of ascorbic acid, activated charcoal and cysteine on callus growth of leaf and stem explants of *Glycyrrhiza glabra.*

Group (n = 6)	Treatment	Concentration (mg/l)	Growth Index	
			Leaf explants	Stem explants
1	Activated charcoal	100	2.15 ± 0.07	2.12 ± 0.06
2		500	2.42 ± 0.12[a]	2.30 ± 0.10[a]
3		1000	2.40 ± 0.12[a]	2.25 ± 0.08[a]
4	Ascorbic acid	50	2.04 ± 0.09	2.10 ± 0.10
5		100	2.33 ± 0.14[a]	2.40 ± 0.14[a]
6		150	2.18 ± 0.09[a]	2.20 ± 0.10[a]
7	Cysteine	50	2.05 ± 0.13	2.12 ± 0.22
8		100	2.25 ± 0.34[a]	2.36 ± 0.35[a]
9		150	2.15 ± 0.55	2.22 ± 0.15[a]
10	Control		2.00 ± 0.06	2.01 ± 0.05

Readings are mean ± SE. (n = 6)
a : significant ($p<0.05$) Vs control

2.3 Callus Proliferation

Modified MS basal medium supplemented with growth regulators (BA, Kn, IAA, NAA) either alone or in different combinations were used for proliferation of explants. All the cultures were transferred to fresh medium after 3-4 weeks duration. The callus growth, observed by using the Growth index, was evaluated after 4 weeks of incubation. Callus was regularly sub cultured on the fresh medium after every 4 weeks of incubation.

The leaf and stem explants were divided into ten groups containing five explants in each group in the following manner: Group 1: medium containing activated charcoal 100 mg/l, Group 2: medium containing activated charcoal 500 mg/l, Group 3: medium containing activated charcoal 1000 mg/l, Group 4: medium containing ascorbic acid 50 mg/l, Group 5: medium containing ascorbic acid 100 mg/l, Group 6: medium containing ascorbic acid 150 mg/l, Group 7: medium containing cysteine 50 mg/l, Group 8: medium containing cysteine 100 mg/l, Group 9: medium containing cysteine 150 mg/l, Group 10: control i.e. medium without any adsorbent or antioxidant. The effects of adsorbent and antioxidants were studied by observing the growth indices and the color of the callus as compared with that of the control. The growth indices were observed in terms of fresh weight as reported in earlier studies [12, 13].

3. Results

The explants in the control group showed browning after 12 - 15 days of growth and a decreased growth rate. Activated charcoal inhibited the browning of the callus however it was not fully checked. The most favorable level was 500 mg/l, which brought maximum inhibition in browning with growth index of 2.42 and 2.30 respectively for leaf and stem. The subsequent higher level controlled browning but at the same time suppressed the callus growth. Browning was also inhibited by ascorbic acid, the best being shown by at the concentration of 100mg/l with growth index of 2.33 and 2.40 respectively for leaf and stem explants respectively. Cysteine did not show any effective control of callus browning though it didn't affect adversely the callus growth. The growth indices of all the groups are shown in table - 1.

4. Discussion

Major problems in callus culture studies are mainly due to alterations in the activities of enzymes phenolase and peroxidase [14]. Increased phenolase and decreased peroxidase activities due to more pro oxidative oxidation of phenolic compounds and non-availability of substrate for peroxidase activity are the main factors involved in browning of callus tissues [15]. Antioxidants and adsorbents added in tissue culture media affect the growth, color and texture of callus cultures as reported in earlier studies [16]. In our study use of ascorbic acid and cysteine effectively promoted the growth of callus and ascorbic acid significantly prevented browning which may be due to its antioxidant activity that prevented the formation of oxidative by products responsible for browning [17]. Activated charcoal also prevented browning which may be due to its adsorbent properties. Other studies have also proved ascorbic acid as an effective adsorbent to control browning [18,19]. The use of ascorbic acid as a growth promoter and an effective antioxidant in controlling browning is also reported in earlier studies [20,21]. Hence from our study we can conclude that ascorbic acid and activated charcoal can be used as antioxidant and adsorbent to prevent browning in the tissue culture studies of liquorice.

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