Effect of sodium sulfate on in vitro organogenesis of tobacco callus

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Abstract. Callus of tobacco (*Nicotiana tabacum* L. cv. Wisconsin 38) was grown on callus-proliferating (CP) and shoot-forming (SF) media with elevated sodium sulfate (Na$_2$SO$_4$) concentrations either in the light or dark for more than one year. An increase in Na$_2$SO$_4$ concentration resulted in a decrease in callus growth index, an increase in percent dry weight of callus tissues grown on both media, and a decrease in both number of calli forming shoots and number of shoots per callus in SF medium. The CP callus grown in the light spontaneously began to form shoots after the 5th monthly transfer, and spontaneous root formation occurred after the 16th transfer in the presence of 0.75 and 1.0% Na$_2$SO$_4$. Both water ($\psi$) and osmotic ($\psi_s$) potentials of the callus increased with increasing Na$_2$SO$_4$ concentration; and callus exhibited greater $\psi$ and $\psi_s$ in the light than dark for both CP and SF media.

Introduction

Soil salinity is a growing problem in cultivated land throughout the world, particularly where agricultural production is heavily dependent upon irrigation. While physical methods have been traditionally used to alleviate the problem, more recent efforts have focused on producing salt tolerant plants via breeding and genetic manipulation [8]. In addition to conventional methods of plant breeding by which improvement of plants to salinity stress has been relatively successful [15], plant tissue culture techniques offer alternative means of producing salt tolerant plants.

Salt tolerant cell lines of several crop plants have been selected using plant tissue culture [16, 20]. Selected cell lines of rice [5] and alfalfa [4] respectively were capable of growth in the presence of 1 and 2% NaCl. In tobacco and pepper, cell lines tolerant to 1 and 2% NaCl were able to retain this salt tolerance even after they were grown for several passages in salt-free medium [6]. The salt tolerance trait was not only retained in the cells through mitotic division, but also was transmitted to subsequent generations, through plant regeneration and seed production. Plants regenerated from tolerant cells of haploid *Datura innoxia* also exhibited salt tolerance in the resulting callus cultures [24]. It was also reported that plants regenerated from tobacco callus tolerant to 0.64% NaCl showed salt tolerance for the two

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subsequent generations tested and could be irrigated with water containing 3.34% NaCl [14]. However, plant regeneration from salt-tolerant cells has been rather limited. In most cases, by the time the cell selection was made the cells had lost their regenerative capacity.

The purpose of this study was to determine the behaviour of tobacco callus, cultured under shoot-forming and callus proliferating conditions for more than one year, to sodium sulfate (Na$_2$SO$_4$). Na$_2$SO$_4$ was used since it is the predominate salt which accumulates in irrigated and non-irrigated Canadian Prairie soils, and is present at elevated levels in over 500,000 ha in Alberta.

**Materials and methods**

**Culture conditions**

Callus cultures of tobacco (*Nicotiana tabacum* L. cv. Wisconsin 38) was initiated from pith-phloem tissues and maintained on a modified Murashige and Skoog’s (MS) [13] medium following the method of Thorpe and Murashige [23]. Investigations on Na$_2$SO$_4$ effects were conducted by transferring callus (ca 2 x 2 x 4 mm$^3$) to 50ml Erlenmeyer flasks each containing 25ml of either shoot-forming (SF) or callus-proliferating (CP) medium, described below, supplemented with 0, 0.75, 1.0, and 1.5% (w/v) Na$_2$SO$_4$. There were two calli per flask and 10 flasks per treatment. Cultures were transferred monthly and kept at 27 ± 1 °C either in the dark or in the light with a 16 h daily photon fluence rate of ca 80 μmol·m$^{-2}$·s$^{-1}$ (380–800 nm) from Sylvania GRO-lux F40T12 Gro-WS fluorescent lamps.

The SF medium contained MS salts, adenine sulfate (9.8 x 10^{-4} M), L-tyrosine (5.6 x 10^{-4} M), sodium phosphate, monobasic monohydrate (1.2 x 10^{-3} M), myo-inositol (5.6 x 10^{-4} M), thiamin·HCl (1.2 x 10^{-6} M), indole-3-acetic acid (IAA, 10^{-5} M), kinetin (10^{-5} M), sucrose (3%), and Difco-bacto agar (0.9%). The CP medium consisted of MS salts, myo-inositol, IAA, sucrose, and agar similar to those in SF medium, and the following constituents: casamino acid, (acid hydrolysed casein, Difco Lab., Detroit, Michigan) (1%); nicotinic acid (4 x 10^{-6} M); pyridoxin·HCl (2.5 x 10^{-6} M); thiamin·HCl (3.3 x 10^{-7} M); glycine (27 x 10^{-6} M); kinetin (2.5 x 10^{-6} M). Constituents were added to the medium and the pH adjusted to 5.7 before autoclaving at 1 kg/cm$^2$ and 121 °C for 15 min.

Growth of callus in terms of callus growth index (CGI) and percent dry weight was measured for four months at monthly intervals. For CGI, callus was rated using numerical values 0, 1, 2, 3, and 4 which represent dead, poor, fair, good, and excellent, respectively, according to growth and appearance of the callus. Organogenetic response was evaluated in terms of number of shoots per callus, and number of calli that formed shoots or roots.

**Measurement of water and osmotic potential**

Potentials were measured using a Wescor thermocouple hygrometer sample chamber C-52 and a Wescor Dew Point Microvoltmeter HR-33T. Samples